Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Fakulteten för landskapsarkitektur, trädgårds- och växtproduktionsvetenskap

Bioelectromagnetics for improved crop productivity

– An introductory review with pilot study of pre-sowing treatment of tomato

Bioelektromagnetik för ökad produktivitet hos grödor

- En inledande genomgång med pilot-försök av försåddsbehandling av tomat

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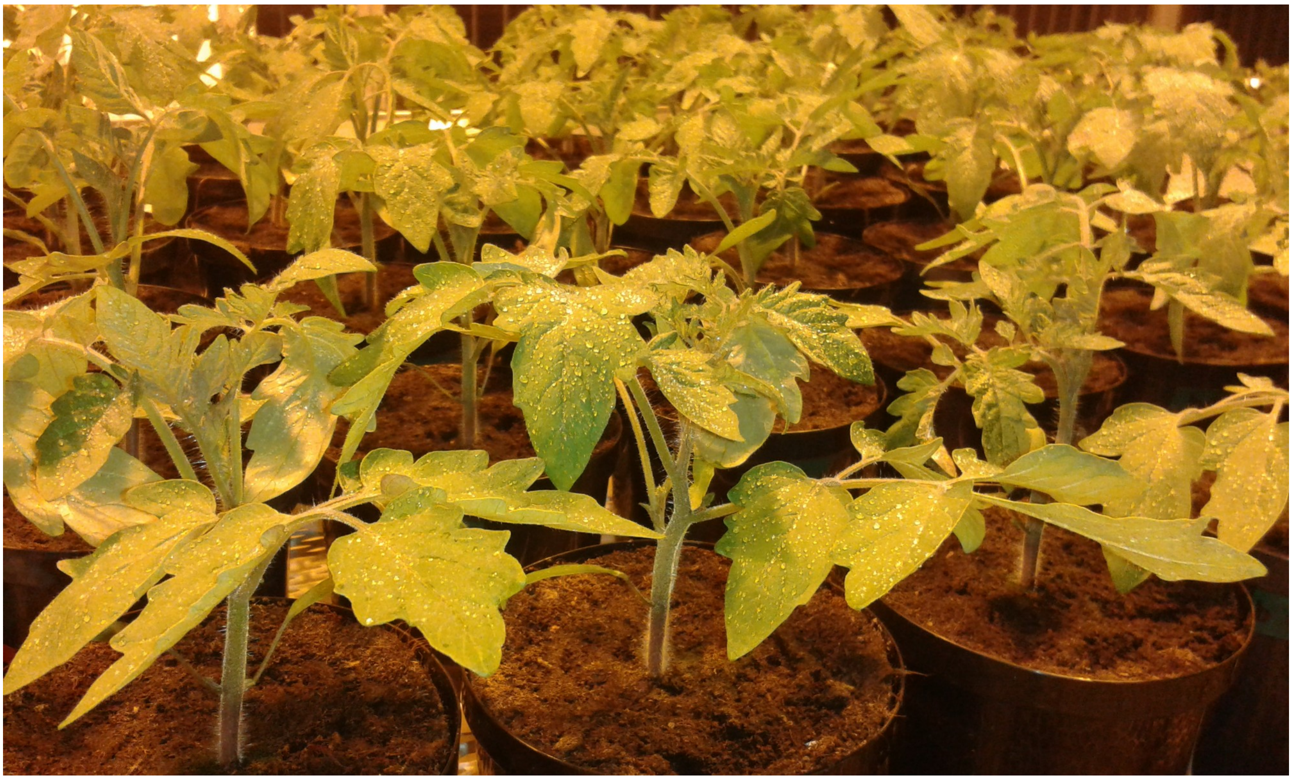

Självständigt arbete • 15 hp
Hortonomprogrammet
Alnarp 2015

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Handledare: Siri Caspersen, SLU, Institutionen för biosystem och teknologi

Btr handledare: Mattias Larsson, SLU, Institutionen för växtskyddsbiologi

Examinator: Håkan Asp, SLU, Institutionen för biosystem och teknologi

Omfattning: 15 hp
Nivå och fördjupning: G2E
Kurstitel: Kandidatarbete i biologi
Kurskod: EX0493

Program/utbildning: Hortonom
Examen: Kandidatexamen i biologi
Ämne: Biologi

Utgivningsort: Alnarp
Utgivningsmånad och -år: Juni 2015
Omslagsbild: Tomater från pilotförsöket
Elektronisk publicering: http://stud.epsilon.slu.se

Nyckelord: *horticulture, electromagnetic treatments, electromagnetic fields, electric fields, magnetic fields, effects, plants, magnetoreception, ion cyclotron resonance, solanum lycopersicum*

SLU, Sveriges lantbruksuniversitet
Fakulteten för landskapsarkitektur, trädgårds- och växtproduktionsvetenskap
Institutionen för biosystem och teknologi

Preface

First of all, I would like to extend a thank you to all the people who has helped me make this work possible. Especially my supervisor ***Siri Caspersen*** (SLU), who has been a huge support and motivation throughout the whole process of this work and offered much of her cunning and time in order for this to become a reality. I would also like to thank ***Mattias Larsson*** (SLU), for the help in the planning of the project and the experiments. I would like to thank ***Rachel Muheim***, of Lund University, for making the electromagnetic treatments possible by providing the materials and facilities used for the exposures, as well as inspiration and insights into the phenomena of biological magnetoreception. Furthermore, I would like to thank ***Simon Jeppson***, of Nord-Gen, for the support and provision of materials used in the seed germination tests. I would also like to thank ***Flemming Yndgaard***, for the help with the statistical analyses of the experimental data. Last but not least, i would like to thank ***Partnerskap Alnarp***, for the funding of the experiments. Without the help of these people, this work would not have been possible.

I would also like to mention that I am neither a physicist nor an expert in the science of plant bioelectromagnetics. It is a highly advanced and complex subject, and to delve into it the way I have done the past six months has been extremely interesting but sometimes also very frustrating. There is a vast amount of literature in the subject, with many reported effects and proposed theories. Even so, there is still no general consensus in many areas of the subject. In the literature, the terminology and quality of the actual physics also vary, making it difficult to discern what fields has been used, their intrinsic qualities and how they have been applied. It is because of these difficulties, and the striking confusion that I believe many horticulturalists experience when trying to understand these subjects, that I felt this work was needed. In this work I wanted to offer an inspiring and easy to follow introduction into the subject, describe some mechanisms for biological interaction between electromagnetic fields and plants and assess the potential use of bioelectromagnetics for improved crop productivity. It is supposed to make further browsing of the literature easier for the reader, and possibly separate some facts from fiction in a study veiled in controversy. Though, as said, I am no expert and do not claim to know all the answers, or to offer all the various mechanisms in a highly precise manner. Should there be something I have missed or misinterpreted, feel free to make contact and help me in my own understanding of this puzzling field of study. I hope you will enjoy the reading, and that this might be the introduction you need to start pursuing the exciting world of interactions between electromagnetic fields and plants.

Anton Samuelsson
anton.samuelsson@live.se

Alnarp, Sweden
June 2015

Abstract

Various electromagnetic (EM) treatments has been investigated for their potential in improving crop productivity over the past century. There is today an increasing amount of scientists advocating EM treatments as an organically compatible method for improving plant growth and yield. However, failure to produce repeatable effects and defining causal mechanisms has made it a study of much controversy and debate. The subject requires in-depth interdisciplinary knowledge, making it inaccessible for the majority of biologists, horticulturists and growers. In this combined literature review and pilot study, these issues are addressed by providing a comprehensive introductory review of the topic plant bioelectromagnetics and its potential horticultural usability. A vast amount of literature has been reviewed to assess the nature of electromagnetic fields (EMF), what effects of horticultural relevance has been observed, what the fundamental mechanisms behind studied effects might be, and ultimately the potential of using EM treatments for improved crop productivity. A pilot study investigating the effects of an EM pre-sowing treatment of tomato, *Solanum lycopersicum,* seeds is also presented both as a means of assessing the usability of EM treatments and providing an example of a study in bioelectromagnetics. In the pilot study, a 50 Hz non-uniform sinusoidal EMF of 160, 40 and 9 mT was used to treat the seeds for 15 or 30 minutes, with un-exposed seeds as controls. The exposure was also set in three background static magnetic field (SMF) conditions; one where Ion Cyclotron Resonance conditions for calcium were met (65.8 µT), one where they were not (68.5 µT) and one where only the natural geomagnetic field was present (46.9 µT). The results indicate that a background SMF of 65.8 µT has a significantly inhibitory effect on germination of *S. lycopersicum* ($p<0.01$), while the EMF exposure had no significant effects on germination or subsequent growth. It should however be noted that this data is indicative and needs further validation with better experimental conditions. The literature review found that EM treatments have shown many horticulturally interesting effects on plants, and that EM treatments has the potential for horticultural use. However, since they are hard to predict and reproduce it is proposed that extensive species-specific and exposure-specific research should be conducted prior to field application. Many biological effects and mechanisms has also been described and proposed, but much is still debated and more research is needed in these areas since they are key in improving the predictability and accuracy of EM treatments.

Sammanfattning

Under det senaste århundradet har olika elektromagnetiska (EM) behandlingar undersökts för deras potentiellt gynnsamma effekter på grödors produktivitet. Idag har EM behandingar föreslagits av flera forskare som en möjlig ekologiskt anpassad metod för ökad tillväxt och skörd hos växter. Svårigheter att upprepa resultat och förklara bakomliggande orsaker har dock gjort ämnet mycket kontroversiellt och omdebatterat. Elektromagnetiska studier kräver djupa interdisciplinära kunskaper vilket gör dem otillgängliga för många biologer, hortonomer och odlare. I denna kombinerade litteraturstudie och pilotstudie addresseras dessa frågor genom en omfattande inledande genomgång av ämnet växtinriktad bioelektromagnetik samt dess potentiella hortikulturella användbarhet. En stor mängd studier har bearbetats för att bedöma EMF's natur, vilka effekter av hortikulturell relevans som har studerats, vilka fundamentala mekanismer som påstås ligga bakom dessa effekter, och slutligen användbarheten av EM behandlingar för ökade skörder. En pilotstudie som undersökte effekten av en EM försåddsbehandling av tomat, *Solanum lycopersicum,* presenteras också som ett exempel på en EM studie och som en del av användbarhetsbedömningen. I studien behandlades fröerna med ett 50 Hz sinusoidalt EMF av styrkan 160, 40 och 9 mT i 15 och 30 minuter. Behandlingen utfördes även I tre olika bakomliggande statiska magnetfält (SMF); ett där villkor för cyklotron-resonans för kalcium var uppfyllt (65,8 µT), ett där det ej var uppfyllt (68,5 µT), och ett där endast det geomagnetiska fältet var närvarande. Resultaten indikerar att ett bakomliggande SMF på 65,8 µT hade en signifikant inhiberande effekt på groningen av *S. lycopersicum,* medan det EMF'et inte visade någon påverkan på groning eller tillväxt. Det bör dock poängteras att dessa data endast är vägledande och bör valideras under bättre experimentella förutsättningar. Litteraturundersökningen visar att EMF kan ge upphov till många effekter av intresse för hortikulturell verksamhet och har potential för hortikulturell användning. De är dock svåra att förutspå och upprepa, varpå det föreslås att utförlig forskning bör bedrivas för varje enskild art och behandlingstyp innan applicering i fält. Många biologiska effekter och bakomliggande mekanismer har också beskrivits och föreslagits, men mycket är fortfarande omdebatterat och mer forskning behövs då de är nyckeln till att öka förutsägbarheten och noggranheten av EM behandlingar.

Abbrevations

AC = Alternating Current

AP = Action Potential

EF = Electric field

ELF = Extremely Low Frequency

EM = Electromagnetic

EME = Electromagnetic Energy

EMF = Electromagnetic Field

EMR = Electromagnetic Radiation

EMS = Electromagnetic Spectrum

DC = Direct Current

GMF = Geomagnetic Field

ICR = Ion Cyclotron Resonance

IPR = Ion Parametric Resonance

MF = Magnetic Field

PEMF = Pulsed Electromagnetic Field

ROS = Reactive Oxygen Species

RW = Radiowave

SEF = Static Electric Field

SMF = Static Magnetic Field

VP = Variation Potential

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1 Introduction

1.1 Overview

1.1.1 Background

Bioelectromagnetism is a discipline that examines the electric, magnetic and electromagnetic (EM) phenomena that arise in biological tissue (Malmivuo and Plonsey, 1995). It basically studies the interaction between electromagnetic fields (EMF) and biological entities; be it exogenously applied or intrinsic EMF's, the behaviour of excitable cells, biological sensory mechanisms for EMF's or the electric currents of nerve cells. The discipline is strongly tied to the general theory of electromagnetism. The study of ***Bioelectromagnetics***, on the other hand, focus only on interactions between exogenously applied EMF's and biological entities.

Bioelectromagnetics has been studied as a means of improved crop productivity for the past century, with mixed claims of its effects, usefulness and potential (Pietruszewski, 2011; Volkov, 2006). In the early 1900's, large-scale agricultural studies involving static electric fields (SEF) showed great promise with greatly increased yields (Kinahan, 2009; Volkov, 2006). However, when the studies failed to produce repeatable results and no cause-effect mechanisms could be defined, the science eventually became too controversial and costly to continue. However, as of the past decades, technological and theoretical advancements and the need for environmentally friendly cultivational techniques has yet again awakened an interest in the subject. With a new focus on static magnetic fields (SMF) and time-varying EMF's, EM techniques are again proposed as methods to improve quality and quantity of both plants and yields (Bilalis et al., 2012; Dhawi, 2014; Pietruszewski, 2011; Tanaka et al., 2010).

However, literature and studies in the subject often requires an in-depth knowledge of theoretical physics, technical engineering and biology. In horticultural science this is rarely the case, with general confusion on the subject and a remaining controversy as a result. The difficulty of defining biological mechanisms, economic costs and the general inconsistency of results has also made many scientists weary of conducting large-scale studies on the subject of plant bioelectromagnetics and its horticultural applications (Volkov, 2006). Even though many potentially useful effects has been found, bioelectromagnetics is still a question-mark for the many and the study of a few.

This work seeks to erase that questionmark and offer the inter-disciplinary knowledge needed for a basic understanding of plant bioelectromagnetics and the biological interactions between EMF's and plants, as well as assess its potential use for improved crop productivity.

1.1.2 Aim, objective, issues & audience

The aim of this work is to clarify, make available and increase the interest for the study of plant bioelectromagnetics. By assessing its potential horticultural usefulness and providing the fundamental frame-work needed for the study, development and use of plant bioelectromagnetics, the hope is to bring the study into the public sphere and turn it into a bigger and more serious area of research for the potential benefits of horticulture.

The objective is to deliver a comprehensive introductory review of plant bioelectromagnetics and assess its usability in horticulture.

Issues sought to be answered are:

- *What are EMF's and where can they be found?*
- *What effects of horticultural relevance has been found in crops exposed to EMF's?*
- *What biological effects & mechanisms are proposed to account for these effects?*
- *What potential do bioelectromagnetics show for horticultural use?*

This work is directed to an audience of biologists, horticulturalists and growers which sometimes need more inter-disciplinary knowledge to fully comprehend the different phenomena attributable to plant bioelectromagnetics.

1.1.3 Demarcation

This work will not look into the technical parts of bioelectromagnetics, nor will it investigate specific types of application or exposure systems using EMF's. It will only look at the terminology and nature of electromagnetism, the effects of and possible interactions between EMF's and plants, and investigate its potential usability for improved crop productivity. Hence, it will neither look into various EM treatments associated with diagnosing, heating or disinfecting plant material. Only EMF's in the lower end of the electromagnetic spectrum (EMS) will be investigated, with a focus on static and extremely low frequency (ELF) EMF's. The usability will also be assessed by conducting a pilot-study with electromagnetic (EM) treatment of tomato seeds.

1.1.4 Disposition

Given the width of the subject of plant bioelectromagnetics, many different areas of study will be addressed in this work. Thus, this work is structured with a bottom-up approach, describing one area of study at a time, each section building upon the last. This work is based on both a thorough literature review and a practical execution of a pilot study with an EM pre-sowing treatment of tomato seeds. To separate the theoretical from the practical, the entire literature review is presented in the Introduction, culminating with the pilot study in the Material & Methods and Results section. Both will however be represented in the discussion to connect them with the stated issues of this work.

