# PRECISION METHOD FOR MEASUREMENT OF CHARACTERISTICS DURING AN ELECTRIC WEEDING TREATMENT

A thesis presented in partial fulfilment of the requirements of the degree of:

Master of Engineering (Mechatronics)

At Massey University, Manawatu, New Zealand

Hamish Penny

2021

# ABSTRACT

A method is proposed that allows measurement of electrical phenomena during an electric weeding discharge, to allow development of a viable non-herbicide weeding option for agriculture. Electric weeding – killing plants with high voltage electricity – has been researched since the 19th century, but the mechanism for plant death is not yet fully defined. There has also been little research into the localised electrical effects and how the treatment could be optimised based on the plant type and environmental conditions. The proposed method allows separation of the system into the 'plant' and the 'root-soil' sub-systems, and allows measurement of the division of the voltage and energy between them. For plants where the dimensions of the stem can be mechanically measured – such as many dicotyledon (broadleaf) weeds – the method allows calculation of the electric field strength, current density, and energy volume density within the plant tissue. It is suggested that these parameters can be associated with plant survival rates to develop understanding of the mechanism for death and to optimise the energy and time of electrical weeding systems that are developed in the future.

# ACKNOWLEDGEMENTS

To complete this project a range of skills were required, many of which I didn’t possess when it began. In developing understanding of horticultural science and the biology of plants, Dan Bloomer of my sponsoring company Page Bloomer Associates has provided significant support, which I really appreciate. My two supervisors, Morio Fukuoka and Steven Le Moan, have also provided valuable support throughout the project and provided the freedom to explore and be creative. I would also like to thank my family, and in particular my partner Georgia, for their support and encouragement throughout the project.

# TABLE OF CONTENTS

**ABSTRACT** .......... **II**

**ACKNOWLEDGEMENTS** .......... **III**

**TABLE OF CONTENTS** .......... **IV**

**LIST OF FIGURES** .......... **VI**

**LIST OF TABLES** .......... **VI**

**1 INTRODUCTION** .......... **1**

1.1 WHAT IS ELECTRIC WEEDING? .......... 1
1.2 CONTEXT .......... 1
1.3 THE GOAL .......... 1
1.4 PROJECT AIMS .......... 1
1.5 HYPOTHESIS .......... 1

**2 OVERVIEW OF RELEVANT ELECTRICAL THEORY AND NOTATION** .......... **2**

2.1 VOLTAGE, CURRENT AND RESISTANCE .......... 2
2.2 CAPACITANCE .......... 2
2.3 ENERGY .......... 3
2.4 CURRENT AND VOLTAGE DIVISION .......... 3
2.5 NOTATION .......... 4

**3 LITERATURE REVIEW** .......... **5**

3.1 PRIOR WORK IN ELECTRIC WEEDING .......... 5
  *3.1.1 Historical Research Interest* .......... *5*
  *3.1.2 Gap in Knowledge* .......... *5*
  *3.1.3 Surface Flashover* .......... *6*
3.2 COMPARABLE MEASUREMENT SYSTEMS .......... 6
  *3.2.1 Electric Weeding Measurement Systems* .......... *6*
  *3.2.2 Detection of Electroporation* .......... *7*
3.3 STRUCTURE AND ELECTRICAL CHARACTERISTICS OF PLANT TISSUE .......... 8
  *3.3.1 Plant Structure* .......... *8*
  *3.3.2 Electrical Circuit Model* .......... *9*
3.4 ELECTRICAL EFFECT ON TISSUE .......... 10
  *3.4.1 Thermal Damage Mechanism* .......... *11*
  *3.4.2 Cell Permeabilisation (Electroporation)* .......... *12*
  *3.4.3 Reactive Oxygen Species Generation* .......... *15*
3.5 ROOT AND SOIL IMPEDANCE .......... 15
3.6 SUMMARY .......... 16

**4 METHOD DEVELOPMENT** .......... **18**

4.1 OVERVIEW OF DEVELOPMENT PROCESS .......... 18
4.2 CRITERIA FOR SELECTED METHOD .......... 18
4.3 OPTIONS CONSIDERED .......... 19
4.4 INITIAL PROPOSED METHOD .......... 19
4.5 ELECTRICAL THEORY .......... 20
4.6 CONSIDERING SURFACE IMPEDANCE .......... 22
4.7 SYSTEM SPECIFICATIONS .......... 24

4.8 PART SELECTION ........ 24
4.9 CALCULATIONS ........ 24
*4.9.1 Resistance ........ 24*
*4.9.2 Energy ........ 24*
*4.9.3 Electric Field Strength ........ 25*
*4.9.4 Current Density ........ 25*
*4.9.5 Energy Volume Density ........ 25*

**5 VALIDATION EXPERIMENT ........ 26**

5.1 ELECTRIC APPLICATION DEVICE ........ 26
5.2 PARTS USED ........ 26
5.3 SETUP ........ 26
5.4 RESULTS AND DISCUSSION ........ 29
5.5 CONCLUSION AND IMPROVEMENTS ........ 32

