Direct Application of Energy to Soil for the Management of Soilborne Pathogens and Plant-Parasitic Nematodes

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INTRODUCTION

Alternatives methods to soil fumigation are needed, especially those that minimize buffer sizes and re-entry periods. One such alternative may be the pre-plant treatment of soil with electrical current, developed by LisiGlobal in Richland, WA. This method involves delivering highly concentrated electrical pulses directly into the soil. These pulses are tailored to affect specific structures in the target organism and damage or disrupt the normal function of those structures. The amount of electricity can be controlled in a way analogous to rate of application, dose, and concentration used for common management practices.

MATERIALS AND METHODS

Equipment set-up: The Directed Energy System (DES) was used to deliver energy treatments. The total system consists of the Power Supplies, control and monitoring, and the pulse forming system (**Fig. 1a**).

Meloidogyne hapla: *Meloidogyne hapla* was added to soil to achieve an initial inoculation density of 10,000 eggs/pot. Each pot contained 5,000 cm^3 of a sandy loam soil mix moistened with 430 ml water. A total of 48 pots were prepared. The soil in half of the pots was treated prior to planting with low, medium, or high energy (Table 1), or left untreated (**Fig. 1b**). All 48 pots were planted with a 2-week-old tomato 'Rutgers' transplant. Soil in the second set of 24 pots was treated with low, medium, or high energy (**Table 1**), or left untreated two weeks after planting (**Fig. 1c**). All of the treatments were replicated six times and pots were arranged in a randomized design in the greenhouse. Two months after planting, plants were destructively harvested. At this time shoot and root weights were determined. *Meloidogyne hapla* eggs were extracted from roots and then counted.

Globodera ellingtonae: Field soil containing cysts of *G. ellingtonae* was obtained from Powell Butte, OR. Approximately 500 cm^3 of infested soil was added to pots and moistened with 50 ml water; a total of 24 pots were prepared. Soil in pots was then treated with low, medium, or high energy (**Table 1**), or left untreated; each treatment was replicated six times. After treatment, soil was removed from pots and air-dried prior to extraction of cysts from soil using a USDA cyst extractor. Cysts (n = 10) were hand picked and placed in individual wells of a 96-well plate. To each well a 10% potato root diffusate solution was added. After 1 week the number of hatched second-stage juveniles (J2) was determined. The number of unhatched eggs was determined by liberating remaining eggs in cysts and counting. Percentage hatch was determined as hatched J2/hatched J2 + unhatched eggs.

Soilborne pathogens: Inoculum of *Phytophthora cinnamomi* was prepared by growing an isolate in V8 juice-amended vermiculite in a fungal spawn bag for 1 month at 20C. Microsclerotia of *Verticillium dahliae* were produced on potato dextrose agar overlaid with cellophane, sieved to 35 µm, and then mixed with sand to create stock inoculum. The pathogens were then added to a sandy loam soil at densities of 100 and 10 propagules/g soil for *P. cinnamomi* and *V. dahliae*, respectively. Inoculated soil, 500 cm^3, was added to pots for a total of 24 experimental units for each pathogen; no additional water was added to pots. Soil in pots was treated with low, medium, or high energy (Table 1), or left untreated; each treatment was replicated six times. After treatment, the soil for *V. dahliae* was air dried (*P. cinnamomi* soil was not air dried) and population densities of the pathogens were determined by dilution plating on PARP and NP10 for *P. cinnamomi* and *V. dahliae*, respectively.

Statistical analysis: Difference between treatments was determined using analysis of variance. Means were separated using Tukey's least significant differences ($P < 0.05$).