To start off, electromagnetism will be introduced from a purely physical point of view to help in the understanding of the nature of EMF's and how they are described in the literature. After that, earthly occurring EMF's and their relevance for life is discussed. Then, an in-depth review of plant bioelectromagnetics follows. In this section, the history and findings of horticultural bioelectromagnetics for improved crop productivity is presented, a short introduction to the electrophysiology of plants, and then what biological effects & mechanisms are presumed to account for the observed effects. In the following section Materials & Methods, methodology for the pilot study is presented, while in the Results-section, the outcome of the pilot study is presented. The work ends with a discussion of the data of the pilot study, observed effects of EMF's on plants and its potential usability in horticulture, proposed biological mechanisms and the current scientific understanding for EMF interactions with plants, as well as general conclusions as of the stated issues of this work.

1.1.5 The pilot study

The pilot study was conducted as a means of investigating the possible horticultural use of EM treatments, as well as providing an example of a bioelectromagnetic study. Tomato, *Solanum lycopersicum,* was chosen for the study due to it being of commercial interest and its genome being fully sequenced (The Tomato Genome Consortium, 2012). Furthermore, the F1-hybrid variety *Solanum lycopersicum* 'Super Sweet 100' was chosen due to its uniform genetic makeup and fast indeterminate growth. The EM treatment chosen was a 50 Hz sinusoidal EMF since similar treatments has shown enhancing effects on germination and early growth of *Solanum lycopersicum* (De Souza et al., 2006, 2005; Efthimiadou et al., 2014; Jedlicka et al., 2014). The time-varying EMF exposure was also set in three different SMF environments, discussed in further detail in the following text.

The study was split up in two parts; germination tests and a cultivation experiment. The main objective of the germination test was to determine if differences in the background SMF environment has an effect on the outcome of the time-varying EMF treatment. This since many studies lack a recording of the local geomagnetic field (GMF), and it thus is difficult to rule it out as a possible parameter affecting end results (Maffei, 2014). An interesting theory involving the combination of SMF's, like the GMF, and time-varying EMF's is that of the Ion Cyclotron Resonance (ICR) hypothesis *(See section 1.4.6)*. It postulates that energy-transfer of EMF's occur when the combined EMF and SMF fulfil conditions for cyclotronic resonance *(See Section 1.2.4)* of biologically important ions. In *Raphanus sativus* seedlings, it has been observed that both germination and subsequent growth were significantly affected when cultivated with ICR-conditions for calcium fulfilled (Smith et al., 1995). Therefore the EMF exposure was set in both the ambient GMF and an artificially produced SMF set to be either in or out of tune with the cyclotronic resonance of calcium.

The main objective of the cultivation experiment was to determine if the pre-sowing EMF exposure in itself had any subsequent effects on growth-parameters and flowering, not considering the background SMF. Hence, plants used in this experiment were exposed to EMF's only in the ambient GMF.

1.2 Terminology and physics of electromagnetic fields

To avoid repetition, the entire following section on Terminology and physics of electromagnetic fields is referenced in advance to the books of Ulaby et al. (2010), Barut (2013) and Atkins and Jones, (2010) unless otherwise stated.

1.2.1 Electromagnetism & its components

What is electromagnetism and electromagnetic fields?

Electromagnetism is a very broad term that describes one of the four fundamental interactions of nature; the other three being gravity, the weak interaction of nuclei and the strong interaction of nuclei. Electromagnetism accounts for many everyday phenomena, such as chemical bonding, electricity, magnetism and light. It describes the interaction between charged particles in the form of ***the electromagnetic force***, also known as ***the Lorentz Force***. Historically, EF's and MF's was thought of as different forces, and for practicality both can be described with numerous separate equations. However, in its literary meaning the term EMF include both EF's and MF's, since they both are aspects of the electromagnetic force, and seldom appear without the presence of the other. When an EMF is nearly constant in time, namely static, it may in some cases however be viewed as either a pure EF or a pure MF to the observer. This has divided the study of classical electromagnetics into three branches; ***electrostatics,*** the study of static electric fields (SEF), ***magnetostatics*****,** the study of static magnetic fields (SMF) and ***electrodynamics*** (the study of time-varying EMF's).

Taking this in consideration, it comes to no surprise that terminology often varies when reviewing the literature, including the terms EF's, MF's and EMF's in various combinations. For instance, the term time-varying MF literally depicts the same thing as the term time-varying EMF, but different scientists present them differently. An example can be seen when comparing the studies of Halgamuge et al., (2009) and Jedlicka et al., (2014). In their studies, Halgamuge et al. refer to AC (Alternating Current) EMF's and Jedlicka et al refer to MF's, while when reading their studies one notice that they both depict the exact same type of EMF. This complexity may confuse the reader as of which fields has been used. It is therefore a good rule of thumb to view all fields as having both electric and magnetic components, and that the terminology often simply refer to how these fields are produced and which of the two aspects that has been measured.

In this work, a division will be made between static and time-varying EMF's, where static electric fields (SEF) and static magnetic fields (SMF) refer to near constant EMF's with a more prominent electric or magnetic component. Unless otherwise stated, time-varying EMF's refer to all kinds of EMF's with variable components, since they generally have both electric and magnetic components. The term EMF alone still refers to the umbrella-term including all above mentioned fields. In the following text, an attempt is made to make clear both the nature and different components of EMF's from a simplistic traditional point of view.

Electric components

In a sub-atomic scale, particles of opposing charge *attract* each other, while particles of the same charge *repel* each other *(See table 1)*. These interactions are described through ***electric***

fields (EF), E *(See illus. 1)*. When a surplus of positively or negatively charged particles appear on a macro-level, the resulting charge is described as a ***voltage, V***. Between sources of differing voltage a macroscopic EF is created. The strength of the EF is determined by the difference in voltage and the ***distance, d,*** between them. In this EF, sub-atomic particles can be forced into movement, seeking to even out the imbalance in charge between the two sources. Thus, positively charged particles are attracted to the source with a negative surplus, while the negative does the opposite. The polarity (direction) of the EF is generally depicted as going from the more positive to the more negative voltage. A SEF can be described with the following equation:

$$E=\frac{V}{d}$$

For a particle to move in the field, it needs to travel through the medium the field is acting upon. Depending on the conductive properties of the medium, different field strengths will be needed to force the particle into movement. The conductiveness of a material is described with its ***resistiveness, R***. The movement of charged particles is described as a ***current, I***. This relationship is described with ***Ohm's Law***:

$$I=\frac{V}{R}$$

Table 1: How electric charges interact

Charge	+	-
+	repels	attracts
-	attracts	repels

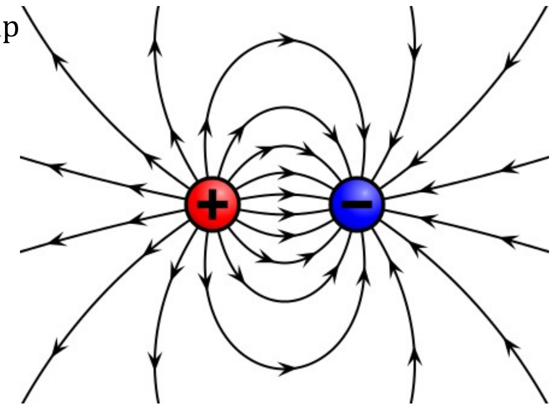


Illustration 1: Electric field lines as a result of opposing charges. Source: www.creative-commons.org. SVG due to Geek3

Magnetic aspects

When a charged particle are in movement, a ***magnetic torque*** is created. Contrary to electric charges, particles with the *same magnetic torque attract*, while particles of the *opposite torque repel (See table 2)*. These interactions are described through ***magnetic fields (MF), B*** *(See illus. 3)*. When a surplus of magnetic torque in one polarity appears on a macro level, as in the case of a flowing current, the resulting torque is described as a ***magnetic polarization*** or ***magnetization, M.*** Between two sources of magnetic polarization a macroscopic MF is created. The strength of the MF between two sources depend on the level of magnetic polarization and distance between them. Usually, MF's are measured in Teslas (T), in terms of its ***magnetic induction, B***, defined as:

$$B=\frac{V*s}{m^2}$$

The polarity of a MF is dependent on the polarity of the current, following ***the right hand rules*** for negatively charged particles, and ***the left hand rules*** for positively charged particles *(See illustration 2).* These rules can also be inverted when considering a circular current, where the MF will have a different polarity within and without the circle due to the magnetic torque. This give rise to something commonly known as a ***magnetic north and south pole,*** also referred to as a ***magnetic dipole*** *(See illustration 3).* Considering sub-atomic particles carrying charge, their ***magnetic spin*** refers to their inherent rotation (making them a circular current) creating a magnetic dipole. MF lines are generally depicted as going from the north pole to the south pole.

Table 2: How particles interact according to their magnetic torque or polarity

Torque	↺	↻	Pole	N	S
↺	attracts	repels	N	repels	attracts
↻	repels	attracts	S	attracts	repels

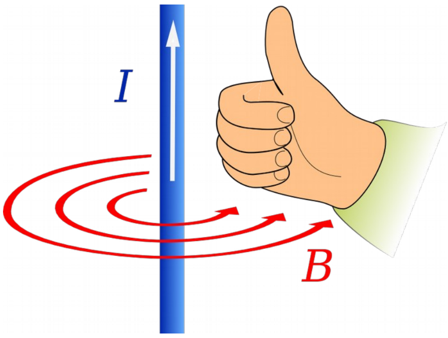


Illustration 2: Depiction of the right hand rule, where I is the moving current and B is the created magnetic field. Source: creative-commons.org. SVG due to jfmelero

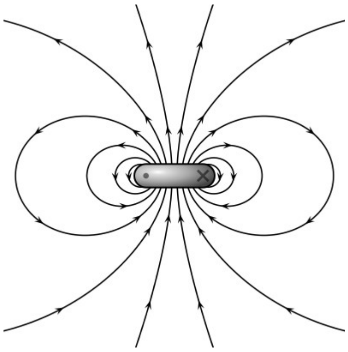


Illustration 3: Depiction of a circular current, creating a magnetic north and a magnetic south pole. Source: creative-commons.org. SVG due to Geek3

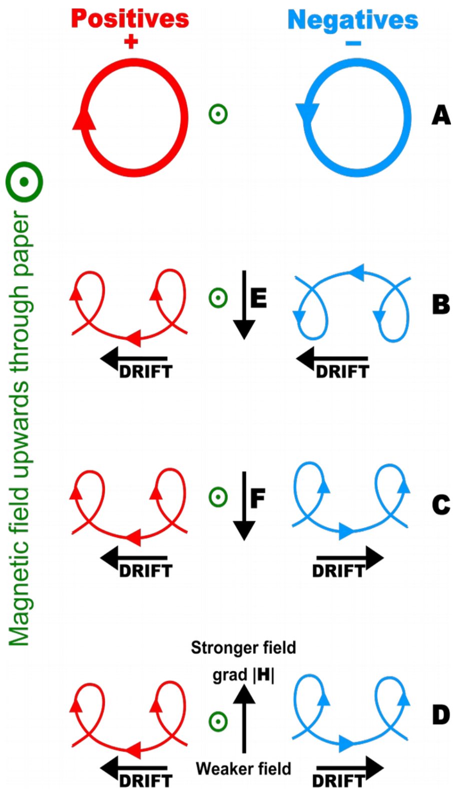


Illustration 4: Depiction of how charged particles drift from a SMF with (A) no disturbing force, (B) an electric field, E, (C) an independent force, F, or (D) an inhomogeneous magnetic field gradient, H. Source: creative-commons.org. Original SVG due to Stannered, derivative SVG by Maschen.

The electromagnetic force

The forces that EF's and MF's exerts on a charged particle can be difficult to describe. The different parameters continuously affect and interact with each other and thus constantly change over time and space. In *illustration 4,* a few examples of how these interactions affect the resulting movement of a charged particle is visualized. The interactions between particles, MF's and EF's is described with **the Lorentz force**:

$$F = q\,[E + (v \times B)]$$

Where F is the *resultant force*, q is the *charge*, E is the *EF intensity,* v is the *velocity of the particle* and B is the *MF intensity.*

1.2.2 Qualities of electromagnetic fields

Why is the quality of an electromagnetic field important?
Given the width of the subject of electromagnetism, it is important to remember that there is also a wide variety of mechanisms and phenomena that can be attributed to this field of study. This calls for a detailed knowledge of the ***quality*** of the EMF being studied, and a basic knowledge of how this is categorized in the literature. For instance, the mechanisms behind a refrigerator-magnet and a cellphone-antenna are both EM in their nature. Though, the quality of their respective EMF's differ to such a degree that the effects of the former cannot be attributed to the latter. Hence, it may prove difficult for scientists to draw general conclusions about EMF's without accounting for its quality in detail, and that cross-referencing between effects of EMF's of different qualities often just isn't possible.

Field Distribution
The nature of a field can be described as either ***uniform*** or ***non-uniform,*** depending on the distribution of field properties in the studied medium. In a *uniform* field, every point in the field are of equal intensity and polarity, whilst in a *non-uniform* field they may differ in intensity and polarity. It is also commonly referred to as ***homogeneous*** or ***heterogenous*** fields, as evident in the study by Fathi et al. (2006).

Field Frequency
Fields with a *near constant intensity* are termed ***static fields.*** For a SEF's this means that charges are stationary, while for a SMF, this means that the current is steady and continuous. They are often referred to as ***DC fields*** (Direct Current) in the literature, as present in the study of Halgamuge et al., (2009). This is the case of ordinary refrigerator-magnets.

Fields with a *changing intensity* are termed ***time-varying fields.*** They may be ***transient,*** meaning there is random disturbances in intensity, or ***continuous,*** meaning there is a well-defined and repetitive shift in intensity. The number of phase-shifts in continuous fields over a given time is described as the ***field frequency***, given in Hz (cycles per second). Terms like ***alternating, oscillating*** *or* ***AC fields*** (Alternating Current) are often used to describe continuous time-varying fields with a rhythmic shift in both intensity and polarity, as evident in the studies by Eşitken and Turan (2004) and Halgamuge et al. (2009). Microwave-ovens commonly use continuous time-varying EMF's with a frequency of 2.45 GHz to heat food (GSMA, 2015).

Field magnitude, amplitude, polarity & polarization
In SEF's and SMF's, the field ***magnitude*** describes the intensity of a field, while the ***polarity*** describes the axial orientation of the field as it propagates in time. In time-varying EMF's, ***amplitude*** describes the intensity of the field while ***polarization*** describes the nature and orientation of the time-varying electric and magnetic components in time and space.

Field waveform
In time-varying EMF's, changes in amplitude & polarity may be different in their nature, which can be visualized as a ***field waveform***. This is basically a conceptual graphic of the field

amplitude and polarity over time. Waves are also classified into different ***types.*** The simplest type of wave is the ***plane wave***, in which oscillations appear in only one orientation, the plane of energy-transfer. ***Transverse waves*** depicts waves in which oscillations appear perpendicular to energy-transfer*. One common plane waveform is the *sine-wave*, which depicts a two-dimensional oscillation of intensity and polarity over time. An alternative to this is the *rectified sine-wave*, which nullifies the polarity-change of the field but maintains a rhythmic change in amplitude. Another common wave-form is the *pulsed wave,* similar to the rectified sine-wave, but where the change in amplitude appear in short rhythmic bursts. Different waveforms are presented in *Illustration 4.*

EM waves are transverse in their nature, involving particle displacement perpendicular to energy-transfer *(See illustration 5)*. However, in the literature, the waveform of EMF's are often described as simple plane waves such as *sinusoidal, rectified, squared or pulsed,* depending on the measured two-dimensional visualization of the field.