**6 FINAL PROPOSED METHOD ........ 33**

6.1 OVERVIEW OF CHANGES ........ 33
6.2 PARTS USED ........ 34
6.3 CONTACT METHOD ........ 34
6.4 SEMI-AUTOMATED PROGRAM ........ 35
6.5 MEASUREMENT PROCESS ........ 35
6.6 SUMMARY ........ 36

**7 FINAL EXPERIMENT ........ 37**

7.1 SUMMARY ........ 37
7.2 SETUP ........ 37
7.3 DATA INTERPRETATION ........ 38

**8 RESULTS AND DISCUSSION ........ 39**

8.1 SUMMARY OF RESULTS ........ 39
8.2 SOIL MOISTURE EFFECT ON RESISTANCE ........ 40
8.3 MEASURING DIAMETER ........ 41
8.4 AREA AND RESISTANCE FOR BEANS ........ 41
8.5 ELECTRICAL PARAMETER CALCULATIONS ........ 42
8.6 SOLUTIONS TO GRASS DIAMETER MEASUREMENT PROBLEM ........ 43
8.7 MEASUREMENT OF ROOT-SOIL SYSTEM ........ 44
8.8 CONTACT BETWEEN ELECTRODE AND PLANT ........ 44
8.9 MULTIPLE POINTS OF PLANT CONTACT WITH SOIL ........ 44
8.10 FUTURE USES OF METHOD ........ 45
*8.10.1 Association of Parameters with Plant Survival ........ 45*
*8.10.2 Optimisation for Time and Energy Efficiency ........ 45*
*8.10.3 Defining When Flashover Occurs ........ 45*

**9 CONCLUSION ........ 47**

**10 REFERENCES ........ 48**

# LIST OF FIGURES

Figure 1 Schematic of the experimental setup (a) and the equivalent circuit model (b) for measurement of impedance change during potato tissue ablation [22] .................................................7
Figure 2 Grass plant crown ..................................................................................................................8
Figure 3 Proposed electrical representation of a plant cell in electrical weeding literature. ...................9
Figure 4 Proposed electrical representation of a plant cell in electroporation literature. .....................10
Figure 5 (a) Single dispersion, and (b) Double dispersion, Cole impedance Model ...............................10
Figure 6 (a) untreated soybean, (b) soybean exposed to 1042°C flame for 130 milliseconds, (c) elodea leaf treated at 52°C, (d) elodea leaf treated at 54°C [30].....................................................................11
Figure 7 Thermal damage to pea plant cells before (a) and after (b) treatment [10] ............................12
Figure 8 Electron microscope images of corn leaf tissue chloroplast. (a) Untreated mesophyll chloroplast (29,000 X). (c) Flamed mesophyll chloroplast (20,000 X) [31]............................................12
Figure 9 Onion cells exposed to neutral red dye [34] ............................................................................13
Figure 10 Pathways of electrical conductance [15] ...............................................................................16
Figure 11 Measurement diagram ..........................................................................................................20
Figure 12 Resistive voltage divider ........................................................................................................20
Figure 13 Capacitive voltage divider......................................................................................................21
Figure 14 Plant surface tissue impedance consideration. A is point of interest, B is point of connection to measurement device, R is surface tissue resistance and C is surface tissue capacitance .................23
Figure 15 Ruler attached to electrode ...................................................................................................27
Figure 16 Grounding electrode and shunt resistor ................................................................................28
Figure 17 Photo of measurement setup ................................................................................................28
Figure 18 Waveform shown after pulse .................................................................................................29
Figure 19 Plant size vs root-soil resistance graph ..................................................................................30
Figure 20 Contact method diagram.........................................................................................................34
Figure 21 Graphical user interface .........................................................................................................35
Figure 22 Setup during treatment...........................................................................................................37
Figure 23 Soil moisture percentage vs root-soil resistance ...................................................................40
Figure 24 Cross-sectional area vs resistance for beans .........................................................................42
Figure 25 Electrical parameters by treatment .......................................................................................43

# LIST OF TABLES

Table 1 Summary of Validation Experiment Results ...............................................................................30
Table 2 Results for Plant Two in Bag Ten ...............................................................................................31
Table 3 Approximate Dimensions............................................................................................................31
Table 4 Final parts list ............................................................................................................................34
Table 5 Summary of Final Experiment Results ........................................................................................39

# 1 INTRODUCTION

## 1.1 WHAT IS ELECTRIC WEEDING?

Electric weeding is a method proposed to solve the age-old agricultural problem of removing plants that are growing where they are not wanted. The method derived from the observation that applying a high-voltage electric field to plant tissue can have a destructive effect and, in some cases, can lead to plant death. The first electric weeding system was patented in the 19th century [1], since when there has been consistent research interest using various approaches. In most cases a high voltage electrode is connected to plant tissue above ground and an earthing electrode is connected to the soil nearby, with current passing through the plant, root system and soil.