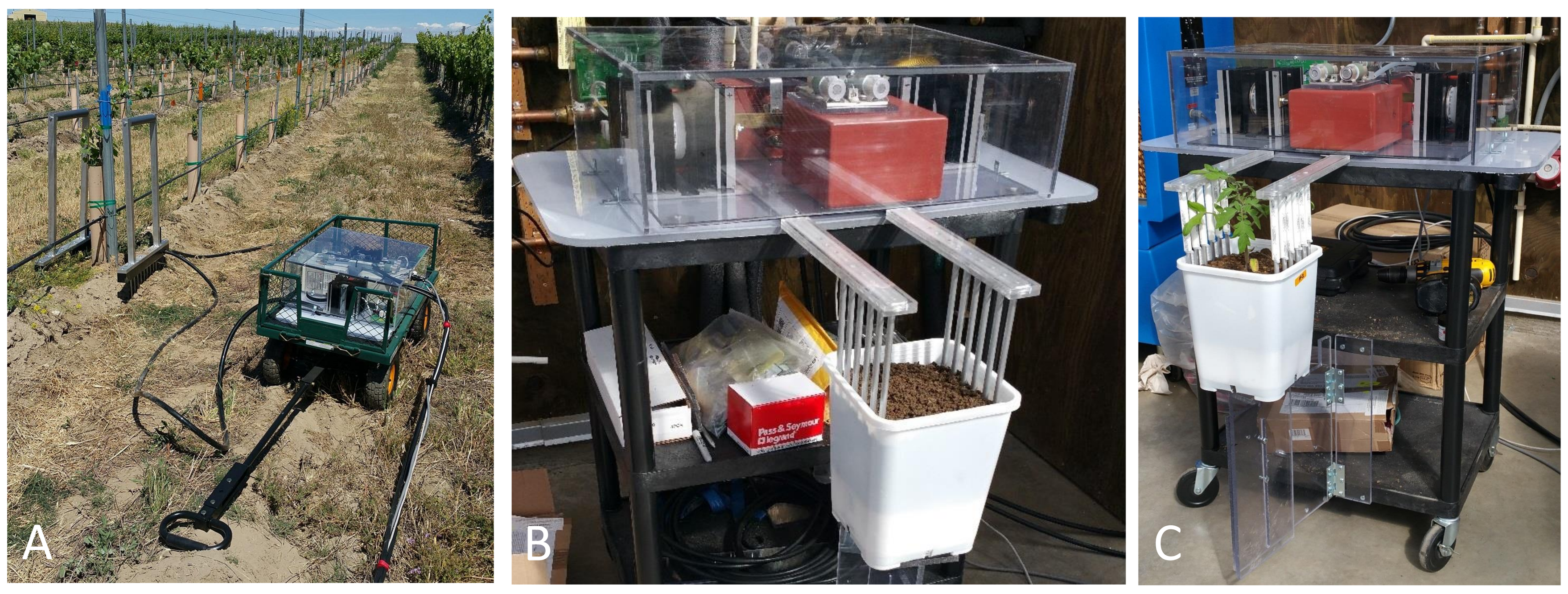

Figure 1: The Directed Energy System (DES): **A)** in-field unit consisting of Power Supplies (large gray boxes), control and monitoring (PC, control box and oscilloscope) and the pulse forming system (equipment in the acrylic box); and greenhouse-unit used for **B)** pre-plant and **C)** post-plant treatment of soil containing *Meloidogyne hapla*.

Table 1: Amount of energy to which plant-parasitic nematodes and soilborne pathogens were exposed to using the Directed Energy System (DES).

Organism	Low	Medium	High
	---------------------- joules/cm^3 soil --------------------------		
Meloidogyne hapla (pre)	2.2	13	25
Globodera ellingtonae	20	30	70
Soilborne pathogens	4	6	11

RESULTS

Meloidogyne hapla

Treatment of soil containing eggs of *M. hapla* with energy prior to planting tomato resulted in a significant reduction in final egg densities compared to the untreated control (Fig 2). When plants infected with *M. hapla* were treated with energy, a reduction in final egg densities was also observed (Fig. 2). At all energy doses, this reduction was significantly greater than that observed with the pre-plant treatment. Across energy treatments, there were 90 to 99% fewer *M. hapla* eggs at the end of the experiment in plants treated post-plant vs. pre-plant.