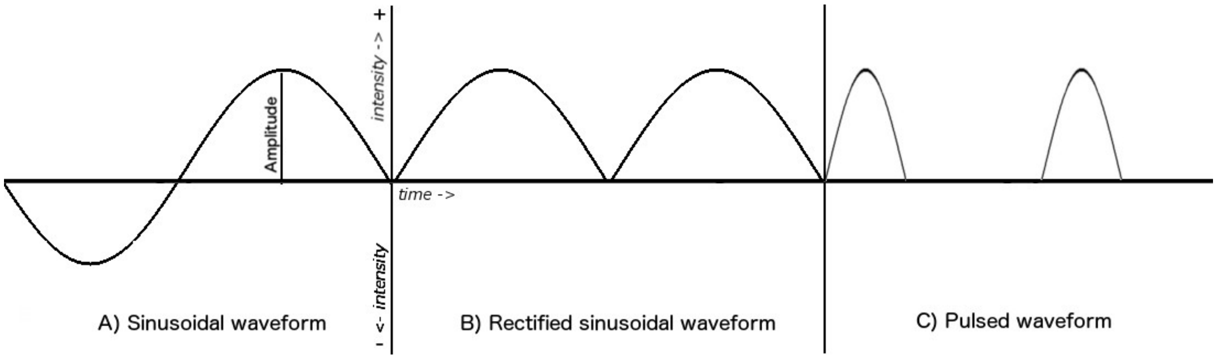


Illustration 4: Two-dimensional visualization of A) a sinusoidal waveform, B) a rectified sinusoidal waveform, and C) a pulsed waveform.

1.2.3 Electromagnetic Radiation

What is electromagnetic radiation?

Electromagnetic radiation (EMR) refers to a type of radiant energy considered the part of an electromagnetic field (EMF) that has *gained enough distance from its own source to propagate independently*. It is sometimes simply referred to as electromagnetic energy, or EME. In classical electrodynamics it is described as the ***electromagnetic wave*** *(See illustration 5)*, travelling at the speed of light, losing amplitude by an inverse-square law of power and propagating without the need of a physical medium. In quantum mechanics the energy is also quantified into particles, or packets of energy, called ***photons***. Photons are massless particles, but they still respond to gravity. The energy-level of a photon corresponds to the wave-length of the electromagnetic wave, which in turn depicts the field frequency *(See Section 1.2.2)*. A higher frequency means a higher energy-level. Visible light, and all the colours therein, is EMR of very specific energy-levels.

* For a visual depiction of different types of waves, please visit http://www.acs.psu.edu/drussell/Demos/waves/wavemotion.html

The electromagnetic spectrum

The electromagnetic spectrum (EMS) is basically all the possible wavelengths of EMR. It may be categorized in different ways, but categorization is generally according to their energy-level and general function for humans. A traditional classification would include *radiowaves (RW), microwaves, infrared waves, visible waves, ultra-violet waves*, *X-rays* and *gamma-rays (See Illustration 6).* Borders of these classes are not clearly defined and cross over each other.

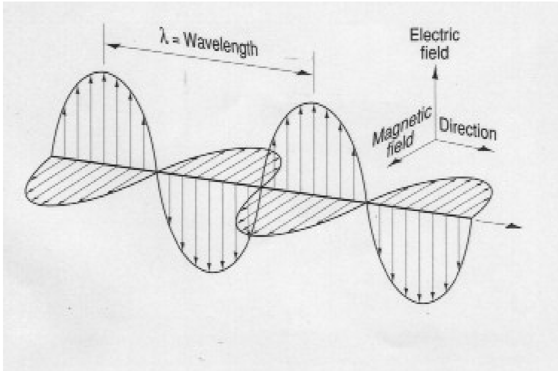


Illustration 5: Depiction of an electromagnetic wave with electric and magnetic components. Source: NASA

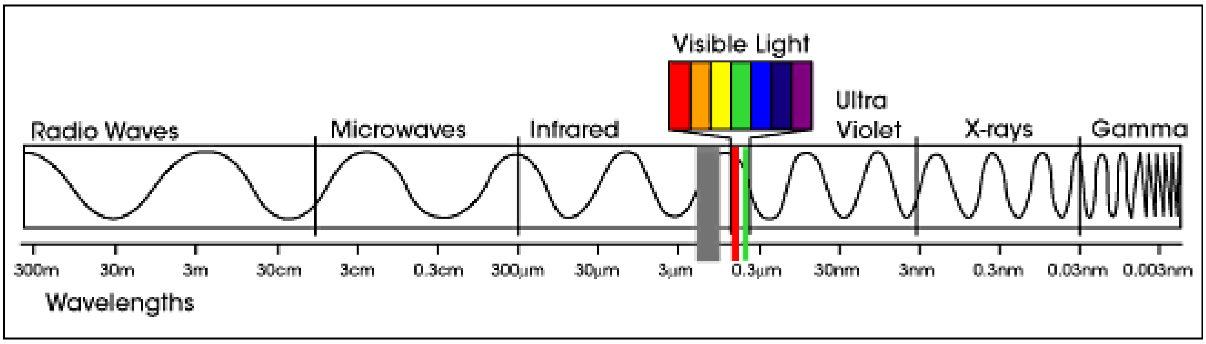


Illustration 6: Depiction of the electromagnetic spectrum, with wavelengths of different frequency bands. Source: NASA

The increasing amount of studies on the subject of EMR and human health as of the past few decades has required a higher resolution classification of the lower parts of the RW band of the EMS. As evident in the study by Consales et al. (2012), very common additions to the EMS has thus been the categories of *Direct Current* (DC) and *Extremely Low Frequency* (ELF), situated before the start of the RW frequency band. These fields are more often depicted in frequency, rather than wavelength. While DC waves have a frequency of near zero, ELF waves are related to power-lines and electrical devices with a frequency of 1-300 Hz. This classification makes it easier to match different quality fields with possible biological effects and ultimately avoid cross-referencing in the literature.

Interactions with matter

The energy, or wave-length, of EMR interacts with matter in several ways. For example, when EMR reach the electron-cloud of an atom, its energy may be *temporarily absorbed*, thus exciting the electrons to a higher energy-state for a short duration. This is known as ***photoexcitation.*** Eventually the electrons fall back into its ground state, and the energy is reflected back as EMR of similar quality as the initial input. Depending on the discrete structure of the atom, the energy may also be *permanently absorbed*. This may occur when the energy-level of the photons *exactly match* the energy-requirements to force the electrons into a higher, and stable, energy-state. The EMR is then instead converted into thermal energy. This is the case with all coloured materials – they absorb EMR at certain energy-levels, or wavelengths, while others are reflected. One of the greatest inventions of nature is based on this principle - namely photosynthesis. The chlorophyll molecules behind this phenomena absorb visible EMR in the blue and red spectrum, turning it into chemically bound energy, and reflects visible EMR only in the green spectrum (Taiz and Zeiger, 2010).

EMR can be broadly divided into two separate categories based on its ability to disrupt matter: ***non-ionizing radiation*** and ***ionizing radiation*** (European Commission, 2005). The former does not carry enough energy to liberate electrons from their accompanied atoms, and is considered to have mainly kinetic and thermal effects on matter. This is the case for the parts of the EMS ranging from the RW frequency-band to the lower end of the UV frequency-band. The latter carries enough energy to liberate electrons and ionize their accompanied atom and thus breaking molecular bonds. This can lead to mutations of DNA and tissue damage in living organisms. This is the case for EMR ranging from the higher end of UV frequency-band to the gamma ray frequency-band of the EMS. The term ***radioactivity*** refers to unstable atoms which during their decay emit certain types of ionizing radiation, thus possibly making them harmful for living organisms.

1.2.4 Cyclotronic resonance

In a uniform SMF, charged particles start to rotate due to the Lorentz force, a very important phenomena connected to the ***Ion cyclotron resonance*** described in *Section 1.4.6* (Halgamuge et al., 2009). The connection between charge, mass and rotation is described with the following equation:

$$\omega = 2\pi f = \frac{ze}{m} B_{DC}$$

were ω describes *angular frequency*, f is the *frequency,* z is the *multiplier of charges*, e is the *electric charge*, B is the *magnetic induction* and m is the *mass of the particle*. This equation can also be translated into:

$$\frac{2\pi f}{B_{DC}} = \frac{ze}{m}$$

This is a very important equation, telling us how specific particles respond to a SMF according to its charge-to-mass ratio, and can be used in combination with time-varying EMF's of a harmonic frequency to accelerate particles (Halgamuge et al., 2009).

By using a time-varying EMF parallel to the SMF oscillating at the same frequency as the rotating particle, the energy of the particle will increase. This effect occurs in both harmonics and sub-harmonics of the rotational frequency of a given ion, with decreasing energy-transfer at higher harmonics and lower subharmonics.

1.3 Electromagnetic fields on earth

1.3.1 The geomagnetic field & the magnetosphere

Origin and nature of the geomagnetic field

On earth, an intrinsic planetary magnetic field known as ***the geomagnetic field*** (GMF) has been present for at least 3.2 billion years (Tarduno et al., 2007). This field varies in intensity across the globe, but normally stay within 25-65 μT (National Geospatial-Intelligence Agency, 2015). Current consensus is that the GMF originate from turbulent flows of electrically conductive fluids in the outer core of the planet, rotating due to the dynamo-effect (British Geological Survey, n.d.). The GMF have mainly static, or long wave properties, but so called ***micropulsations*** of the GMF, short term oscillations in field amplitude, give the field minor time-varying properties (Jacobs, 1970). The polarity of the geomagnetic field is not permanent, but "flips sides" at irregular intervals of around 200 000 – 300 000 years (British Geological Survey, n.d.). These re-occuring events are referred to as ***geomagnetic pole reversals***.

Interacting with the solar wind, the GMF gives shape to earth's so called ***magnetosphere*** *(See illu. 6)*. In the magnetosphere, energetic ions create an electric current surrounding earth, referred to as ***the ring current*** (Daglis et al., 1999). ***Geomagnetic storms***, temporary world-wide increase of the GMF intensity, is a phenomena greatly tied to interactions between the ring current and the solar wind (British Geological Survey, n.d.), as well as the internal dynamics of the atmosphere-ionosphere-magnetosphere system (Kozyra and Liemohn, 2003). Smaller magnetic storms, known as substorms, tend to re-occur according to the rotation-period of the sun of 27 days, while larger storms are connected to the sun-spot cycle of approximately 11 years (British Geological Survey, n.d.).

Importance of the GMF for life

The GMF has been a crucial factor for the evolution of life. It shields the planet from the ionizing EMR of the solar wind, which would otherwise make the planet uninhabitable (Yong and Wei-Xing, 2014). It also significantly affect climate change and local weather-conditions on earth (Bucha, 2012; Dergachev et al., 2012; Genevey et al., 2013; Knudsen and Riisager, 2009; Rossi et al., 2014). During the transition-period of a geomagnetic pole-reversal, which can take up to several thousand years, the GMF is also weakened. This has not only been linked to an increase of ionizing EMR reaching earth, but recently also a quadrupled oxygen-escape in the atmosphere (Wei et al., 2014). An increasing amount of studies show correlations between increased rates of extinctions and speciation during geomagnetic pole reversals (Raup, 1985; Wei et al., 2014; Yong and Wei-Xing, 2014), although no general consensus on the subject has been reached (NASA, 2011). Further investigations on whether these findings is fundamentally of climatic, radiative or geomagnetic origin done by Dubrov, (2013) indicate that the GMF may indeed be the determining factor in these evolutionary processes.

Many organisms also use the GMF for orientation and navigation. ***Magnetoreception***, as it's termed, has been found in bacteria, fish, birds, reptiles, insects & mammals (Pazur et al., 2007; Solov'yov and Schulten, 2012), and lets the organisms sense and orient themselves along the GMF lines. Another very interesting aspect of the GMF is the daily fluctuations that appear due to the planetary rotation. The shadow side of earth usually has a slightly weakened MF due to interactions with the solar wind, thus creating a small daily oscillation of the GMF intensity (British Geological Survey, n.d.). This daily oscillation could possibly help to entrain the circadian clock of various organisms, where cryptochromes associated with the circadian rhythm may act as magnetoreceptors *(see Section 1.4.4).*

1.3.2 Other sources of electromagnetic fields

Naturally occuring EMF's

Some natural earth minerals may also be sources of SMF's. These are referred to as ***ferromagnetic,*** meaning they have the possibility to become permanently magnetized. These minerals often include elements like iron, cobalt and nickel (University of Cambridge, n.d.). Other naturally occurring minerals are termed ***paramagnetic***, meaning they have the possibility to become temporarily magnetized. Thus, they are attracted to, and enhance, externally applied magnetic fields (like the GMF). These minerals are more common and may include elements like magnesium, molybdenum, lithium and tantalum (University of Cambridge, n.d.).

Another very interesting finding is also that transient EMF's can be observed prior to ***earthquakes,*** a phenomena proposed to be linked with the anomalous animal behaviour often found prior to these events (Philippetis, 2009).

A very common source of SEF's is that of ***thunderstorms.*** They appear when there is a build-up of charged particles in the atmosphere contrary to earth. It has been proposed that plants respond to these fields by increasing their metabolism in order to fully make use of the incoming rainfall (Volkov, 2006). This is discussed further in *Section 1.4.3*.

Artificial fields

Today, we use these electromagnetic principles in most of our technology. The list of clever inventions is increasingly long, and it comes to no surprise that the vast amounts of different technologies also affect the EM environment that we have evolved in. There is today few places in an industrialized society where one can completely escape all the different EMF's produced by our technology. How these fields affect biological systems is controversial and debated by many (Consales et al., 2012; European Commission, 2005). As will be presented in the following section, a wide range of biological phenomena has been studied after exposure to EMF's. However, since most of these technologies produce non-ionizing EMF's in the ELF and RW-band of the EMS, they are often considered to be safe for humans (European Commission, 2005). These technologies has not only been evaluated for their effects on humans, but also their effects on plants and their potential use for improved crop productivity. This will also be discussed in the following section.

1.4 Bioelectromagnetics & plants

1.4.1 History and findings of horticultural bioelectromagnetics

Theories of bioelectromagnetism has led to many clever inventions, like the electrocariography (EEC), in 1895, and electroencephalography (EEG), in 1934, as well as novel EM treatments to accelerate healing of bone fractures (Volkov, 2006). While these are common and accepted health-care devices for man, less is available in the agricultural and horticultural sector. Large scale studies of horticultural and agricultural importance first began in 1904, when Karl Lemström conducted experiments with crops exposed to strong SEF's, greatly increasing yields (Lemström, 1904). In his studies, Lemström noted an average yield increase of 45% compared to controls. This led to the UK and US setting up committees to investigate the phenomena (Kinahan, 2009), and the new technique was termed ***Electro-culture*** (Blackman, 1924; Briggs, 1926). However, stimulation did not always work, and difficulties to explain causal mechanisms and sustain repeatable results, as well as economic costs and electrical hazards, led to an abrupt end to most horticulturally oriented studies in the 1930's, with a few studies appearing in the 1960's and 1980's (Kinahan, 2009; Volkov, 2006). The few studies being conducted either presented electro-culture as a means for enhancement for plant growth and reproduction (Pohl and Todd, 1981), or a lethal technique only suitable for weed management (Diprose et al., 1984). It has been proposed that plants use SEF's as cues of incoming thunderstorms – and thus nutritious rainfall (Volkov, 2006). Interestingly, the conflicting results of the early electro-culture experiments can be correlated with the local weather conditions of the field studies. The researchers conducting studies in drier areas often shut down their electrical circuits when the weather forecast was rain, meaning their plants thus quickly would deplete their resources in the in between dry periods when the SEF's were present (Volkov, 2006).

Studies with SMF's of horticultural importance are said to have begun in the 1930's, when a 100 % increase in elongation rate of wheat seedlings was observed by Savostin (as referenced by Flórez et al., 2007; Peñuelas et al., 2004; Pietruszewski, 2011). However, it wasn't until the 1960's that studies with SMF's really started to flourish (Pietruszewski, 2011). For instance, an auxin-like effect on plant roots was detected, and the term ***magnetotropism*** was coined (Audus, 1960). Further studies extended to tomato ripening, were similar auxin-like effects was found (Boe and Salunkhe, 1963). Since then, studies has accumulated on how SMF's and time-varying EMF's affect growth parameters and yields of plants, and EM treatments are now again proposed as cultivational methods for increasing crop yields (Bilalis et al., 2012; Dhawi, 2014; Pietruszewski, 2011; Tanaka et al., 2010).