## 1.2 CONTEXT

This project brings together knowledge from electric weeding, horticultural science and automation engineering to develop a research method that can be used to understand the electrical phenomena occurring during electrical weeding. The output is intended to be used by plant/weed science specialists and provide a basis in electrical engineering theory to support future development of an electric weeding system. Although voltage application with sufficient magnitude and duration has demonstrated the ability to kill weeds, the localised electrical effects on tissue haven't been measured so it hasn't been possible to understand and optimize an output for a specific weed in certain conditions to maximise effectiveness and time/energy efficiency. The background of the author is in electrical engineering, so an effort has been made to develop understanding of relevant aspects of the plant science field. The descriptions of electrical engineering theory provided in this work are intended to be understandable and useable for specialists in the field of plant/weed science.

## 1.3 THE GOAL

The goal has been to develop a practical and affordable method to record electrical parameters such as electric field strength, current density and energy density within localised regions of the plant-root-soil system. This method will enable development of a precision approach to electric weeding where electrical parameters can be adapted to the plant and environmental factors presented. This will allow future development of electrical weeding technology that can be an effective non-herbicide weeding option for farmers around the world.

## 1.4 PROJECT AIMS

- Design and justify a novel method to measure electric field, current density and energy density in localised regions during an electric weeding discharge.
- Complete an experiment to prove that the method can work accurately and reliably

## 1.5 HYPOTHESIS

*That the practicality, affordability and range of parameters that the method can measure allows it to be used to understand the electrical characteristics of an electric weeding treatment*

# 2 OVERVIEW OF RELEVANT ELECTRICAL THEORY AND NOTATION

To aid readers without a background in electrical engineering to understand some of the concepts described later in this thesis, some relevant fundamental electrical theory is explained here.

## 2.1 VOLTAGE, CURRENT AND RESISTANCE

Electricity is fundamentally based on the movement or storage of charge. Charge is a characteristic of protons and electrons, with protons positive and electrons negative. Conductive materials such as metals have neutral molecules arranged in a structure where the electrons have a weak enough attractive force to the positive atomic nuclei that they can move and transport charge through the material. Solutions with ionic (charged) molecules such as salt water can transfer charge with a change in the concentration of the ionic molecules. As like charges (such as the negative electrons) will always repel each other, a material or solution with mobile charge carriers will reach some sort of charge equilibrium in the absence of external influence.

When charge is moved through an external force such as magnetism (such as a mechanical electric generator) or an electrochemical reaction (such as a battery), the charge forces change out of this equilibrium state and start to move. For a metal, electrons will move from the region of the material with a higher density of electrons – and more negative charge – to the region with a lower density of electrons. This is where we come to the concepts of voltage, current and resistance. The change in forces on the charge that causes movement is the voltage. This is the difference in the potential of the electric charge from one point to another. As mentioned, this voltage causes charge to move, but any movement of charge will cause heating in the material it is moving through (the conductor). This loss limits the rate of the movement of charge and is called the resistance. The rate of charge flow (charge per second) is the current. When the resistance to charge flow is halved then the same voltage will cause the current – the amount of charge flow per second – to double. This relationship is defined by the fundamental electrical relationship:

$$V = IR \tag{1}$$

So voltage (V) equals current (I) multiplied by resistance (R). The only slight trick is that, although negative electrons are one of the most common charge carriers, a positive voltage represents a positive charge (lack of electrons) and a positive current represents the flow of positive charge (so opposite to the movement of negative electrons).

## 2.2 CAPACITANCE

When opposite positive and negative charges are near enough to experience an attractive force but are physically constrained from moving nearer, potential energy is stored in this electric (charge based) field. Again, the difference in charge is called a voltage, but because there is no way for the charge to move it is said that the resistance is infinite and therefore the current is zero. When two metal plates are brought very close together and a voltage is created by removing charge from one plate and adding charge to the other, the positive and negative charges on each plate will attract and store energy in this electric field. The total amount of charge that is stored on the plates for a given voltage (difference in the potential of the electric charge – also the electric force that would cause current to flow if a conductive material was connected between the plates) is called the capacitance.

Doubling the area of the plates would mean for the same voltage, double the charge could be stored, so the capacitance is doubled. The mathematical relationship is:

$$Q = CV \tag{2}$$

So charge (Q) equals capacitance (C) multiplied by voltage (V). Although these charge storage devices – called capacitors – are made artificially and are a common design element in electrical circuits, any conductor will have the capability to store some charge within the physical structure when a voltage is applied. As described in detail later in Section 3.3.2, a biological cell membrane is able to store a significant amount of energy with charge and so is said to have significant capacitance.

## 2.3 ENERGY

A concept that brings together these various fundamentals is energy. Energy is governed by the physical law that it cannot be created or destroyed, but can only change in form. When a voltage causes a current to pass across a resistance, such as a high-voltage electrode causing current to pass through ionic plant tissue, electrical energy is lost as heat (thermal energy) in the material. The energy is related to the current and resistance through the equation:

$$E = I^2Rt \tag{3}$$

Where E is the energy, I is the current, R is the resistance and t is the time. It is important to note that the current value is squared in this relationship. This is often expressed as energy per second (power), which looks like:

$$P = I^2R \tag{4}$$

Where P is power.