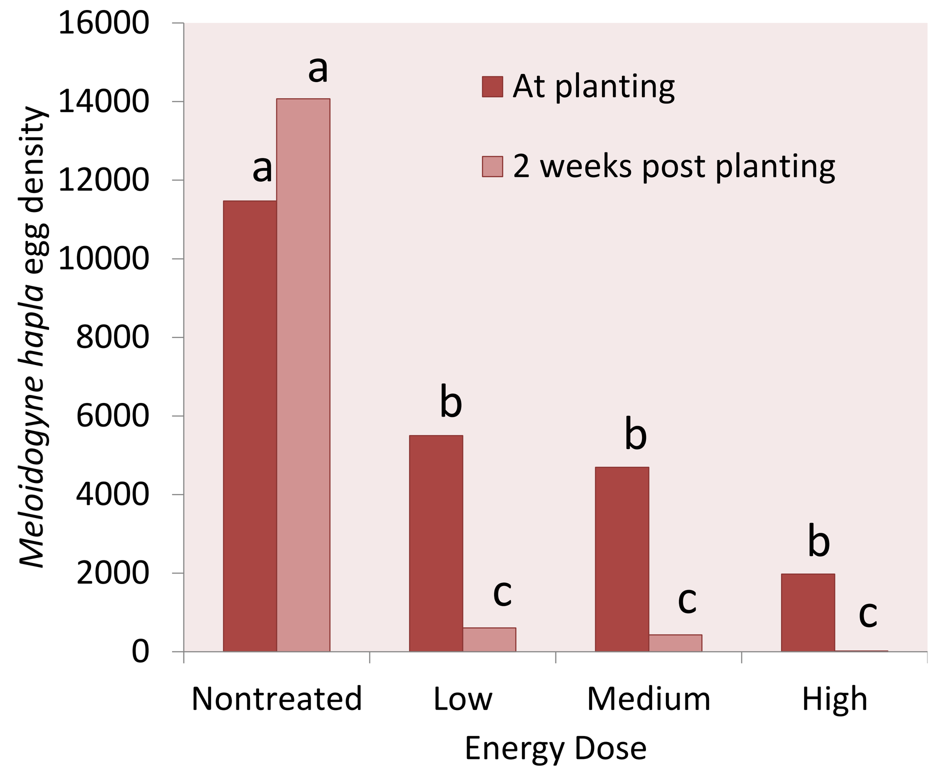

Figure 2*: Meloidogyne hapla* egg densities in roots of tomato plants after exposure to energy pre- or post-plant. Means followed by the same letter are not significantly different ($P \geq 0.05$).

Globodera ellingtonae

Table 2: Percentages of cysts which hatched and egg hatch of *Globodera ellingtonae* after exposure to varying levels of energy. Means followed by the same letter are not significantly different ($P \geq 0.05$).

Energy treatment	Cysts in which hatch occurred	Egg hatch
	--------------------- % --------------------	
Low	90 a	56 a
Medium	72 a	55 a
High	12 b	7 b
Untreated	90 a	43 a

Only the highest dose of energy resulted in a reduction in the number of cysts in which hatch occurred (Table 2). The same trend was observed for egg hatch, with a 84% reduction in egg hatch at the high dose compared to hatch in the untreated control.

Soilborne Pathogens

Treatment of soil containing *P. cinnamomi*, regardless of energy dose, resulted in a significant reduction in ppg of this pathogen (Table 3). A similar response was not observed for *V. dahliae*.

Table 3: Propagules per gram soil (ppg) of two soilborne pathogens after exposure to varying levels of energy. Means followed by the same letter are not significantly different ($P \geq 0.05$).

Energy treatment	*Phytophthora cinnamomi*	*Verticillium dahliae*
	-------------------- ppg -------------------	
Low	14 a	24 a
Medium	12 a	22 a
High	15 a	15 a
Untreated	33 b	23 a

CONCLUSIONS

- At similar energy doses (2.2 to 25 joules/cm^3 soil), M. hapla population densities were reduced more effectively when soil/plants containing nematodes were treated post-plant rather than pre-plant. It appears that plant roots are a better conductor of energy than soil.
- Much higher levels of energy, 70 joules/cm^3 soil compared to 2.2 joules/cm^3 soil, were required to elicit a reduction in egg hatch of *G. ellingtonae* compared to *M. hapla*. The protective cysts surrounding the eggs may act as a barrier to energy infiltration.
- *Phytophthora cinnamomi* survival was affected at a energy dose similar to that of *M. hapla* (11-13 joules/cm^3 soil).
- *Verticillium dahliae* microsclerotia was the most difficult to control organism to control with DES. Much higher doses of energy, > 11 joules/cm^3 soil, will be required to control this soilborne pathogen.
- The DES system is currently being evaluated in the field.

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