SMF seed treatments has been found to increase germination rate and biomass in a huge number of crops, for example *Helianthus annuus* (Vashisth and Nagarajan, 2010), *Cicer arietinum* (Vashisth and Nagarajan, 2008), *Oryza sativa* (Carbonell et al., 2000; Flórez et al., 2004), *Zea mays* (Flórez et al., 2007), *Solanum lycopersicum* (Martinez et al., 2009; Poinapen et al., 2013), *Nicotiana tabacum* (Aladjadjiyan and Ylieva, 2003), *Phaseolus vulgaris* and *Triticum aestivum* (Cakmak et al., 2010). Similar results has been found when using time-varying EMF's on similar crops. For instance, increased germination rate and early growth characteristics from time-varying EMF seed exposure has been found in *Zea mays* (Bilalis et al., 2012)

Solanum lycopersicum (De Souza et al., 2006, 2005; Efthimiadou et al., 2014; Jedlicka et al., 2014), and *Gossypum ssp.* (Bilalis et al., 2012; Leelapriya et al., 2003). Several of above mentioned studies also show an increased yield after EM seed treatment, making them interesting for horticultural use. For instance, in *Gossypum ssp.* a doubling of yield was found in seeds exposed to sinusoidal ELF EMF's (Leelapriya et al., 2003), and an average yield increase of 19.4-28.5 % was found in *Solanum lycopersicum* seeds exposed to rectified ELF EMF's (De Souza et al., 2006).

EMF's has also been investigated for horticultural applications in later growth stages. For instance, in cuttings of *Origanum vulgare* a pulsed electromagnetic field (PEMF) treatment was better at inducing root formation than hormonal treatments (Bilalis et al., 2012). In *Fragaria x ananassa,* sinusoidal ELF EMF's applied during cultivation increased yields, both by fruit weight and number of fruits, as well as accumulation of most mineral nutrients (Eşitken and Turan, 2004). In *Phoenix dactylifera,* similar findings with increased mineral contents were observed after a SMF treatment (Dhawi et al., 2009). In Vitro cultures of *Coffea arabica* seedlings exposed to ELF EMF's also showed increased net photosynthesis, plant vigour and development (Isaac Aleman et al., 2014). In Vitro cultures of *Phalaenopsis ssp.* protocorm-like bodies exposed to the north pole of a SMF's increased in both fresh and dry weight compared with controls (Tanaka et al., 2010). In *Allium cepa,* a weak SMF changed both the chlorophyll, protein and lipid content of the plant, with possibilities of both increasing and decreasing the content (Novitskaya et al., 2001, 2006).

Furthermore, several studies have also found an increased stress-tolerance of various sorts in plants and seeds exposed to SMF's and EMF's. For instance, Chen et al., (2011) found that *Vigna radiata* seeds exposed to SMF's showed less toxicological effects from cadmium. Anand et al. (2012) found that *Zea mays* seedlings showed less water stress after seed treatment with SMF's. Ružič and Jerman (2002) observed that weak sinusoidal ELF EMF's applied during cultivation alleviated the harmful effects of heat stress in *Lepidium sativum.*

However, when reading above mentioned studies, a common statement is that the underlying mechanisms for observed effects are poorly understood. Hence, this literature review also look deeper into what biological effects and mechanisms has been linked to SEF, SMF and time-varying EMF exposure. However, to fully understand the basic mechanisms for EM interaction with plants, one must first understand the electrochemical nature of plant physiology. This is discussed in the following section.

1.4.2 Plant electrophysiology

For the ease of the reader, the following section on Plant Electrophysiology is referenced in advance to the books of Volkov (2012, 2006)*, unless otherwise stated.*

The electrochemistry of excitable cells & plant electric signalling

All living cells actively build up gradients of different ions within and without their membranes using various pumps and ion channels (Taiz and Zeiger, 2010). The membrane hinders the movement of ions from one side to the other, meaning the ions will be accumulating on either side of the membrane. Since ions in a liquid medium strive toward electric and osmotic neutrality, meaning they will naturally disperse as evenly as possible in the medium, an electrochemical charge will build up over the membrane. This electrochemical charge is referred to as a ***Membrane Potential*** (MP) (Taiz and Zeiger, 2010). This MP can be measured as a voltage, meaning there is a SEF's acting across the membrane. Plants are especially good in building strong MP's, as they have hydrogen ion-pumps that actively produce strong differences in pH within and without the membranes, where animals instead would use sodium-pumps for the same activity (Sze et al., 1999). The ***Resting Potential*** (RP) refers to the strength of the MP when the cell is inactive (Taiz and Zeiger, 2010).

In the cell membrane, there are gated ion channels that can be opened on a given signal. These channels might be ***ligand-gated***, meaning they require a certain molecular "key" to open them (like hormones). Others might be ***voltage-gated***, meaning they will open when a certain voltage-threshold is acquired (Armstrong and Hille, 1998). When a gated ion channel is opening, the built-up MP is converted into a current consisting of the ions that quickly enter the cell (Armstrong and Hille, 1998). This swiftly ***depolarizes*** the membrane, a signal that will open more voltage-gated channels and thus increase the ion current further until all gates are open. The influx of ions eventually reverses the MP, which in turn closes the voltage gated channels (Armstrong and Hille, 1998). The cell then actively restores its RP, something referred to as ***repolarization,*** by an outflow of other ions (Armstrong and Hille, 1998).

These swift changes in voltage are referred to as ***Action Potentials*** (AP), and are signals that has the possibility to spread to adjacent cells, creating a chain-reaction that may spread long distances within the plant. Another type of electrochemical signal is that of ***Variation Potentials*** (VP)***,*** which is a slower change in voltage resulting from hydraulic waves transmitted through the plant tissue.

Plant long distance electric signaling

Plant cells are interconnected via two different networks; the ***symplast*** and the ***apoplast*** (Taiz and Zeiger, 2010). The former consists of the living inside of each cell, connected via plasmodesmata into an inter-cellular network. The latter consists of the outsides of cells, namely the cell walls and dead tissues. An increasing amount of studies show that the main route of electrical long distance signaling with AP's is through the symplast and the phloem, while VP 's mainly travel through the apoplast and xylem.

Calcium – the ultimate messenger?

Calcium is a very important ion in plant biology since many of the electrophysiological phenomena are connected to this ion. While different molecular "keys" like hormones, microbe-associated molecular patterns and nutrients may activate signalling in plants, the fundamental response to many stimuli is an influx of calcium ions into the cell. Aside from being part of the ***signal transduction*** through membrane depolarization, calcium also acts as a so called ***second messenger*** within the cell (Berridge et al., 2000). In the cell calcium binds to a certain protein known as ***calmodulin,*** a sort of calcium switch. Calmodulin reacts to cytosolic calcium by either activating or deactivating other molecules, thus regulating many processes within the cell (Chin and Means, 2000). Different types of information are conveyed using the different signalling processes and networks previously mentioned, creating oscillations in cytosolic calcium (Smedler and Uhlén, 2014). These oscillations are known to encode information to the plant genome in order to properly respond to different stimuli (Allen et al., 2000; Evans et al., 2001; Love et al., 2004). One could thus argue that intracellular communication mainly stem from the rhythmic presence or non-presence of calcium within the cytosol, while electrical inter-cellular communication is a process tightly bound to voltage-gated ion channels and AP's.

Interactions with external electromagnetic fields

All plant electrophysiological phenomena can be affected by external EMF's since they involve movement or build-up of charged particles and other EM mechanisms. This is similar to animal muscles responding do externally applied currents causing twitching. Given the electrophysiological nature of plants, everything from genetic expression to nutrient availability may be affected. In the following section, some studied effects and proposed biological mechanisms for EM interactions will be discussed.

1.4.3 Biological effects & mechanisms of static electric fields

Introduction

Since plants use electric signals for much of inter-cellular communication, it comes to no surprise that SEF's can have many diverse effects on plants. The following text seeks to address some of the effects and mechanisms behind observed effects of SEF's.

Effects on genetics, biochemical processes & cellular signalling

When using SEF's, plant responses seem to be linear up to a point of saturation, and then suddenly decline due to membrane electroporation. In experiments with *Arabidopsis thaliana,* SEF's increased growth up to 10 kV/m, but above this value had no added effect (Okumura et al., 2014). This is similar to the effects studied in the early electro-culture trials of 1900-1930 (Blackman, 1924; Briggs, 1926; Lemström, 1904). A sustained increase in synthesis of growth factors has been reported in many organisms as a response to SEF's (Aaron et al., 2004).

It is assumed that this effect is due to disturbances of transmembrane signalling (Aaron et al., 2004). One proposed mechanism is that SEF's activates voltage-gated calcium-ion channels and increases the membrane permeability to calcium following SEF exposure (Volkov, 2006). An influx of calcium, being a second messenger for many cellular activites, would then affect enzyme-activity and thus the overall metabolism and genetic expression of the plant (Volkov, 2006). However, the change of genetic expression may not solely stem from disturbances of transmembrane signalling. In experiments with *Allium ascalonicum,* SEF's was shown to increase reactive oxygen species (ROS) in leaves, coupled with an increased production of enzymatic and non-enzymatic anti-oxidants in the symplast (Cakmak et al., 2012). This again could be the result of an increase in membrane permeability, possibly creating a leakage of ROS from peroxisomes, or that SEF's induce additional formation of ROS.

It is also an alternative explanation of why plants increase the production of growth factors as a response to SEF's, since ROS are key signalling molecules for plants, with strong links to processes of growth, development and stress-responses (Mittler et al., 2004).

Polarity & electrotropism

The polarity of SEF's is of importance, mainly in the establishment of the plant polarity. Studies on zygotes of the sea-weed *Fucus* has shown that rhizoids appear in the direction of the current (Novák and Bentrup, 1973). This has been confirmed to occur naturally by endogenous SEF's in many polarizing cells such as pollen grains, moss spores and animal zygotes and an externally applied field would thus initiate the polar development (Volkov, 2006). This is speculated to originate in voltage-gated ion channels that locally increase calcium uptake which stimulates metabolism in that specific area (Volkov, 2006). The establishment of polarity in higher plants seem to be controlled by an intimate relationship of polar auxin transport and electrical currents, where areas of higher auxin content is correlated with a more positive charge, although this relationship is not fully understood (Volkov, 2006). This phenomena of plant movement or curvature in response to SEF's are known as ***electrotropism***.

1.4.4 Biological effects & mechanisms of static magnetic fields

Introduction

Just as SEF's affect many parts of the plant electrophysiology, so does SMF's. Since all moving charges and magnetic minerals in the plant are attracted or repelled by external SMF's, observed effects are very diverse in their nature. The following text seeks to address some of the effects and mechanisms behind observed effects of SMF's.

Effects on water & nutrient uptake

SMF's not only affect the electrophysiology of plants, but also the solubility, electrolyte availability and hardness of water (Alimi et al., 2007; Baker and Judd, 1996; Cai et al., 2009; Fathi et al., 2006; Pang and Deng, 2008; Szcześ et al., 2011). Some studies has shown increased growth characteristics of plants watered with magnetized water (Carbonell et al., 2000; Maheshwari and Grewal, 2009). SMF's is also known to affect water absorption rate of plants (Es'kov and Rodionov, 2010; Reina et al., 2001; Reina and Pascual, 2001). In studies with *Phoenix dactylifera*, an increased concentration of calcium-, magnesium-, manganese-, iron-, sodium-, potassium- and zinc-ions was measured after seedling treatment (Dhawi et al., 2009), indicating that externally applied SMF's may increase uptake of important nutrients.

Effects on genetics & biochemical processes

Since all ions and free radicals present within a plant are paramagnetic and responsive to an external SMF, many effects observed after a SMF treatment are thought to stem from interactions with biochemical processes that involve ROS, proteins, various enzymes and paramagnetic elemental particles (Dhawi, 2014). In studies with *Helianthus annuus*, a SMF treatment was coupled with an increase in enzyme activity of a-amylase, dehydrogenase and protease, possibly explaining the often observed increase in germination rate (Vashisth and Nagarajan, 2010). They also reported an improved seed coating integrity, reduced cellular leakage and electrical conductivity of seeds. In another study, *Helianthus annuus* seeds exposed to SMF's were also found to increase mutation rates at certain SMF intensities (Kiranmai, 1994). According to Dhawi, (2014) SMF's are thought to increase the lifetime of ROS, which may induce oxidative stress in the cell as they accumulate. This has been proposed as a possible mechanism for the genetic effects and abnormalities sometimes seen after a strong SMF treatment, where ROS may damage or alter the genetic material (Dhawi, 2014). However, in experiments with *Allium ascalonicum*, weak SMF's showed both an increase of enzymatic and non-enzymatic anti-oxidants in the symplast, without a corresponding rise in ROS levels, indicating that ROS accumulation, and thus genotoxicity, may be dependent on SMF intensity (Cakmak et al., 2012).

Magnetic susceptibility

An important aspect of magnetic treatments seems to be that of the plant ***magnetic susceptibility*** (Pietruszewski, 2011). This is basically a measurement of how much magnetically interactive materials are present within the plant. One proposed mode of measurement is that of ***diamagnetic susceptibility,*** meaning the ratio between diamagnetic and paramagnetic particles in the plant tissue (Peñuelas et al., 2004) Since diamagnetic particles are weakly repelled by SMF's, they act as magnetic insulation and weakens applied SMF's. Paramagnetic particles, on the other hand, is magnetically permeable and enhances applied fields by attraction. Studies with magnetically treated plants has shown that plant diamagnetic susceptibility is directly correlated to root growth, where a lower diamagnetic susceptibility showed greater inhibition of growth (Peñuelas et al., 2004). This indicates that plant growth can be directly affected through magnetic interactions with paramagnetic particles within the plant, without any plant receptor necessarily being involved.

Polarity & magnetotropism

Polarity seems to play a vital role in plant responses to SMF's. In several cereal crops, seed germination was increased when applied SMF's were parallel to the field lines of the GMF, while no effect was observed when field lines were crossed (Pittman, 1963). This effect has been reproduced with artificial SMF's, where cereal seeds germinated faster when parallel to SMF lines (Yano et al., 2001). This was also concluded in studies with *Solanum lycopersicum*, where seed orientation and SMF intensity was found to be more critical to germination characteristics than relative humidity (Poinapen et al., 2013). In experiments with *Raphanus sativus*, roots responded significantly when exposed to the south pole of a magnet but insignificantly when exposed to the north pole, indicating importance of polarity (Yano et al., 2001). Furthermore, roots of Zea mays preferred to grow along north-south SMF lines (Kato, 1988). This phenomena of plant movement or curvature in response to SMF's is known as ***magnetotropism***.

Magnetotropism was coined as a new tropism in 1960 (Audus, 1960). *Statholites*, known to be important organs for gravitropism (Taiz and Zeiger, 2010), is the proposed magnetoreceptor for this response. Statholites are starch containing amyloplasts with diamagnetic properties. They generally segment at the bottom of cells accumulating auxin, which is known to affect cell elongation (Taiz and Zeiger, 2010). When applying an external SMF, other paramagnetic cell components will be accumulated toward the MF gradient, thus sedimenting the statholites away from the gradient, as evident in the study by Schwarzacher and Audus (1973). From these phenomena, one could argue that statholites are both part of the gravitropic and magnetotropic responses seen in plants.

Ferromagnetic particles & magnetoreception

A very common magnetoreceptor used by magnetotactic bacteria is that of ***magnetosomes*** (Pazur et al., 2007). Magnetosomes are cellular compartments where ferromagnetic minerals like magnetite or greigite are biologically constructed (Pazur et al., 2007). Microorganisms use them as magnetic compasses for orientation, but since they also have been found in various animals (including humans), it has been suggested that this might be involved in animal magnetoreception aswell (Cadiou and McNaughton, 2010). This arise the question of whether plants also could use magnetite for magnetoreception. In plants, magnetite is found in ***phytoferritin***, which is synthesized *de novo* by plants (Briat et al., 1999). Phytoferritin is not only ferromagnetic, but also has the highest electrical conductivity of any cell material, making it a strong candidate for simple plant memory processes (Størmer and Wielgolaski, 2010). Studies done with several crop plants has showed that some plants are also capable of absorbing nanoparticles through their roots, including magnetite-compounds, thus accumulating it over time in their tissues (Cifuentes et al., 2010). The amount of ferromagnetic particles absorbed by plant roots would then result in a general increase of plant magnetic susceptibility, and hence the effect of externally applied SMF's.