## 2.4 CURRENT AND VOLTAGE DIVISION

When a circuit (conductive material connecting points of different voltage) contains a variety of resistances throughout its structure the current and voltage will no longer be evenly distributed. If a plant stem has some tissue with high moisture content and free ions (such as xylem) so lower resistance to the movement of charge, and other tissue with lower moisture and higher resistance, more charge will move (current) in the lower resistance region. Any time there are multiple paths for charge to move, the magnitude of the current will be higher in paths with lower resistance. As the energy equation is current squared, a region of lower resistance will have greater heat generation.

If a conducting path contains multiple regions of different resistance in a series combination, then the current through the series of resistive regions will be the same – as the same amount of charge has to flow through the whole system– but the voltage across each region will vary. As the voltage is equal to the current multiplied by the resistance and the current is equal, a region of higher resistance will have a higher voltage drop than a region of lower resistance. For example, if a plant has constant resistance and the resistance of soil varies, then for the same voltage applied at the top of the plant, soil of higher resistance will have a greater voltage across it. As the total voltage drop across the plant and soil is still the same the voltage across the plant will be lower.

Both current and voltage division have formulae to allow calculation of the values for a specific network of resistances. Where relevant these and other equations are included and explained later in the thesis. The important points are that:

- More current will flow through parallel paths of lower resistance
- More voltage will drop across series regions of higher resistance

## 2.5 NOTATION

### Voltage

Expressed as V with units Volts (V). In many papers referenced in this thesis the voltage per unit distance (V/cm) is the unit used, which is the electric field (see below).

### Electric Field

Often denoted with E, which is confusingly also used for energy. Unit is Volts per centimetre (V/cm), so it represents the difference in electric potential across a given length of conductor. In this thesis the electric field strength is denoted as $E_f$ to more clearly differentiate from energy.

### Current

Expressed as I with units Amperes (A). A representation used in some referenced papers and in this thesis is current density J, representing current per unit area with units A/cm$^2$.

### Energy

Expressed as E with units Joules (J). A representation of energy volume density (J/cm$^3$) will be used in this thesis. Power in Watts (W), which is joules per second may also be used.

### Capacitance

Expressed as C with units Farads (F).

### Impedance

Impedance is the combination of resistance (R) and capacitive reactance (X), and all three are expressed with units Ohms ($\Omega$). As both a resistive and capacitive circuit element can inhibit the flow of charge, the combination of these (impedance) is commonly used to define this parameter.

# 3 LITERATURE REVIEW

This section is intended to give a review on existing work in electric weeding and related fields as well as a theoretical background for the method that has been developed. Sections 3.1 and 3.2 cover existing research in the field of electric weeding and where related fields have developed solutions to similar problems, as well as where the gaps in knowledge have been up to this point. The structure of plant tissue and how this relates to electrical theory is explained in Section 3.3, possible electrical effect on tissue is explained in Section 3.4, and impedance in roots and soil is explained in Section 3.5.

## 3.1 PRIOR WORK IN ELECTRIC WEEDING

This section covers the historical research into electric weeding, including the methods and approaches employed. The gaps in knowledge and the relevant observation of surface flashover are also covered.

### *3.1.1 HISTORICAL RESEARCH INTEREST*

The crest of research for electrical weeding was in the 1970's and 1980's, particularly in Soviet countries, Japan, the UK, Europe and the USA [2]–[4]. During this period two distinct methods for electrical weeding were identified by researchers. The approach preferred by Soviet countries used high voltages in the range of 30kV to 80kV and short pulses in the microsecond to millisecond range. Western and Japanese researchers have preferred using lower voltage ranging from 5kV to 20kV with longer duration discharge [3]. At lower voltage, the kill method is believed to be the thermal destruction of plant cells, while higher voltage pulses appear to cause some other physiological damage. Although both approaches proved some capability to kill plants, and the lower voltage approach claimed to be safe, neither led to commercial success. The main issues were an extremely high energy requirement for the lower voltage method, electrical safety concerns and competition from the relative effectiveness of herbicides at that time [5], [6]. The only equipment to become commercially available in this period of research was a weeder targeting sugar-beet bolters produced by the Lasco Corporation (USA) in the 1980's [7]. The height of the undesirable bolting plants relative to the main crop was well suited to the electrical systems that had been developed. Recently there have been several new patents published and some new systems are starting to become commercially available [8], [9].

Much of the earlier research was empirical but there has been work done to improve theoretical understanding. Diprose et al. (1980) claimed to observe the thermal damage to plant cells a system had caused, while Soviet researchers have further investigated the effect on plant cells, as well as modelling soil resistance and the impact of different pulse shapes [11]–[13]. There has been extensive research in some other areas relevant to electrical weeding where understanding is required for another industry. For example, measuring the conductivity of soil is very useful to land mapping and to grounding systems protecting transmission towers from lightning strikes [14], [15]. The impact of thermal and electrical stress on plant cells has also been researched to develop alternative weeding methods [16]–[18].

