USE OF ELECTROMAGNETIC RADIATION & ELECTROPORATION AS EXTERNAL NON-IONIZING STIMULANTS FOR SWITCHGRASS SEED GERMINATION

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ABSTRACT

In the present study, Shelter Switchgrass seeds were selected and tested to see the influence of electromagnetic radiation (EMR) and electroporation on increased germination percentage. 50 to 55 seeds along with electroporation buffer were placed in a custom designed gene pulser cuvette and fixture, to concentrate EMR energy. For EMR experiments, two setups were used, one emitting 2.4 GHz or 12.5cm wavelength, the other emitting 0.9 GHz or 33.3cm wavelength. Each experiment had a set duration and different cuvette power levels (0.05W, 0.5W, and up to 5W). Average cuvette field Intensity (E, units kV/m, V/m) was estimated using specialized engineering software. The number of trials varied from 3 to 5 in the EMR experiments, including the control. For electroporation experiments, seeds were exposed to voltages of 2.5kV/cm and 3.0kV/cm for 1ms pulses for 3 trials, where there were 15 pulses for 2.5kV/cm and 30 pulses for 3.0 kV/cm trials. After the seeds were treated they were dried and plated in Petri dishes on top of filter paper (20-25 seeds a plate, 2 plates per trial). The plates were watered every day and excess water was removed to prevent fungus. Each experiment was concluded after 10 days in the greenhouse, where germination data was calculated, and results were analyzed.

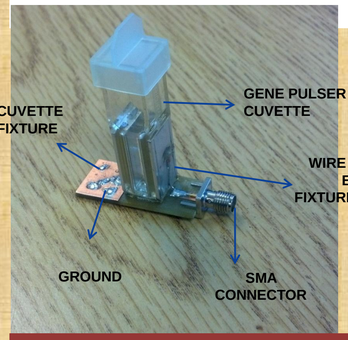


Figure 1: Design of Cuvette connected to custom built fixture for Electromagnetic Radiation

INTRODUCTION

Currently, there is an increasing demand to provide the world with biofuels not only because it is a renewable and inexhaustible source of fuel but also because it has a very low emission rate per gram (Monti, A. et al. 2012). Seed germination is already known to be affected by both the electric and magnetic fields from the earth itself, without the application of external electromagnetic radiation. Switchgrass (*Panicum virgatum* L.) is a highly efficient biofuel feedstock, harvested for its biomass to make ethanol from its cellulose. Its ability to grow in marginal soil and thrive in diverse climates across North America sparks great interest because it does not have to compete with food crops (Fike et al. 2006). Switchgrass, however, has a very low germination rate thereby lowering its cost efficiency as a biofuel feedstock. Bio-stimulation has been greatly considered, as a means to increase germination percentage. Biological stimulation plays an important role in optimizing Switchgrass crops in terms of the maximization of yield, promotion of plant growth, and protection against exogenous agents that cause plant disease. Bio-stimulation can be accomplished by applying various techniques, but we will only be testing stimulation by electromagnetic radiation and gene pulser electroporation system. These methods are more particularly beneficial because they do not require any toxic chemicals and residuals. Shelter Switchgrass seeds were chosen because of their known low germination percentage.

Figure 5: Experiment two of Electroporation after 10 Days in the Greenhouse

Figure 6: Plating the seeds after treatment

MATERIALS & METHODS

EMR

For this experiment the 0.4cm cuvette and fixture were hooked up to a Network Analyzer to determine the engineering specs in terms of how effective the EMR energy transfer to the cuvette is. We then connected the fixture to a radio frequency (RF) setup, amplifying the power generated from a signal generator adjusted to 0.05W, 0.5W, or 5.0W. For the first experiment we used a 2.4GHz amplifier, and 50 seeds for each trial. The seeds were placed in the cuvette and approximately 750ml of electroporation buffer was added. The cuvette fixture was then radiated for 10 minutes (no radiation for the control). 5 trials were performed for the 0.05W and 0.5W power settings. The second EMR experiment was performed using a 0.9 GHz amplifier, and 55 seeds were used for each trial. Due to the longer wavelength the duration was cut in half to prevent heat stress. This time five trials were performed for the 0.05W, 0.5W, and 5.0W power settings. After treatment seeds were taken from the cuvette and dried; then transported to the greenhouse to be plated in petri dishes on top of filter paper. For the first experiment 40 seeds were plated for each trial (20 seeds per plate), and for the second experiment 50 seeds were plated for each trial (25 seeds per plate). The seeds were given fresh water every day and after 10 days germination data is recorded.