Radical-pair theory, cryptochromes & magnetoreception

The radical-pair theory suggests that organisms sense SMF's through certain molecules forming radical-pairs. A radical is basically a molecule with an unpaired electron, making it paramagnetic. In radical-pair forming molecules, it is suggested that or two unpaired electrons appear in vicinity of each other. When an external MF is present, these electrons align with the applied field, thus magnetizing them and making them more stable. As mentioned in *Section 1.3.1*, many organisms has the ability to sense SMF's. In migratory birds, certain molecules known as ***cryptochromes*** has been proposed to be a potential source for magnetoreception (Liedvogel and Mouritsen, 2010). Interestingly, cryptochromes is present in both animals (including humans) and plants (Chaves et al., 2011; Sancar, 2000).

Cryptochromes are blue-light receptors, activated by certain wavelengths of EMR in the blue parts of the visible band of the EMS, and are integral parts of the circadian clock (Chaves et al., 2011). They form radical pairs while in their active state, theoretically makes magnetoreception possible. In *Arabidopsis thaliana*, experiments investigating the phosphorylation of cryptochromes have shown that this process is significantly enhanced by externally applied SMF's (Xu et al., 2014, 2012). Activated cryptochromes thus become more stable by exposure of external SMF's, and affect the cellular activities of plants for a longer period of time. Theoretical calculations and experimental data point to an increased activation of around 10 % to a SMF intensity of 50 μT (Solov'yov et al., 2007; Solov'yov and Schulten, 2012).

Cryptochromes as a magnetoreceptor is feasible from an evolutionary perspective, since they are involved in entrainment and regulation of the circadian rhythm (Taiz and Zeiger, 2010). As stated in *Section 1.4.1*, the field intensity varies slightly over the day as the earth rotates. This daily oscillation would be a steady cue for the diurnal processes of the plant, working synergistically with the EMR of the sun.

Connections with the Ion Cyclotron Resonance Theory

SMF's has also been proposed to show such variable biological effects because of interactions between applied SMF's and endogenous time-varying EMF's (Liboff, 2010, 1997). This is a part of the Ion Cyclotron Resonance hypothesis, which is discussed in further detail in Section 1.4.6.

1.4.5 Biological effects & mechanisms of time-varying electromagnetic fields

Introduction

Many of the studies on biological mechanisms behind effects of time-varying EMF exposure has been conducted on animals, hence much of the reference material in this section come from animal studies. It is however very unlikely that mechanisms would vary significantly between plant and animal cells (Volkov, 2006). Much of the experimental data is inconsistent and varies greatly between studies (Phillips et al., 2009). Even so, many biological effects seem to be ***frequency independent*** (Lai, 2001)***,*** appearing across a wide range of the EMS. Other effects seem to be ***frequency dependent,*** where contradictory effects may appear in a small windows of frequency, as evident in the studies by Blackman et al., (1994) and Smith et al. (1995). In the following text mainly frequency independent effects will be discussed, while in *Section 1.4.6*, some frequency dependent effects will be discussed.

Effects on genetics & biochemical processes

Since the DNA molecule possess characteristics of a fractal antennae, namely electrical conductivity and self-symmetry, it has been proposed that DNA may directly interact with time-varying EMF's in the ELF and RW range of the EMS (Blank and Goodman, 2011). In the ionizing range of the EMS, direct interactions with DNA would be similar but much more complex (Blank and Goodman, 2011)***.*** However, since the energy content of ELF and RW EMF's is too low to directly break molecular bonds, it is believed that interactions with genetic material is of secondary nature rather than direct (Phillips et al., 2009).

Important to notice is that in the majority of studies, non-ionizing time-varying EMF's is rarely causing genetic mutations, but rather alters the functions of the genetic material (Lai, 2001). Interestingly, literature reviews investigating the relationships between genetic expression, genotoxicity and different time-varying EMF's in the ELF and RF range has found that the effects may be very similar even though the energy content of the different waves differ a thousandfold (Lai, 2001). Many studies has found an increase in breakage of DNAstrands following time-varying EMF exposure (Ivancsits et al., 2002; Lai, 1996; Lai and Singh, 2004). According to Lai (2001) the oxidative status of the cell may explain much of the biological and genotoxic effects observed in EM studies. Following that ROS- and iron-scavangers has been shown to effectively block the genotoxic effects of time-varying EMF's, it has been suggested that cells with a higher metabolic activity and iron-content is more susceptible to DNA damage from time-varying EMF's (Phillips et al., 2009). Since iron works as a catalyst for the conversion of the ROS hydrogen peroxide, a mitochondrial product, into the more potent ROS hydroxyl, it has been suggested that time-varying EMF's may enhance this conversion (Phillips et al., 2009). Hence, cells containing high amounts of anti-oxidants and less iron should be less susceptible to genetic damage from time-varying EMF's. In a study of the effect of ELF EMF's on wetted *Picea abies* seeds, it was found that the effect of ELF EMF exposure was inhibitory to germination under acidic conditions, indicating that stress factors may enhance damaging effects of time-varying EMF's (Ruzic et al., 1998). However, there are some studies that indicate that genetic effects can be induced by time-varying EMF's without the presence of ROS (Alcaraz et al., 2014; Ferreira et al., 2006; Furtado-Filho et al., 2014). This could possibly be explained by synergistic effects between EMF's and other mutagens (Lai, 2001).

It is likely that the increased stress tolerance observed in many plant studies after time-varying EMF exposures is due to the above mentioned stresses of the plant material, thus increasing tolerance to stronger stresses (Ružič and Jerman, 2002). In *Solanum lycopersicum,* stress related mRNA transcription factors accumulated rapidly after RW EMF exposure (Roux et al., 2007). In studies on *Spirodela oligorrhiza*, the universal stress signal alanine increased after ELF EMF exposure, whereas an addition of anti-oxidants decreased this effect, indicating that ROS plays a vital role in this process (Ben-Izhak Monselise et al., 2003). Moreover, studies on *Brassica napus* and *Zea mays* showed increased activity of stress-related anti-oxidant enzymes in some tissues after ELF EMF exposure (Shabrangi and Majd, 2010). Furthermore, studies conducted on several *Triticum ssp. s*pecies also showed an increase in enzyme activity of glutathione S-transferase after ELF EMF exposure, thus promoting a higher resistance to pathogens, oxidative stress and heavy-metal toxicity (Rochalska and Grabowska, 2007).

Effects on cell signalling & structure

Just as with SEF's, an increase in growth factors has often been noted after a time-varying EMF exposure (Aaron et al., 2004). One proposed mechanism is, again, interactions with voltage gated calcium-ion channels and calmodulin (Pall, 2013). It has been shown that non-ionizing EMF's can increase intracellular calcium concentrations, and some effects seen on biological systems after EMF exposure has also been reported to disappear when voltage gated calcium-ion channels are blocked (Pall, 2013). However, often the inhibition of effect is only partial, and it has been suggested that the main causal agent for voltage gated channel activation is by membrane ion leakage caused by increased membrane permeability (Volkov, 2006). Many studies also indicate that certain receptor-proteins are involved in the effects observed in studies with non-ionizing EMF's. In studies with rat behaviour, both ELF and RF electromagnetic fields has shown similar behavioural effects (Lai et al., 1998; Wang and Lai, 2000), while the blocking of certain opiate receptors also blocked the behavioural effects of both EMF's (Lai et al., 1992; Lai and Carino, 1998).

It has also been found that EMF's induce a redistribution of cell surface receptors (Cho et al., 1994), and changes in cytoskeletal organization (Cho et al., 1996). In studies with RF EMF's on *Allium cepa,* mitotic aberrations, thought to stem from impairment of the mitotic spindle, were noted in root meristimatic cells (Tkalec et al., 2009).

Considering pulsed fields

PEMF's has been shown to cause increased membrane permeability and so called electroporation of cell membranes (Knorr and Angersbach, 1998). In experiments with PEMF's on *Arabidopsis thaliana*, strong fields pulsed for 100 nanoseconds completely killed the plants due to electroporation of plasma membranes, whereas 10 nanosecond treatments of weaker strength showed increased growth (Eing et al., 2009). Similar results has been found in other plants, like *Haloxylon ammodendron* (Su et al., 2015). It has been shown that treatments can be effective a long time after treatment, with at least one study showing the greatest effect as late as two weeks after exposure (Songnuan and Kirawanich, 2012).

The increased growth when using short pulses of EM fields can possibly be due to changes in genetic expression of the plant due to cytosolic oscillations of ions, coupled with an increase of ROS following the increased membrane permeability. EF's activate voltage-gated ion channels in the plasma membrane, and thus disturb or regulate intercellular ion-fluxes (Volkov, 2012). PEMF's do so at short regular intervals and thus initiate calcium release into the cytosol creating oscillations of cytosolic calcium, although the amplitude of calcium release often decreases as treatment progresses (Sauer et al., 2002). Calcium oscillations are known to encode complex information for many plant cells (Evans et al., 2001). For instance, in *Arabidopsis thaliana* it has been shown that oscillations of calcium both encode diurnal information and instructions for stomatal opening and closing (Allen et al., 2000; Love et al., 2004). The frequency of pulsation would thus possibly produce differing effects depending on the calcium oscillations created.

1.4.6 Combined fields: Ion Cyclotron Resonance & Ion Parametric Resonance

Ion Cyclotron Resonance Hypothesis

While many observed effects of time-varying EMF's seem to occur over a wide range of frequencies, some observed effects seem highly frequency dependent. One proposed mechanism for frequency dependent effects is the ***Ion Cyclotron Resonance*** (ICR) ***Hypothesis*** (Liboff, 2013). Cyclotron resonance is a well-known and well studied phenomena, used for accelerating ions in particle-accelerators (as evident in the study by Darquennes et al., 1990) and mass spectroscopy (Baldeschwieler and Woodgate, 1971). It is a naturally occuring phenomena, known to cause heating of the ionosphere (Lysak et al., 1980; Norqvist et al., 1996; Ungstrup et al., 1979). However, as a biological phenomenon, the ICR hypothesis was first proposed in 1985 by Abraham Liboff (as referenced by Liboff, 2013), and has been further improved upon since then (Liboff and McLeod, 1988). Because of the controversy of the subject, it is sometimes simply referred to as ***Combined Magnetic Field*** (CMF) exposures in the literature, even though they commonly follow the ICR principles (Liboff, 2013). *Theoretical basis and equations for cyclotronic resonance can be found in Section 1.5.4.*

The ICR Theory suggest that energy-transfer from time-varying EMF's can occur in biological systems when the frequency of the applied time-varying field matches the cyclotronic resonance of an ion moving in a circular or helical path due to the natural GMF (or another SMF) (Lin, 2012). This has been proposed as a natural means of cellular regulation, where endogenous EMF's of the cellular network interact with the GMF to activate or inactivate biologically important ions (Liboff, 2010). In the natural condition of the GMF, many biologically important ions has a cyclotronic resonance that fall in the ELF-range of the EMS, thus giving a possible explanation to the many inconsistencies in biological studies conducted with ELF-EMF (Lin, 2012). Since the GMF intensity varies over the day and between different locations on earth, studies with the exact same frequency of ELF-EMF's, but conducted in different locations and time of day, would produce differing effects.

Several studies has shown that time-varying EMF's in cyclotronic resonance with biologically important ions may create adverse biological effects. In experiments with radish seedlings, *Raphanus sativus var.* Belle, the tuning of combined EMF's to calcium speeded germination, whilst a potassium-tuning slowed germination, and a magnesium-tuning left it unaffected (Smith et al., 1995). In flax stems, the gravitropic response turned negative with a calcium-tuning while turning positive with a potassium-tuning (Belova and Lednev, 1999). In studies on *Hordeum vulgare,* etiolated seedlings grown under ICR conditions for calcium showed decreased growth compared to controls (Pazur et al., 2006). In studies on rat behaviour, a calcium-tuning showed a decreased activity of the rats while a magnesium-tuning showed an increased activity (Zhadin et al., 1999). In experiments on the aquatic *Amphora coeffeaformis* motility, a calcium-tuning showed increased motility, while a potassium-tuning showed decreased motility (McLeod et al., 1987). However, this last experiment was not repeatable in trials conducted by other scientists (Prasad and Miller, 1994).

In studies investigating calcium binding proteins in *Arabidopsis thaliana,* it was shown that increases of cytosolic calcium only occured at the beginning and end of an EM treatment tuned to the cyclotronic resonance of calcium (Pazur and Rassadina, 2009). This study indicate that an ICR treatment only is effective for the first 20 minutes of exposure, which ultimately would make long-term exposures ineffective. Other interesting findings show that the ICR effect can be detected even at *ultra-low* intensities of applied EMF's, more than a 100-fold weaker than the GMF itself (Alberto et al., 2008; Comisso et al., 2006; Pazur, 2004; Zhadin et al., 1998).

The ICR Theory was first proposed as a mechanism for activating gated ion channels at specific frequencies (McLeod et al., 1992). Further extensions to the ICR theory suggests that ion-binding proteins rather than gated ion channels is the key mechanism behind the ICR effect, which is discussed in the following sub-section.

Ion Parametric Resonance Hypothesis

In 1991, an extension to the ICR theory was proposed by Lednev (Lednev, 1991). This is known as the ***Ion Parametric Resonance Hypothesis*** (IPR). The model for IPR not only take the ion's charge-to-mass ratio and the SMF magnitude into account, but also the amplitude of the applied time-varying EMF, claiming that biological effects at ICR-conditions occur only at certain ratios of SMF to time-varying EMF intensity (Greenebaum and Barnes, 2006). Following critique (Adair, 1998, 1992, 1991), the model has been defended and further improved upon (Blanchard and Blackman, 1994; Engström, 1996; Machlup, 2007), as well as quantified (Engström and Bowman, 2004). According to one of the newer calculations, the greatest biological effect are said to appear when the ratio between SMF and time-varying EMF intensity are 1,84 (Blanchard and Blackman, 1994).

The theory suggests that "ion-trapping" molecules, such as the before mentioned calmodulin, in fact are ionic oscillators modulated by the presence of a SMF (Greenebaum and Barnes, 2006). By applying a properly calibrated combination of SMF's and time-varying EMF's with the correct intensities, ions could effectively be released from their ionic traps, resulting in increases of biologically available ions. A simple analogue to parametric resonance is a child swinging – as long as the pumping motions of the child is in resonance with the frequency of the swing, energy and swinging will increase, eventually throwing the child off the swing. If resonance is lost, the energy and swinging will decrease and eventually stop.

This model is said to have greatly increased the repeatability of frequency dependent studies with combined EMF's, and may serve as an explanation for inconsistencies seen in some early ICR studies (Liboff, 2013). For instance, studies on human PC-12 cells has showed great prediction of effects using this model (Blackman et al., 1995, 1994).

Feasibility and considerations of ICR and IPR

Extensive modeling and testing has shown that both the ICR and IPR model are feasible, but only in "unrealistically low viscosities" (Halgamuge and Abeyrathne, 2010). Moreover, both theories has been criticized and questioned do to a lack of replicated experimental evidence and some theoretical contradictions (Greenebaum and Barnes, 2006)Hence, further theoretical development and empirical research might be needed in order to fully understand what mechanisms might be involved and why effects from combined EMF's may appear in small resonance windows.

Furthermore, since ICR and IPR according to their theories may affect all available ions in a solution, it is sometimes the case that not only one selective ion should be affected by the combined EMF's (Smith et al., 1995). One must therefore take into consideration the different charge-to-mass ratios of all available ions, study the harmonics of the different cyclotronic resonances, and find a proper harmonic that doesn't interfere with other ions but the one that is being studied. On the other hand, it would then also possible to find harmonics that selectively affect several ions simultaneously, thus possibly creating new biological effects.

2 Materials & Methods

2.1 The pilot study

2.1.1 Experimental setup & exposure system

Image 1: Picture of the experimental electromagnet.