### *3.1.2 GAP IN KNOWLEDGE*

One of the gaps in knowledge for published work in electric weeding has been a clear idea of the mechanism of plant damage. Some research has shown there is insufficient energy for thermal damage to be the only contributing factor to plant death [4], suggesting another factor is involved.

Even when higher energy is used it seems likely that an alternative factor to thermal will be involved, and for any approach it is possible that multiple effects combine to produce plant death. Partly due to this knowledge gap, almost all of the published electrical weeding development work uses a trial and error approach rather than using understanding to optimise the electrical parameters used. Without a mathematical definition of relationships between electrical parameters and plant death, if conditions change outside what has already been directly tested it is difficult to adapt parameters to suit. If this gap can be filled and a higher level of understanding developed, electrical weeding technology has the potential to become a significant non-chemical alternative to herbicides.

### *3.1.3 SURFACE FLASHOVER*

Although the identified effects on plant tissue are enhanced with increasing electric field strength, it has been observed in work with pulse discharges that there is a certain limit where current will flow across the surface of plant tissue rather than through it [12], [19]. This is associated with a reduction in damage to tissue – supporting the theory of an internal current mechanism of tissue damage – and needs to be considered when defining parameters. The conductivity of plant tissue surface could be expected to change as a result of a range of factors including relative humidity and surface moisture. Defining if and when flashover could occur influences the optimisation of controllable parameters for electrical weeding. Of interest is that flashover was not observed in work exposing 5cm tall seedlings to 15kV in small energy pulses [4]. During experiments conducted by a plant scientist that were observed by the author, flashover was observed using a single high energy pulse of 10kV with similar sized plants, suggesting either a difference in environmental conditions or supporting a logical hypothesis that pulse energy and time are influential.

## 3.2 COMPARABLE MEASUREMENT SYSTEMS

This section covers the research done for measuring the electric effects of high voltage pulses on biological tissues in comparable systems. Although there has been some investigation of these effects for electric weeding, there is a much greater depth of analysis in some other fields such as medicine and food processing. In these fields high voltage is commonly used to destroy cells based on the effect of electroporation (identified as a possible factor for electric weeding in Section 3.4.2). Although the scale of these experiments is typically smaller than electric weeding, the measurement systems to identify electrical parameters have many similarities.

### *3.2.1 ELECTRIC WEEDING MEASUREMENT SYSTEMS*

There has been some work done by Russian researchers to determine the electrical parameters during a discharge and associate these with the degree of damage to the plant. A 5-65 V square pulse was applied to a very small (unspecified) region of tissue and the impedance of the tissue was measured [20]. Based on the impedance change from before the pulse to after the pulse a critical electric field of 14 V/mm was identified, meaning the tissue length between electrodes must have been between 0.4 and 4.6 mm (the distance corresponding to this field strength of 14 V/mm at max and min voltage of 65 and 5 V). The critical current density was also calculated as 1.5 A/mm$^2$. In this work the critical damage values were derived from the change in impedance, which is associated with death of plant cells in electroporation research from other fields. However, the very small scale experiment was destructive, so plants could not be observed to associate long-term damage and plant death with the results. The critical electric field of 14 V/mm is equivalent to 140 V/cm, which is low compared to electroporation research but is in a plausible range. This work could also not isolate any separate parts of the system for analysis. In another study, high voltage of 3000 V was applied to weed plants, with some treated in pots [12]. The parameters representing the electrical circuit – as shown in Figure 3 – were derived, but the study could not measure these parameters for a plant stem while the plant was in soil. This study did observe a flashover effect, as described in Section 3.1.3.

### *3.2.2 Detection of Electroporation*

Electroporation is a field of research based on the observed increase in permeability of a biological membrane when exposed to a strong electric field. To induce this effect, voltage can be applied to single cells or to a whole region of biological tissue – plant or animal. The theory and applicability of the field to electric weeding are described in Section 3.4.2. Some of the most heavily researched applications for electroporation include drug delivery to the inside of cells during the period of increased permeability, and extensive exposure to the point of cell destruction for cancer ablation and food processing [21]. The use of electroporation for permanent cell destruction is most relevant to electric weeding, and several methods for detection of electric effects during and after pulses have been proposed in the research conducted for this application.

Typically, research to detect the effects of an electroporation treatment will first use a macroscopically isotropic and homogenous tissue such as potato or a region of animal liver. In one example, four electrodes were inserted in a square pattern into potato tissue as shown in Figure 1 [22]. Two diagonal electrodes were used to provide an electroporation pulse and the other two electrodes were used to measure the impedance before and after treatment. A reduction in impedance was observed for the treated tissue.