ELECTROPORATION

This experiment was performed with the Bio-Rad Micro Pulser. To prevent arcing dilution of the electroporation buffer is necessary. For this experiment, we did a 3% dilution of the buffer. The changing parameters are voltage and number of pulses. The first experiment was done using three trials (each trial having 55 seeds) at 3.0kV/cm for 15 pulses. The second experiment was done using three trials (each trial having 55 seeds) at 2.5kV/cm for 30 pulses . A control was also set up for these experiments. The treated seeds were then plated and recorded just like the EMR experiment.

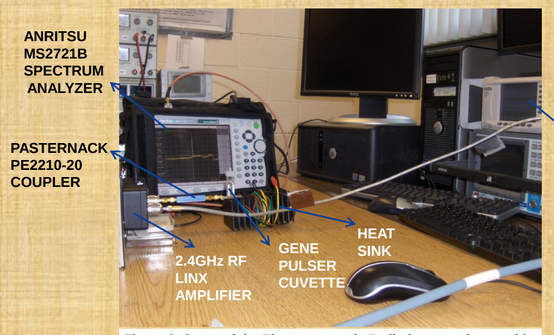


Figure 2: Setup of the Electromagnetic Radiation experiment with the 2.4 GHz Amplifier

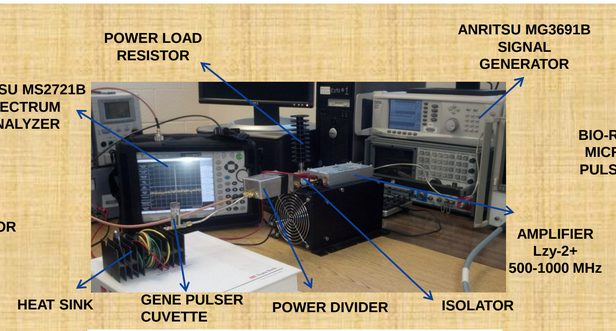


Figure 3: Setup of the Electromagnetic Radiation experiment with the 0.9 GHz Amplifier

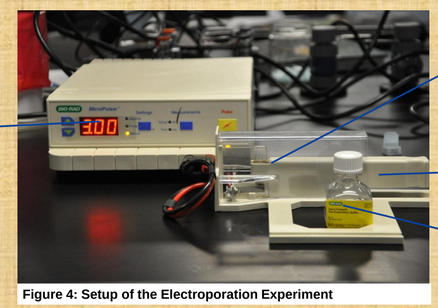


Figure 4: Setup of the Electroporation Experiment

	SIGNAL GENERATOR READINGS	CUVETTE POWER, P	CUVETTE ELECTRIC FIELD , E	SPECTRUM ANALYZER READINGS	DURATION	NUMBER OF SEEDS
EXPERIMENT 1-1	8 dBm	0.5W = 27 dBm	1kV/m	6.89 dBm	10 min	50
EXPERIMENT 1-2	8 dBm	0.5W = 27 dBm	1kV/m	7.21 dBm	10 min	50
EXPERIMENT 1-3	8 dBm	0.5W = 27 dBm	1kV/m	6.93 dBm	10 min	50
EXPERIMENT 1-4	8 dBm	0.5W = 27 dBm	1kV/m	7.27 dBm	10 min	50
EXPERIMENT 1-5	8 dBm	0.5W = 27 dBm	1kV/m	7.10 dBm	10 min	50
Control 1	—	—	—	—	10 min	50
EXPERIMENT 2-1	-2 dBm	0.05W = 17 dBm	300V/m	-2.16 dBm	10 min	55
EXPERIMENT 2-2	-2 dBm	0.05W = 17 dBm	300V/m	-1.96 dBm	10 min	55
EXPERIMENT 2-3	-2 dBm	0.05W = 17 dBm	300V/m	-2.24 dBm	10 min	55
EXPERIMENT 2-4	-2 dBm	0.05W = 17 dBm	300V/m	-1.98 dBm	10 min	55
EXPERIMENT 2-5	-2 dBm	00.5W = 17 dBm	300V/m	-2.18 dBm	10 min	55
CONTROL 2	—	—	—	—	10 min	55