An experimental electromagnet was built using a circular transformator cut up in a similar fashion of that of Huang and Wang (2007), presented in *Image 1*. This was connected in series to a 6-ampere fuse and a configurable AC/AC-transformator with an output voltage set to 64 V, producing a 50 Hz non-uniform sinusoidal EMF in the cut opening of the electromagnet. The sinusoidal EMF was measured using an oscilloscope, and four field intensities was set by placing treatments at an increasing distance of the electromagnet. Time-varying EMF intensities was measured (in peak value) to approximately 160 mT (±5 mT), 40 mT (±2 mT) and 9 mT (±0,5 mT) for exposures, and 0 mT for controls. Two fans was placed on either side of the electromagnet, for heat-dissipation, and a heating plate was used to match the heat for the control treatments.

The electromagnet was placed within a three-dimensional helmholz 4-coil system of Meritt et al.-model (Kirschvink, 1992), capable of producing uniform SMF's. This system was set to either produce a SMF environment of 65.8 µT (being in Ion Cyclotron Resonance with calcium-ions) or 68.5 µT (being off-resonance with calcium-ions), or to produce no SMF at all, thus considered as a natural geomagnetic field (GMF) environment. In the following text, these SMF environments are addressed as ICR, non-ICR or GMF. Furthermore, SMF environments are also considered *artificial*, produced by the helmholz 4-coil system, or *ambient*, produced by the GMF. Calculations for ICR of calcium was based on the study by Pazur et al. (2006).

SMF's was measured with a magnetometer connected to a laptop. The local GMF was measured before each exposure to approximately 46.9 µT, varying only slightly between replicates (±0.05 µT). The coil system was wrapped in copper-containing fabric to shield from possible external EM disturbances during exposure. Two different durations were used for exposure, 15 minutes and 30 minutes. A simple schematic of exposure setup is given in *illustration 4*.

Because of technological constraints, only one time-varying EMF treatment and one SMF environment could be produced at a time. This separated the replicates, as well as exposures conducted in different SMF environments, in time. To minimize this difference, exposures of different SMF environments was done within two hours of each other, while separate replicates were done in separate days but in the same hour of the day as previous treatments. To further neutralize the time difference between replicates, the order of exposure were switched between days *(see Table 3)*.

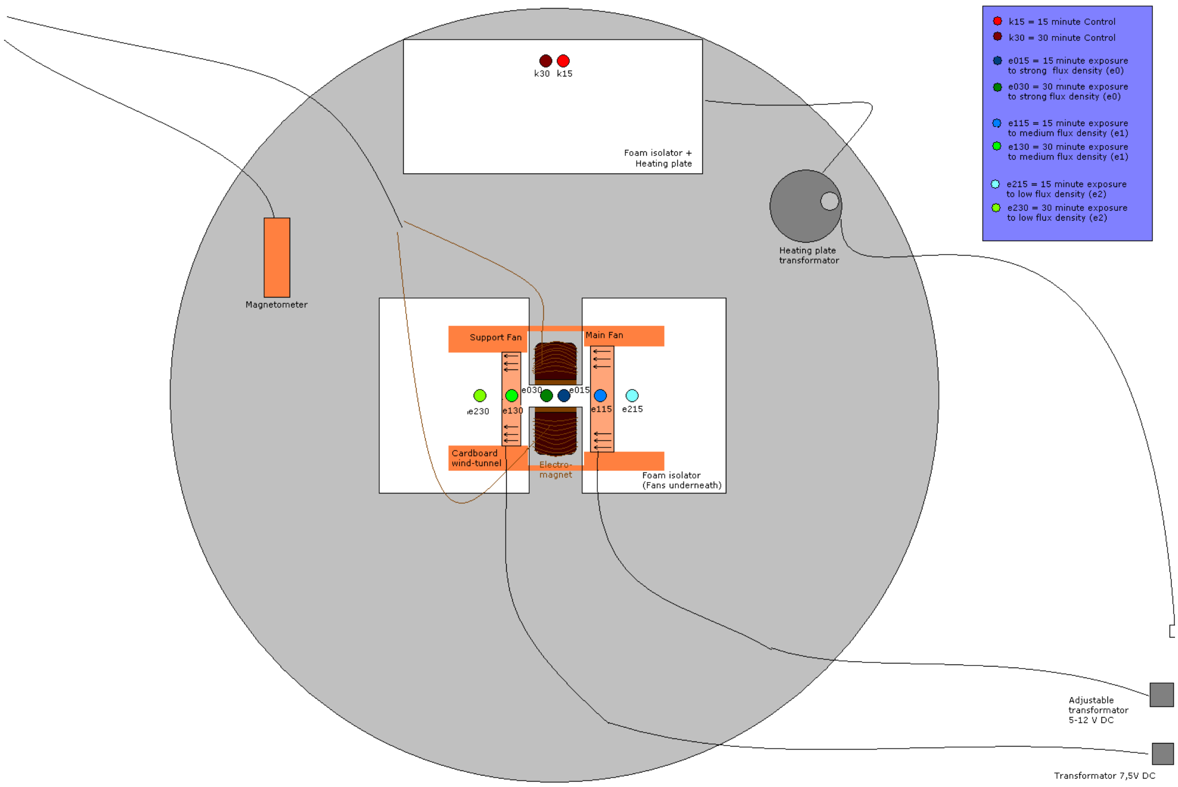


Illustration 7: Schematic of application setup, with positions of electromagnet, magnetometer, heating plate, exposed treatments and controls.

Table 3: Exposure schedule

Day of exposure	SMF environment of first exposure (t=0h)	SMF environment of second exposure (t=+2h)
Day 1	65.8 µT	46.9 µT
Day 2	46.9 µT	65.8 µT
Day 3	68.5 µT	46.9 µT
Day 4	46.9 µT	68.5 µT

Because of this setup, the entire test was split into two separate experiments with only two replicates being used for the germination tests *(See Table 4).* Twenty seeds was used per individual treatment, with a total of 640 seeds per experiment and 1280 seeds in its entirety.

Table 4: Treatment combinations for germination test

Experiment	Replicate	SMF environment (µT)	Time-varying EMF exposure (peak value mT)	Duration of exposure (min)
1	1	65.8 (ICR) / 46.9 (GMF)	160 / 40 / 9 / 0	15 / 30
1	2	65.8 (ICR) / 46.9 (GMF)	160 / 40 / 9 / 0	15 / 30
Sum	2	2	4	2
2	1	68.5 (non-ICR) / 46.9 (GMF)	160 / 40 / 9 / 0	15 / 30
2	2	68.5 (non-ICR) / 46.9 (GMF)	160 / 40 / 9 / 0	15 / 30
Sum	2	2	4	2

In the following cultivation experiment only the treatments exposed to the time-varying EMF in the ambient SMF environment for a duration of 30 minutes was used, which in turn was considered as four replicates *(See Table 5)*. Here, 10 seeds per treatment were randomized for planting, totaling in 160 plants. The cultivation experiment was conducted as a completely randomized block design, each replicate put in its own block.

Table 5: Treatment combinations used in cultivation experiment

Experiment	Replicate	SMF environment (μT)	Time-varying EMF exposure (peak value mT)	Duration of exposure (min)
1	1	46.9 (GMF)	160 / 40 / 9 / 0	30
1	2	46.9 (GMF)	160 / 40 / 9 / 0	30
1	3	46.9 (GMF)	160 / 40 / 9 / 0	30
1	4	46.9 (GMF)	160 / 40 / 9 / 0	30
Sum	4	1	4	1

2.1.2 Germination tests

EM exposure and germination tests were conducted at Lund University (LU). *Solanum lycopersicum* 'Super Sweet 100' seeds were obtained from the seed company Olssons Frö AB. The seed incubator was set to an ambient temperature of 23°C, but since no fan was present, temperature had to be controlled with both an ambient thermometer and IR-thermometer. Incubation was done in complete darkness, except for some occasions when light inlet was unavoidable due to opening and closing of the incubator during EM exposure, and when counting seeds in front of a dimmed computer screen. Seed temperature was measured before and after treatment using an IR-thermometer.

Seeds were collected in bulk and randomized manually using the "counting by hand"-method presented by Kruse and International Seed Testing Association (2004). Seeds of a diameter outside the range of 1.5-3.0 mm were discarded to increase uniformity of exposed seeds. Twenty seeds was counted for each treatment, and put in separate Eppendorf-tubes covered with plastic tape not letting through light. Seeds were soaked in 1 ml of millipore-water 12 hours prior to exposure, and put in a seed incubator to maintain an even temperature over-night. After exposure, the treated seeds were again put in the incubator for 15 minutes before being washed three times with fresh millipore-water. After washing, seeds were put on separate petri-dishes, each dish marked with a randomized serial number to blind the test. Eight ml of millipore-water was added to each petri-dish. When all seeds from one exposure were washed and put on petri-dishes, the entire batch was put in a plastic bag and marked with a letter representing the time of exposure. Two hours after starting the first exposure, the next exposure begun.

Exposed seeds were taken out of the incubator and counted in twelve hour intervals, starting at 48 hours and continuing until 131 hours had passed from initial soaking, making a total of 8 counts per treatment. After their final counting, seeds were put in a styrofoam box for transportation to the Swedish University for Agricultural Sciences (SLU) and subsequent cultivation experiment.

2.1.3 Cultivation experiment

The cultivation experiment was conducted in March at the Swedish University of Agricultural Sciences (SLU), Alnarp. Greenhouse was set at an aeration temperature of 23°C, a heating temperature of 18°C and 70% relative humidity, controlled by spray nozzles. Artificial light was produced 16 hours per day using 5 HPS-lamps placed in between and at the ends of each replicate to cover the entire area of cultivation and minimizing potential corner shading effects. This was done as an addition to the ambient light of the sun, as a means of evening out the seasonal light differences appearing between blocks due to the time-difference of planting (each block being planted with an interval of one day). Light output for each lamp was measured using a flat LI-COR Quantum Q170 light sensor connected to a Li-Cor LI-189 photometer. The sensor was placed right underneath the lamp at the same level as the table used for cultivation and measurements are presented in *Illustration 8*. It was noted that the lamp mounting ramp was curved at one end, increasing the distance of the lamps near replicate 1 *(See Image 2)*.

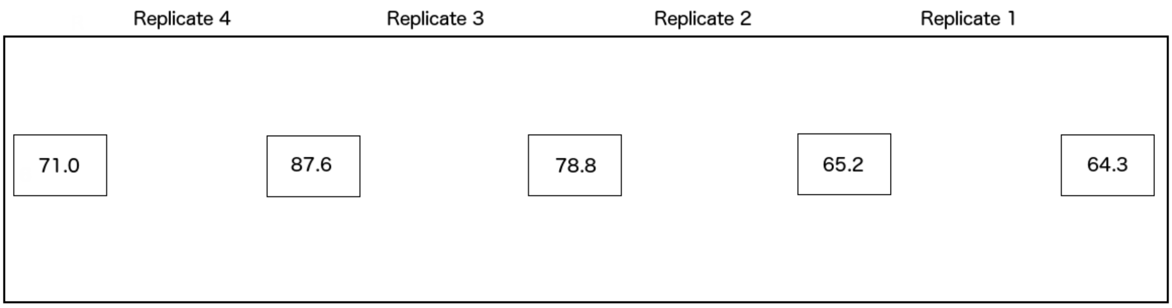


Illustration 8: Light intensity of each lamp across the table of cultivation, given in µmol photons per cm^2 and second

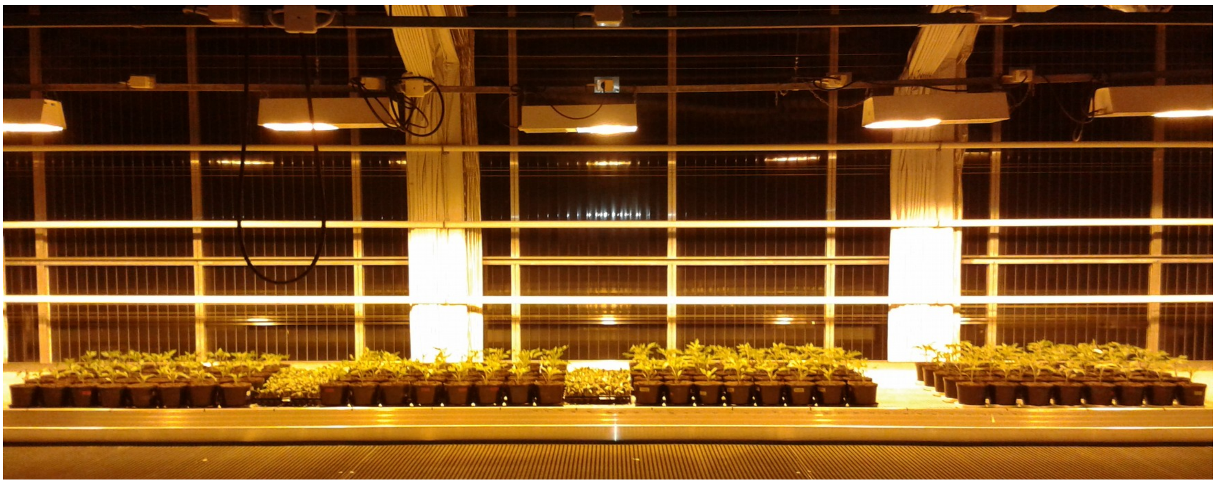


Image 2: Picture of cultivation setup showing the curved light mounting ramp

Four sowing-brims, each with 160 small squares, was filled with manually homogenized commercial soil designed for sowing. Each brim was designated for exposures done in the same day, thus making them separable. All seeds from the 30 minute treatments were selected and planted at a set time of 138 hours after initial soaking. Planting depth was adjusted depending on seed germination, where ungerminated and newly germinated seeds were planted ca 0.5 cm deep, highly developed seedlings so that their tip was barely covered, and fully developed seedlings so that the cotyledons were above soil. After planting, the seedlings were put in the greenhouse and showered daily to maintain soil moisture. No artificial light was being used until 240 hours (10 days) after sowing. Artificial light entrainment of the seedlings was done using a three day cover with fiber cloth, and one day of full exposure.

After the four day light entrainment, seedlings were transplanted into 1,5L pots. The evening before transplanting, manually homogenized potting soil was filled to a box. Using a hygrometer, the humidity of the soil was evaluated to around 20%. A pot was then over-filled and its soil scraped off at the very edge to create a standardized method for filling the pots. The pot was weighed to 657 g. After being watered to field capacity it reached a humidity of around 70% and weight of 1050 g. This weight and humidity was then used as reference for all replicates, aiming at around 20% humidity before starting, filling the pots according to above mentioned method, and modifying to 657 g total weight. However, the hygrometer showed some inconsistencies (±15 %) and did not reach 100 % when put in pure water, so humidity had to be calculated on average. The soil of the third replicate had an obviously higher humidity, approximating 50%, whereas the entire replicate got an addition of 200 g to total weight. This addition was based on both methodology of filling the pots and the approximated weight of the water content. These uncertainties may have led to some variations in soil content of the pots, however these differences should be randomly spread within each replicate. This since each replicate was potted using the same batch of soil and transplanting was completely randomized. Between replicates the variation may have been greater due to different batches of soil being used, with a possible unnoticed difference in soil humidity.

To make possible the use of four replicates, only plants exposed to the EMF in the ambient GMF environment for 30 minutes was selected for transplanting. Of these, 10 out of the 20 available seedlings were randomized for transplanting. Randomization was conducted prior to transplanting using the website *random.org*. If a randomized seedling was not germinated or obviously damaged, the most adjacent seedling was instead selected. The experiment was designed after a completely randomized block design, each replicate serving as a block, and pots being re-randomized each five days to minimize light and temperature differences. Yellow stickers were used to catch potential herbivorous insects, however no insects was detected during the entire experiment.

After transplanting, pots were each given 300 ml of water (except for pots in replicate 3 which got 100 ml due to already wet soil), and another 100 ml five and eleven days later. After that, each replicate was watered with 100-500 ml of water depending on weather-conditions and dryness of the soil. Watering was equal within each replicate but differed slightly between replicates to avoid over- or under-watering. Aside from ordinary watering, each plant was also watered individually 17, 27 and 29 days after transplanting. This was done by weighing the pot and watering until reaching a pre-determined weight. 300 ml of chemical fertilizer was watered out after 21 and 25 days of transplanting, with an N-P-K of 18-6-45. The fertilizer was custom-made using 975 grams of potassium nitrate and 100 grams of ammonium phosphate in a 500 ml concentrate, diluted 1:200 before application.