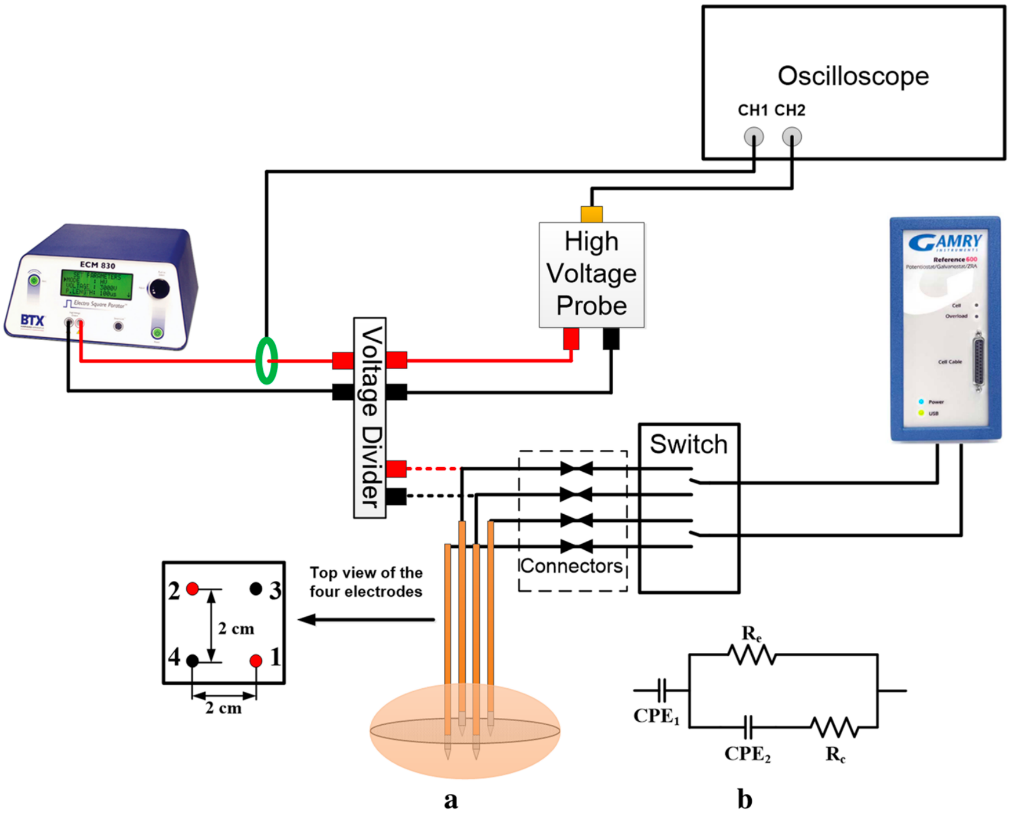


Figure 1 Schematic of the experimental setup (a) and the equivalent circuit model (b) for measurement of impedance change during potato tissue ablation [22]

To assess electroporation in liver tissue, a network of five impedance sensing electrodes has been used [23]. The impedance was measured in a two dimensional plane by using combinations of the five electrodes both before and 10 seconds after the pulse. Again, differences in impedance were observed in areas affected by electroporation. This work has been extended in research directly testing the efficacy of electroporation for pancreatic cancer ablation in humans [24]. The research measured impedance during pulses applied during treatment of 65 patients and associated change in impedance from the first to the last pulse with local recurrence of cancer.

Although there has been work done to measure the electrical parameters – particularly impedance – during electroporation pulses, these measurement solutions work on a different physical scale to a measurement system for electric weeding treatment. In electroporation research the distance between electrodes is typically in the range of 2-10 mm. The minimum region through plant and soil for electric weeding is an order of magnitude greater than this for any practical system, so the maximum voltage that needs to be measured is an order of magnitude higher than for these electroporation systems. At the lower voltages (< 1kV) of electroporation work most oscilloscopes or standard current and voltage measurement devices can provide direct measurements. The challenge of accurate measurement for the higher voltages in electric weeding work has been a significant limitation to measuring the electrical parameters during a treatment.

## 3.3 STRUCTURE AND ELECTRICAL CHARACTERISTICS OF PLANT TISSUE

This section gives a background on the physical structure of plants, how plant tissue can be represented with electrical circuits, and how this assists interpretation of the electrical parameters measured during an electric weeding treatment.

### *3.3.1 PLANT STRUCTURE*

There are two main types of plants of interest for electrical weeding in agriculture: monocotyledons (monocot) and dicotyledons (dicot). The physical structure and biological processes associated with these types of plant mean the electrical parameters are likely to be distributed differently. A dicot typically has broad mature leaves with a netlike vein structure, vascular bundles – nutrient carrying structures – arranged in a ring, and one main root that other smaller roots branch off [25]. A monocot plant has parallel leaf veins, a complex vascular bundle arrangement, and a fibrous root system. Of particular relevance is the capability for monocots to form separate tiller structures. As can be observed in Figure 2, tillers of the same monocot plant have a separate root structure and can survive independently. During a treatment each tiller of a monocot would be exposed to different electrical effects and some could die while others survive. For dicots, where tiller structures do not develop, a part of the plant would not be able to survive independently.