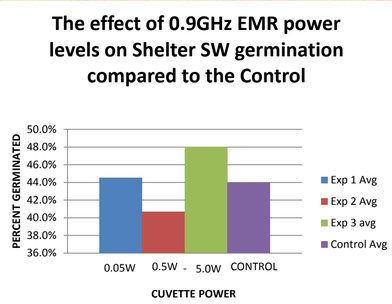
Table 1: Data from EMR Experiments with 2.4 GHz Amplifier

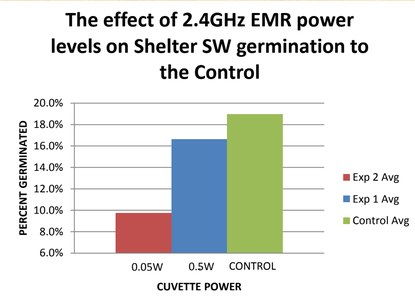
	CUVETTE POWER	SPECTRUM ANALYZER POWER	SIGNAL GENERATOR POWER	CUVETTE FIELD INTENSITY	DURATION	NUMBER OF SEEDS
EXPERIMENT 1-1	.05W= ~17 dBm	(-2.78 dBm)	(-8.5 dBm)	~300 V/m	5 min.	55
EXPERIMENT 1-2	.05W= ~17 dBm	(-3.04 dBm)	(-8.5 dBm)	~300 V/m	5 min.	55
EXPERIMENT 1-3	.05W= ~17 dBm	(-3.18 dBm)	(-8.5 dBm)	~300 V/m	5 min.	55
Control 1	-	-	-	-	5 min.	55
EXPERIMENT 2-1	0.5W= ~27 dBm	8.05 dBm	1.5 dBm	~1.0 kV/m	5 min.	55
EXPERIMENT 2-2	0.5W= ~27 dBm	7.73 dBm	1.5 dBm	~1.0 kV/m	5 min.	55
EXPERIMENT 2-3	0.5W= ~27 dBm	7.64 dBm	1.5 dBm	~1.0 kV/m	5 min.	55
Control 2	-	-	-	-	5 min.	55
EXPERIMENT 3-1	5.0 W= ~37 dBm	17.64 dBm	11.5 dBm	~3.0 kV/m	5 min.	55
EXPERIMENT 3-2	5.0 W= ~37 dBm	17.58 dBm	11.5 dBm	~3.0 kV/m	5 min.	55
EXPERIMENT 3-3	5.0 W= ~37 dBm	17.65 dBm	11.5 dBm	~3.0 kV/m	5 min.	55
Control 3	-	-	-	-	5 min.	55

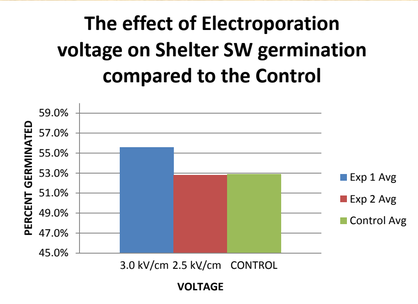
Table 2: Data from EMR Experiments with 0.9 GHz Amplifier

	VOLTAGE	NUMBER OF PULSES	DURATION of PULSE	NUMBER OF SEEDS
EXPERIMENT 1-1	3.0kV	15	1ms	55
EXPERIMEN 1-2	3.0kV	15	1ms	55
EXPERIMEN 1-3	3.0kV	15	1ms	55
Control 1				55
EXPERIMEN 2-1	2.5kV	30	1ms	55
EXPERIMEN 2-2	2.5kV	30	1ms	55
EXPERIMEN 2-3	2.5kV	30	1ms	55
Control 2			1ms	55

Table 3: Data from Electroporation Experiment







SUMMARY

-Germination percentages for experiments were generally very close.
-Power settings 0.05w, and 5.0w did better than the control in 0.9 GHz EM experiments
-Experiment 1 for electroporation with 3.0kv/cm and 15 pulses also did better than the control
-To draw more statistical conclusive results, more experiments will be conducted.

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