During cultivation, notes on plant morphology, height and number of nodes was taken on day 6, 14 and 22 after transplanting. Thirty days after transplanting, final measurements of height, number of nodes and number of flowers were noted. After this, the plants' leaves and flowers, main stem and auxiliary shoots was weighed individually prior to being put in a drying cabinet. After a week of drying, dry weight for each individual part was also noted.

2.1.5 Statistical analyses

The data was analysed using both R and SAS statistical software. For the germination test, analysis included a separate three-factor ANOVA for experiment 1 and experiment 2 (thus making 2 replicates each). The three factors used were static MF environment, time-varying EMF amplitude and duration of exposure. Parameters tested was germination percentage at the 6^{th} and 8^{th} counting. Residual plots were used to affirm validity for the ANOVA.

For the cultivation experiment, analysis included a two-factor ANOVA, were the 10 individual pots per treatment were counted as replicates. The two factors being used was block (actual replicates from exposures) and time-varying EMF amplitude. This was to determine differences between blocks. Furthermore, a one-factor ANOVA were each block was counted as a replicate was conducted, since these were the actual replicates.

Graphs were designed using Microsoft Excel 2011.

3 Results

3.1 The pilot study

3.1.1 Germination tests

Mean values for number of germinated seeds at the 6^{th} and 8^{th} counting were calculated for each replicate and SMF environment across all time-varying EMF treatments and durations of exposure *(see Table 6)*. To analyse the effect of the different EM treatments on germination, germinated seeds from the 6^{th} and 8^{th} counting was used for statistical analysis with ANOVA. No significant differences were found between different time-varying EMF treatments compared to the control only exposed to the background SMF environment. For both countings, statistically significant differences were found between artificial SMF environment (ICR) compared with ambient SMF environment (GMF) in experiment 1 ($p<0.01$) *(see Figure 1)*. No significant differences between artificial SMF environment (non-ICR) compared to ambient SMF environment (GMF) was found in experiment 2 *(see Figure 2)*. The conducted residual plot showed a normal spread, validating the ANOVA. Mean values for number of germinated seeds across time-varying EMF exposures and SMF environments at 6th counting for each experiment is presented in *Figure 1 and 2*.

Table 6: Mean number of germinated seeds (±SD) per replicate and SMF environment across all time-varying EMF treatments and duration of exposure at 6th and 8th counting, n=8

Experiment	Replicate	Artificial SMF environment	Ambient SMF environment
6th counting			
1 (ICR / GMF)	1	7.38 *±2.39*	10.1 *±3.09*
1 (ICR / GMF)	2	7.00 *±4.28*	11.8 *±1.98*
2 (non-ICR / GMF)	1	11.50 *±1.60*	10.0 *±1.77*
2 (non-ICR / GMF)	2	13.12 *±2.70*	13.5 *±1.60*
8th counting			
1 (ICR / GMF)	1	16.1 *±0.83*	17.9 *±1.89*
1 (ICR / GMF)	2	14.4 *±3.34*	17.5 *±1.41*
2 (non-ICR / GMF)	1	17.5 *±1.85*	17.2 *±1.67*
2 (non-ICR / GMF)	2	18.6 *±1.19*	17.2 *±1.13*

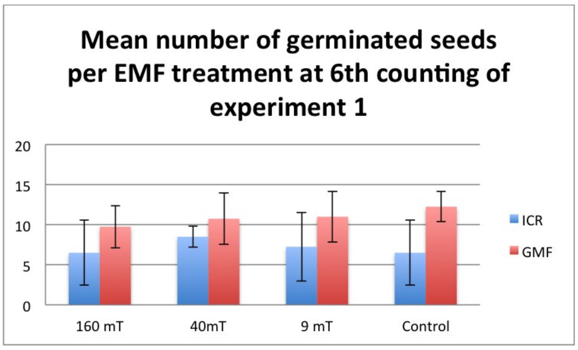


Figure 1: Mean value and standard deviation of number of germinated seeds per time-varying EMF treatment and SMF environment across duration of exposure at 6th counting of experiment 1, n=4.

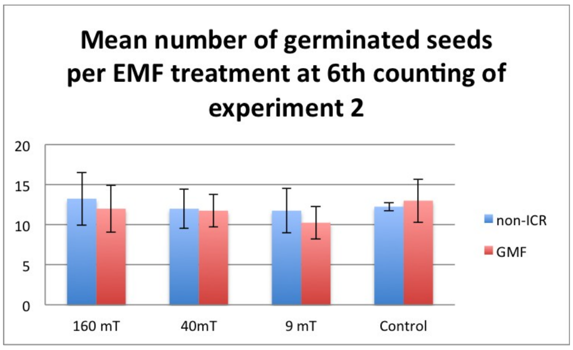


Figure 2: Mean value and standard deviation of number of germinated seeds per time-varying EMF treatment and SMF environment across duration of exposure at 6th counting of experiment 2, n=4.

3.1.2 Cultivation experiment

To analyse the different time-varying EMF treatments, mean values of all parameters were calculated across the four blocks, presented in *Table 7*. The visual observations showed that EM treatments delayed seed case drop to a degree that cotyledons were deformed in several individuals (*See image 3 & 4).* The differentiation between dry and fresh weight of leaves, stem and shoots is presented in *Table 8.* The two-factor ANOVA showed no significant differences between the time-varying EMF treatments and controls without EM exposure, while significant differences between blocks (actual replicates) was found ($p<0.01$). The one-factor ANOVA showed no significant differences between time-varying EMF treatments compared to controls either. Differences between replicates and time-varying EMF treatments in parameters final height, amount of flowers, fresh weight and dry weight is presented in *Figure*

5-8. Growth rate between different treatments were illustrated by plotting the mean value of the four height measurements over time. This is presented in *Figure 9.*

Table 7: Mean values of final measurements (±SD) per time-varying EMF treatment across all blocks, n=4

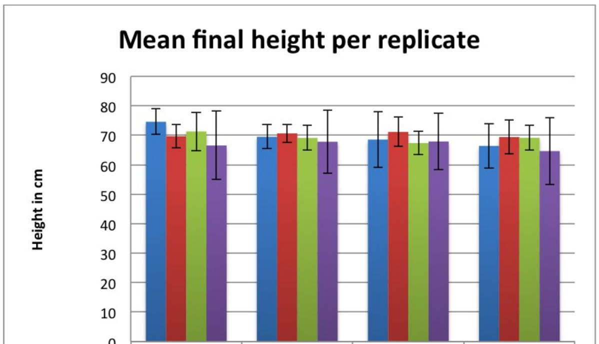
Treatment	Cotyledons deformed	Final height (cm)	Number of flowers	Number of nodes	Total fresh weight (g)	Total dry weight (g)
160 mT	25 %	70.54 *±6.62*	33.95 *±4.99*	16.55 *±0.52*	115.91 *±9.67*	12.81 *±1.45*
40 mT	7.5 %	69.26 *±5.51*	33.95 *±5.61*	16.6 *±0.76*	117.13 *±9.37*	13.13 ±1.37
9 mT	5 %	68.74 *±7.01*	32.15 *±3.80*	16.48 *±0.54*	114.79 *±8.43*	12.80 *±1.23*
Control	0 %	67.40 *±7.20*	31.28 *±3.82*	16.45 *±0.57*	112.91 *±8.41*	12.52 *±1.29*

Table 8: Mean distribution of biomass per time-varying EMF treatment across all blocks

Treatment	Fresh weight lea. & fl. (g)	Fresh weight stem (g)	Fresh weight shoots (g)	Dry weight leaves & flowers (g)	Dry weight stem (g)	Dry weight shoots (g)
160 mT	64,28 56 %	40,73 35 %	10,90 9 %	8,01 63 %	3,61 27 %	1,19 9 %
40 mT	65,25 56 %	39,22 33 %	12,67 11 %	8,20 62 %	3,52 27 %	1,41 11 %
9 mT	63,85 56 %	38,98 34 %	11,95 10 %	8,02 63 %	3,49 27 %	1,29 10 %
Control	63,53 56 %	37,96 34 %	11,41 10 %	7,89 63 %	3,39 27 %	1,24 10 %

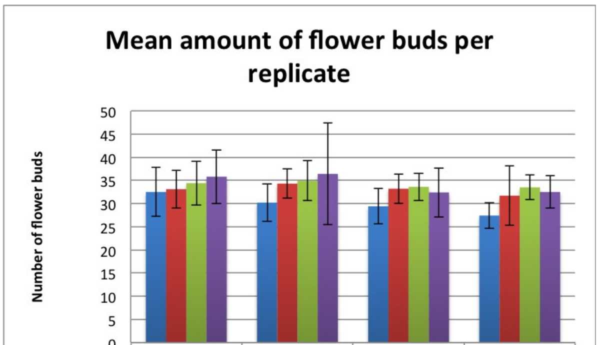
Image 3: Picture of seed coat stuck to cotyledons at plant maturity

Image 4: Picture of deformed cotyledons following delayed seed coat disintegration



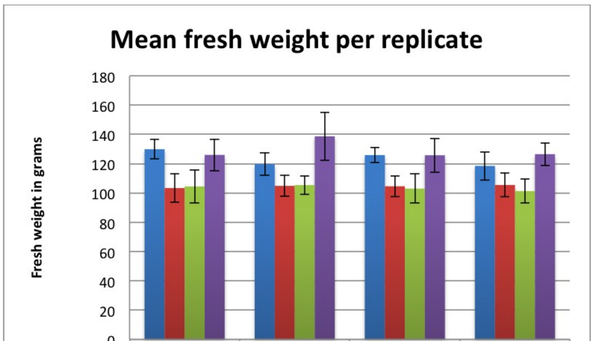
	160 mT	40 mT	9 mT	Control
Replicate 1	74,6	69,50	68,55	66,40
Replicate 2	69,7	70,65	71,15	69,40
Replicate 3	71,3	69,10	67,35	69,15
Replicate 4	66,55	67,80	67,90	64,65

Figure 5: Mean final height measurement for each replicate, including standard deviations, n=10



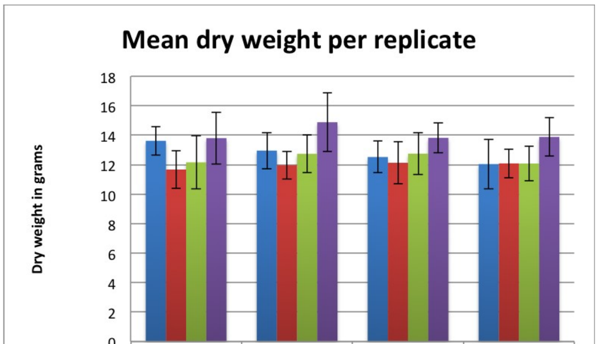
	160 mT	40 mT	9 mT	Control
Replicate 1	32,5	30,2	29,4	27,4
Replicate 2	33,1	34,3	33,2	31,7
Replicate 3	34,4	34,9	33,6	33,5
Replicate 4	35,8	36,4	32,4	32,5

Figure 6: Mean final amount of flower buds for each replicate, including standard deviations, n=10



	160 mT	40 mT	9 mT	Control
Replicate 1	129,859	119,689	125,865	118,465
Replicate 2	103,344	104,835	104,563	105,439
Replicate 3	104,445	105,385	102,962	101,262
Replicate 4	125,987	138,629	125,756	126,464

Figure 7: Mean final fresh weight for each replicate, including standard deviations, n=10



	160 mT	40 mT	9 mT	Control
Replicate 1	13,62	12,953	12,518	12,041
Replicate 2	11,669	11,965	12,128	12,084
Replicate 3	12,157	12,735	12,747	12,076
Replicate 4	13,799	14,878	13,822	13,88

Figure 8: Mean final dry weight measurement for each replicate, including standard deviations, n=10

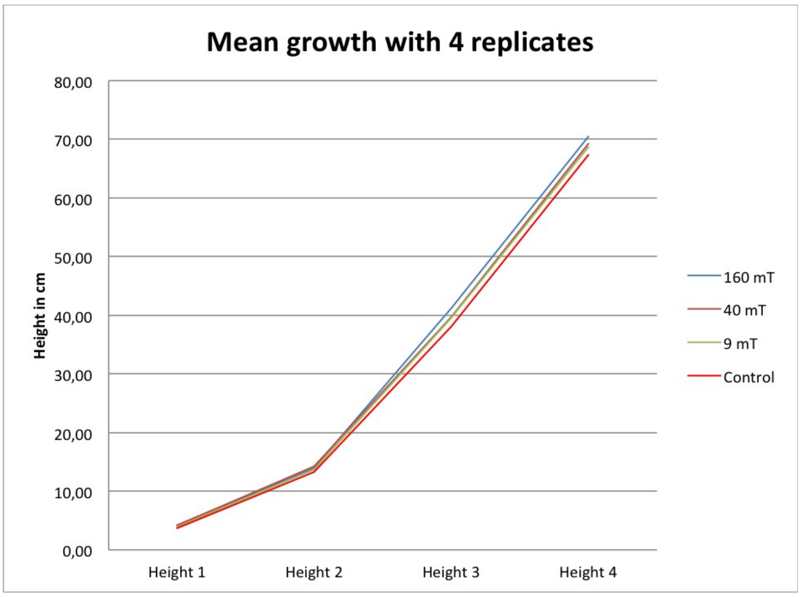


Figure 9: Mean growth development per treatment, across all blocks.

4 Discussion & Conclusions

4.1 The pilot study

4.1.1 Germination tests

Error sources
Due to lacking of certain equipment, only one exposure could be run at once, making replicates appear separately in time. Furthermore, control groups were not exposed to the exact same environment as treatments due to a non-double blind setup. Controls were heated with a warming plate, instead of using a sham control as proposed by Kirschvink (1992). The heating plate may in itself have affected the static magnetic field strength that controls were exposed to, due to it being made of steel. Due to bulkiness of the probe used for the oscilloscope the MF intensity could not be precisely defined. Furthermore, even though control groups were placed at a distance where the oscilloscope could not measure the EMF of the electromagnet, it is possible that the EMF reached control groups at an amplitude not measurable with the oscilloscope. More precise devices should be employed to determine exact amplitudes of applied EMF. The electromagnet also did not produce completely uniform electromagnetic fields in the way that helmholtz-coils do. This could weaken the ICR effect, said to occur when combined electromagnetic fields are completely parallel to each other. The incubator also lacked a fan, possibly affecting heat distribution during the incubation period.

Analysis of results
Since only two replicates were used for the germination tests, it is difficult to draw any concise conclusions from the results. Statistically significant difference showed between replicates, possibly because they were not conducted simultaneously. No significant difference were shown between the electromagnetic exposures compared to control. Interestingly though, statistically significant differences between the intensity of the static MF environment were noted only in experiment 1, when the static magnetic field was tuned to the cyclotron resonance of calcium, showing a delayed germination where ICR-conditions were present compared with GMF-conditions. However, since the control-groups also showed inhibited germination, the static MF environment of 65,8 μT was possibly inhibitory to germination alone. However, since ICR has been demonstrated to work at extremely weak EMF amplitudes (Alberto et al., 2008; Pazur, 2004; Zhadin et al., 1998), it is also possible that the controls were not placed far enough from the electromagnet to completely escape its EMF's. Even so, ICR is proposed to work when combined magnetic fields are applied parallel to each other, which makes the above mentioned theory unlikely because of a certain non-uniformity of the EMF possibly reaching the control groups. It therefore seems more likely that the static MF environment of 65.8 μT itself affected the entire batch of seeds. It has been shown that slight deviations in GMF intensity affect water absorption rate and germination characteristics in seeds (Es'kov and Rodionov, 2010), however it is strange that the effect is not apparent at the very proximate intensity of 68.5 μT. With the current setup using only two replicates, it is also

difficult to rule out that the inhibition was a matter of coincidence and a result from a deviation in the plant material.