FIGURE 2 GRASS PLANT CROWN

Although the exact mechanism for plant death has not yet been defined, it is likely that the requirement to kill a whole plant will require damage to a high percentage of the plant's mass, chemical signalling of programmed cell death, or destruction of a structure to inhibit transfer of nutrients. Existing electrical methods focused on a thermal kill mechanism have claimed the requirement to kill the whole root system, limiting effectiveness in wet conditions when current escapes the roots due to high soil conductivity [3]. However, the mechanism causing plant death during pulse treatment may not have this same requirement, particularly if cell damage can inhibit nutrient transfer through a critical section of the plant such as the crown of a monocot, where electrical current would concentrate before distributing through the roots and soil.

### 3.3.2 *ELECTRICAL CIRCUIT MODEL*

The structure of plant tissue exhibits properties that can be approximated by an electrical circuit. Several different circuits have been proposed in literature, with membranes represented by capacitors or constant phase elements and ionic solution represented by resistors, but with more complex circuits taking into account more minor structures. In all literature identified, a bulk model is used where the individual structures (such as cells) are combined into simple representative circuit elements. One of the simplest circuits has been identified in Russian electrical weeding research and is shown in Figure 3 [20].

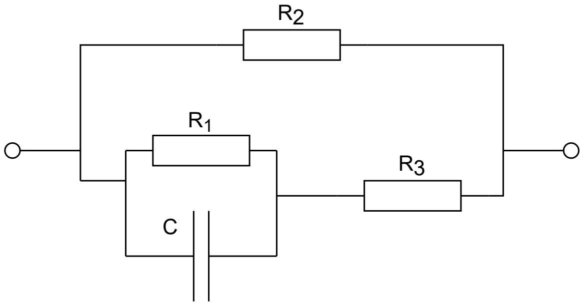


FIGURE 3 PROPOSED ELECTRICAL REPRESENTATION OF A PLANT CELL IN ELECTRICAL WEEDING LITERATURE.

Figure 3 represents the extracellular ionic fluid as parallel resistive path for current, $R_2$. This represents the conductive channels throughout the tissue that can pass around cell membranes. The other three circuit elements in Figure 3 represent the paths for current through cells. C is a capacitor representing the capacitance of the membrane of the cell. Because the membrane is a semipermeable lipid bilayer it can store a significant amount of charge in an electric field [26]. The small ionic channels through the membrane are represented as the resistance $R_1$, which has a high resistance to the flow of current due to the relative size of these channels to the overall cross-section of the membrane. The internal organelles of the membrane are approximated as the resistance $R_3$, although this fails to take into account the capacitance of the organelles. This capacitance is included in a representation used in Figure 4 [27].

In Figure 4 the extracellular ionic fluid, and cell membrane capacitance and resistance are represented with the same configuration as Figure 3, with $R_3$, $C_1$ and $R_1$ respectively. However, Figure 4 separates the electrical properties of the cell interior with the membrane of the vacuole – a large storage organelle within a plant cell – having a separate capacitance and resistance, notated as $C_2$ and $R_5$. The resistances around, in line with, and through the vacuole are notated by $R_4$, $R_2$ and $R_6$ respectively, while in Figure 3 this whole internal structure is simplified to resistance $R_3$.

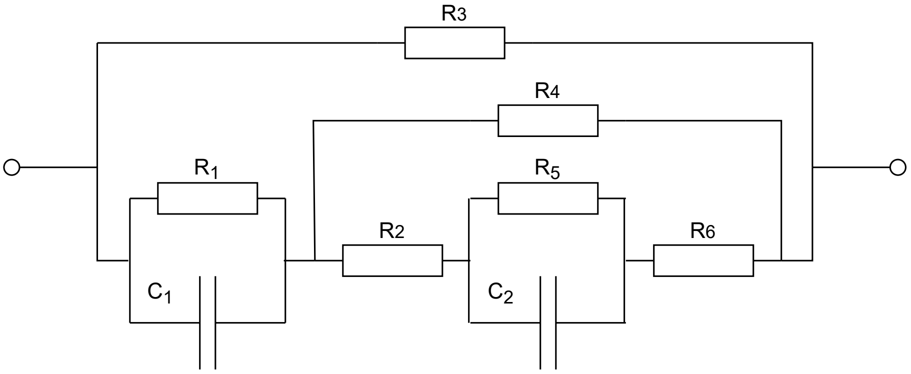


FIGURE 4 PROPOSED ELECTRICAL REPRESENTATION OF A PLANT CELL IN ELECTROPORATION LITERATURE.

Analysis of plant tissue using impedance analysis has shown the two models shown in Figure 3 and Figure 4 to be valid approximations of electrical characteristics. However, there is some error introduced by the fact that the cell membrane does not act as a true capacitor-resistor network. It is much more accurately represented with a constant phase element (CPE) which is an electrical element where the effective capacitance and resistance increase as the frequency of the applied electrical signal increases [28]. This is commonly used where a precise representation of a biological circuit is required. Figure 5 shows two circuits that incorporate a CPE [26]. There is no direct connection between electrical components and physical structures when using these types of representative circuits.