However, in studies with *Raphanus sativus* var. Belle, treatments with weak combined electromagnetic fields tuned to cyclotron resonance of calcium also delayed germination (Smith et al., 1995). In their study, a tuning with potassium instead speeded up germination. In further experiments, a potassium-tuning should also be incorporated in the trial, since a drastic change of effect would strongly indicate that ICR is involved. The control groups need to be placed in a shielded place so that no EMF's produced by the electromagnet may interact with them. They also need to be placed in a similar electromagnet as exposed treatments, but with copper wires being wound in such a way that electric and magnetic field-components nullifies each other as proposed by Kirschvink (1992). Using such a setup, and incorporating more replicates with a greater number of seeds exposed simultaneously, would help in concluding the nature of observed effects.

Furthermore, studies with electromagnetic pre-sowing treatments should always aim at collecting data on what static magnetic fields are present during exposure, something often lacking in the literature (Maffei, 2014). This would help cross-referencing in the literature when investigating the nature of these effects.

Conclusions of germination tests

- The time-varying EMF treatment showed no significant effect on germination of *S. lycopersicum* 'Super sweet 100'.
- The static MF environment of 65.8 μT possibly have an inhibitory effect on the germination parameters of *S. lycopersicum* 'Super sweet 100'.
- The results need to be validated with better equipment and more replicates in other laboratories, and can only serve as indicative in its present execution.

4.1.2 Cultivation experiment

Error sources

Since the cultivation experiment was a direct extension of the germination test, all error sources attributed to the former is also present in the latter. Transportation from Lund University (LU), Lund, to Swedish University of Agricultural Sciences (SLU), Alnarp, meant that replicates may have been exposed to slightly different conditions before sowing, although they were kept in a temperature-proof transport box in complete darkness during transport. During sowing, some germinated seeds had longer radicula than others, making it difficult to plant them without in some way disrupting the root. During transplanting to 1,5L pots, the hygrometer showed some inconsistencies in the readings, possibly contributing to a slight difference in soil content of the individual pots. Furthermore, the inconsistencies of the hygrometer also meant that individual watering had to be conducted using scales instead, meaning that watering could not be conducted in a completely fair way. All plants were grown in the same table, with an even distribution of five HPS-lights above them. HPS-lamps were not equal in light intensity, though this should have been leveled out by the much more intense solar radiation. Replicate 1 and 4 were situated at both ends of the table, giving them a different solar radiation-gradient compared with replicates in the middle of the table. Replicate 1 had morning sun, while replicate 4 had evening sun. Furthermore, during measuring and final weigh-in, an inescapable factor is that of the practical scientific inexperience of the author, contributing to possible human errors.

Analysis of results

No significant differences were noted between treatments. Significant differences were noted between blocks (replicates) instead. When studying the mean values of fresh and dry weight both replicate 1 and 4 had higher values than others. This could be due to corner effects, since they both were situated at the end of the table and hence may have had a higher radiative input and thus daily mean temperature. It is known that the mean temperature and radiation greatly affects the growth and reproduction of tomato plants, and the strongest growing tomatoes is therefore proposed to be situated at the end corners of the table (Hansson et al., 2007). When studying the data on number of flowers, replicate 1 seem to have less flowers than other replicates. This could be because it was the first planted replicate, and also the first to be taken out of the experiment. Since the experiment was conducted in March, with an increasing temperature and day length, all four replicates got a slightly different seasonal environment. Studying the data, one can see an increasing amount of flowers from replicate 1 to 4, indicating that the difference in cultivation period might have an effect. Furthermore, counting the individual flower buds is difficult since many still are tiny at counting, and a possible factor might be that effectiveness of the analyst's ability of counting flower buds increased after some repetition.

The visual observations showed a delayed time of disintegration of seed coats in seedlings exposed to EMF's, which led to a misshaping of cotyledons in many exposed plants. This was an obvious trend within exposed individuals, not showing in any of the control plants. There doesn't seem to be any hard facts stating why seed coats do not fall off their seedlings, however it is generally assumed to occur when seed coats are dry or when not planting deep enough. In this pilot study, all seedlings were planted in the same brim, and showered daily during the first week of planting. Planting was uniform, following a strict method for all treatments. During incubation and transport, seeds from a single exposure were kept in the same plastic bag, with treatments separated in separate petri-dishes. They should therefore have had a very similar humidity throughout germination & been planted at similar depths at sowing. It is possible that observed effects are some sort of methodological artifact, however it seems more likely to be a direct result of the EM treatment. EM treatments has been shown to alter water absorption rate of seeds (Es'kov and Rodionov, 2010; Reina et al., 2001), show auxin-like effects (Maffei, 2014), altering enzyme activity (Shabrangi and Majd, 2010, p. 4; Vashisth and Nagarajan, 2010), and increase stress-related compounds (Ben-Izhak Monselise et al., 2003). Furthermore, studies on *Helianthus annuus* seeds exposed to static magnetic fields of varying intensity showed an increased seed coat membrane integrity, reduced cellular leakage and electrical conductivity (Vashisth and Nagarajan, 2010). It is thus probable that the observed effects are due to modification of seed coat membrane, hormonal disturbances and enzyme activity by applied EMF's, affecting the structure and content of the seed coat itself. Further studies with a deeper biological analysis and further replication are needed to fully validate and address these observations.

The results of the pilot study showed that the EM treatment were ineffective in increasing plant growth and reproductive parameters. However, several similar studies has shown a positive effect on plant growth and yield (De Souza et al., 2006, 2005; Efthimiadou et al., 2014; Jedlicka et al., 2014). Why the treatments were ineffective may have many explanations. One possible explanation is that the seeds, being F1-hybrids, simply were not susceptible to that particular EM treatment. It is also possible that the exposure-conditions were wrong. Since the seeds were exposed to EMF's in a wetted condition, and wetting commenced 12 hours prior to exposure, the old water in the eppendorf-tubes used during exposure may have turned acidic. In studies conducted on *Picea abies*, where similar but much weaker EMF's was used, germination was inhibited at pH levels of 2-3, whilst having no effect or being stimulatory at pH-levels of 6-7 (Ruzic et al., 1998).

It is also possible that the exposure setup was wrong, both by intensity, duration and exposure-type. When cross-referencing the exposure setup with other studies showing increased germination rate and growth characteristics of *S. lycopersicum*, some differences can be found. In one study they used 50 Hz EMF's for 20 minutes on a daily basis, which gave significant increases in germination rate, yield and biomass (Jedlicka et al., 2014). Thus, it is possible that application of time-varying EMF's need to be conducted at regular intervals for a sustained effect. Studies with extremely short 50 Hz EM pre-sowing treatments of *Triticum durum* seeds also indicated that the duration of exposure greatly affects the duration of biological effect (Muszynski et al., 2009). However, studies on *S. lycopersicum* using 60 Hz EMF's has showed significant increases even when only using a single pre-sowing treatment (De Souza et al., 2006). The wave-form used in that study was a rectified sine-wave, contrary to the pure sinusoidal sine-wave used in the present pilot study. Furthermore, studies on pre-sowing treatments of *S. lycopersicum* using PEMF's showed a sustained and significant effect on both plant height and yield (Efthimiadou et al., 2014). Both of these EMF's maintain a constant polarity, which sinusoidal fields doesn't. This makes them similar to treatments with SMF's that remain constant in both polarity and intensity. In studies with SMF's on *S. lycopersicum* seeds, an increase in germination rate and early growth characteristics has been observed (Martinez et al., 2009). It is possible that the polarity is of importance to an EM treatment of *Solanum lycopersicum 'Super Sweet 100',* though it seems unlikely since many studies using sinusoidal EMF's has observed biological effects on other plants (Eşitken and Turan, 2004; Isaac Aleman et al., 2014; Leelapriya et al., 2003).

Important to notice is that the experimental setup was very simple, and replicates could not be conducted simultaneously. Therefore it is also very difficult to ensure validity of the exposure setup and hence if it is applicable to the collected data. Professional validation from the fields of physics and technical engineering would have been needed to ensure that exposures were conducted correctly.

Conclusions of cultivation experiment

- The time-varying EMF treatment had no significant effect on growth parameters of *S. lycopersicum* 'Super sweet 100'.

- The time-varying EMF treatment may have delayed seed coat disintegration from seedlings.
- The results need to be validated with better equipment and more replicates in other laboratories, and can only serve as indicative in its present execution.

4.2 Review of literature

4.2.1 Effects on crop productivity and the potential for horticultural use

EM treatments of various kinds has been explored as a potential agricultural and horticultural technique for increased yields over the past 100 years, yet it is still a poorly understood and controversial subject (Pietruszewski, 2011; Volkov, 2006). As presented in *Section 1.4*, a wide range of physiological and biological phenomena has been observed by exposing plant material to SEF's, SMF's or time-varying EMF's of different quality and quantity. While some observed effects, like increased germination rate (Cakmak et al., 2010; Carbonell et al., 2000; Jedlicka et al., 2014; Leelapriya et al., 2003; Pietruszewski, 2011), growth and yield (Bilalis et al., 2012; De Souza et al., 2006, 2005; Efthimiadou et al., 2014; Eşitken and Turan, 2004; Jedlicka et al., 2014; Leelapriya et al., 2003), nutrient content (Dhawi et al., 2009; Eşitken and Turan, 2004) and stress tolerance (Anand et al., 2012; Chen et al., 2011; Ružič and Jerman, 2002), seems enhancing and positive to crop productivity, other effects may also prove detrimental. Such effects may include genetic mutations (Kiranmai, 1994), oxidative stress (Ben-Izhak Monselise et al., 2003) and developmental aberrations (Tkalec et al., 2009).

At a first glance, it may seem as if effects may be similar across SEF, SMF and time-varying EMF exposures. However, something easily noted when reviewing these studies, is that the studied effects often show non-linear dose-response curves and that these response curves differ across species (even cultivars), different plant tissues, developmental stages as well as different exposure types. An example of non-linear dose response curves in a time-varying EMF exposure can be seen in the study of Efthimiadou et al. (2014), where a 5 minute exposure of *Solanum lycopersicum* to PEMF decreased successful transplanting but 10 and 15 minute exposures increased success rates. An example of difference from a SMF exposure between cultivars of the same species can be seen in the study of Novitskaya et al. (2001), where SMF exposure increased the chlorophyll and protein content in *Allium cepa* 'Arzamasski' but decreased the same parameters in *Allium cepa* 'Ryazanskii'. An example of differences from a time-varying EMF exposure between species and plant tissues can be seen in the study of Shabrangi and Majd (2010). They found that ELF EMF's increased activity of oxidative enzymes in root tissues of *Brassica napus* and decreased it in shoot tissues, while the opposite was apparent in *Zea mays.* An example of differences from SEF and SMF exposure of the same species can be seen in the study of Cakmak et al., (2012), where SEF's induced stress but SMF's increased growth in *Allium ascalonicum.*

From a horticultural usability perspective, extensive exposure-specific and species-specific research would be needed prior to field application in order to ensure repeatable results. These experiments require sophisticated technology and integration of knowledge from several instances, including physics, technical engineering and biology, in order to validate findings and ensure correct exposure-conditions. These difficulties were present in the pilot study; while many studies indicated that EM pre-sowing treatments of *S. lycopersicum* seeds has the potential to have a sustained positive effect on growth and yield, the pilot study showed that they are hard to reproduce without sophisticated methods of exposure and professional expertise. Because of this, it is difficult to state that EM treatments are ready for field use, where both the required knowledge and technology may be lacking. EM treatments definitely has proven a potential for horticultural use, but as of today still needs extensive researching. The main area of research ought to be the underlying biological mechanisms that may interact with SEF's, SMF's and EMF's. With greater insights into underlying mechanisms, scientists may create models for prediction of effects from EM treatments, instead of conducting endless species-specific and exposure-specific research. This would save time and energy in future research and development of various EM techniques for improved crop productivity, as well as enhance the accuracy and repeatability of treatments. This is further discussed in the following section.

4.2.2 Effects on plant biology and the current scientific understanding

A lot has been discovered over the past decades, as of how SEF's, SMF's and time-varying EMF's affect the biology of plants. For example, it has been found that EMF's of various sorts may affect the genetic material (Kiranmai, 1994), electrolyte availability (Pazur and Rassadina, 2009), mineral composition (Dhawi, 2009; Eşitken and Turan, 2004), various biochemical processes (Anand et al., 2012; Novitskaya et al. 2001; Vashisth and Nagarajan, 2010), signalling molecules (Ben-Izhak Monselise et al., 2003), cellular structure (Cho et al. 1994, 1996) and cellular reproduction (Tkalec et al., 2009). When reviewing the literature, one finds an ocean of different approaches, conclusions and theories as of the origin of such effects, and finding consensus proves difficult. Mechanistic theories often include elements like activation of voltage-gated ion channels and receptor proteins, increased membrane permeability, cellular segmentation and direct effects on magnetic content (diamagnetic susceptibility), enzyme activation or inhibition, radical pair formation (cryptochrome magnetoreception), ROS formation, electrolyte energy increase (ICR hypothesis) and interactions with second messengers (IPR hypothesis).

Although debated, many theories has proven experimentally feasible and deepened our understanding of how SEF's, SMF's and EMF's affect the biology of plants. This has improved the accuracy of prediction of EMF's effects on plant material, and may also help in increasing the horticultural usability of EM treatments. For instance, the connections between cellular oxidative status, iron content and genetic damage from time-varying EMF exposure (Phillips et al., 2009) may help in the understanding of how to avoid harmful aspects of an EM treatment. Moreover, in the experimental studies of the ICR and IPR hypothesis, scientists has managed to mathematically predict which combinations of SMF's and time-varying EMF's that will affect the cytosolic release of specific ions (Pazur and Rassadina, 2009), and thus how to produce differing effects when creating resonance with different ions (Pazur et al., 2006; Smith et al., 1995). The findings that the cryptochromes of *Arabidopsis thaliana* showed sensitivity to SMF's provide some understanding as to how weak SMF's can influence something as fundamental as the circadian processes in the plant, and mathematical models has been used to successfully estimate this effect (Solov'yov et al., 2007; Solov'yov and Schulten, 2012; Xu et al., 2014, 2012). Furthermore, the different findings of altered enzyme-activity after EM treatments (Rochalska and Grabowska, 2007; Shabrangi and Majd, 2010; Vashisth and Nagarajan, 2010) may provide a link for future predictions of biological effects of EMF's, where the effects of individual enzymes may be further studied and taken into consideration. The same can be said for the findings of diamagnetic susceptibility, where the ratio of diamagnetic to paramagnetic compounds could be used to predict the intensity needed for an EM treatment to produce an effect (Peñuelas et al., 2004).

Still, a lot of controversy surrounds the subject, and theories like ICR and IPR have received much criticism (Adair, 1998, 1992, 1991; Halgamuge and Abeyrathne, 2010), and many links are still missing. Thus, one cannot underestimate the importance of further research into the biological mechanisms behind biological effects of EMF's, since this is an essential part of solving the puzzles of bioelectromagnetics and its usability for improved crop productivity.

4.3 General Conclusions

- EMF's are force-fields that give rise to phenomena such as light, electricity, magnetism and chemical bonding. They have both electric and magnetic aspects, and are present everywhere in our environment in various forms.
- Various EM treatments of crops have shown enhancements in germination rate, germination percentage, growth characteristics, yield, nutrient content and stress tolerance.
- A wide array of biological effects have been studied in plant material as a response to EMF's, including effects on genetic material, electrolyte availability, mineral composition, biochemical processes, signalling molecules, cellular structure and cellular reproduction.
- Many mechanisms have been proposed, such as alterations to membrane permeability, enzyme activity, cellular segmentation, radical-pair and ROS formation as well as activation of ion channels, receptor-proteins and second messengers.
- The pilot study indicated that the SMF may affect the germination of *Solanum lycopersicum 'Sweet 100',* however the time-varying EMF showed no effect.
- EM treatments have the potential for horticultural use, but is questionable due to difficulties in predicting effects. Extensive research incorporating the knowledge from technical engineering, physics and biology is proposed prior to field application.
- More research into underlying biological mechanisms is proposed to improve the accuracy and usability of EM treatments.

5 References

Licenses for Illustrations

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