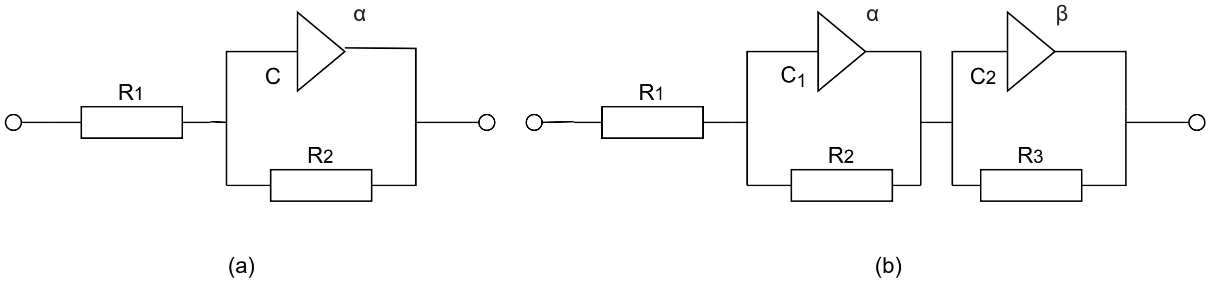


FIGURE 5 (A) SINGLE DISPERSION, AND (B) DOUBLE DISPERSION, COLE IMPEDANCE MODEL

## 3.4 ELECTRICAL EFFECT ON TISSUE

This section gives the possible effects on plant tissue to allow the results to be used and interpreted. One of the goals of developing a measurement system to observe the electrical effects on tissue during a treatment, is to associate these effects with a mechanism of plant death. An overview of the possible connections between electrical parameters and plant death provides background and was used to guide development of the method. There has already been sufficient to work to prove that both temperature and electroporation will have permanent effects on plant tissue. Both of these possible mechanisms could occur at the same time when an electrical pulse is applied. It has been noted that a permanent effect from electroporation can occur with a minimal increase in tissue temperature of 0.55-0.68 °C [29]. However, in this same work they note that the very short pulse duration and the uneven distribution of current in plant tissue mean localised thermal effects on tissue cannot be ruled out. In this section, the existing knowledge on the effects of thermal damage, electroporation and ozone exposure are reviewed.

### *3.4.1 Thermal Damage Mechanism*

A significant quantity of research has been carried out to identify the impact of thermal stress on plant cells and physiological function [30]–[32]. Cell plasmolysis, the loss of water from a cell, has been observed at varying temperatures for all cell types. Other common observations are damage to the cell wall, increases in membrane permeability, and movement of lipids. These thermal effects are observed to be reversable in many cases, but at high enough temperature can cause necrosis (cell death). Thermal effects on tissue increase from around 40°C to 54°C depending on cell type, with necrosis occurring in the range of 47.5°C to 62°C. A significant impact of thermal damage in this temperature range appears to be a decrease in photosynthesis caused by the effects on the chloroplast.

Figure 6 demonstrates the different impact on plant cells of varying thermal treatments, where elodea and soybean leaves were exposed to short duration flame treatment and prolonged soaking in a water bath at different temperatures. Difference in changes to chloroplasts (dark regions) can be observed. After flaming – where an unknown maximum temperature is reached – significant disruption to chloroplasts and nuclei is observed. At the lower temperature of 52°C (c) slight enlargement of chloroplasts is observed, while at 54°C (d) plasmolysis occurs and chloroplasts are damaged, leading to necrosis.

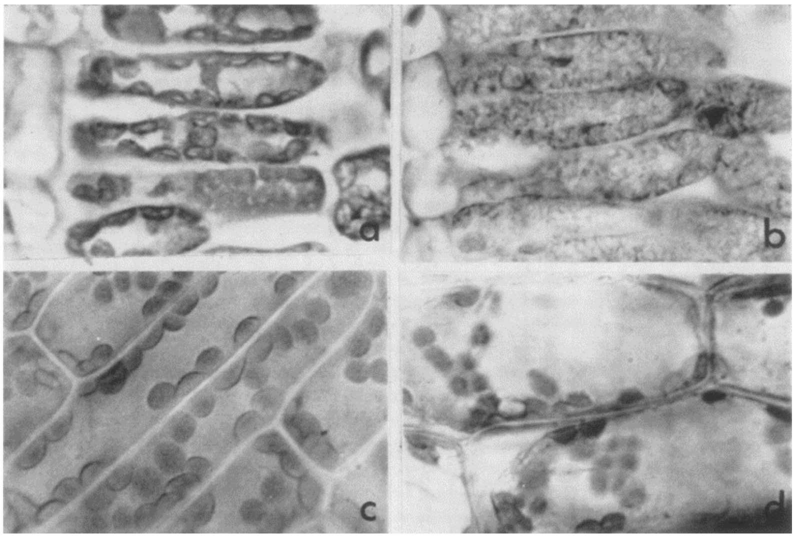


Figure 6 (a) untreated soybean, (b) soybean exposed to 1042°C flame for 130 milliseconds, (c) elodea leaf treated at 52°C, (d) elodea leaf treated at 54°C [30]

In other work observing the root meristematic cells of soybeans, the thermal threshold is lower, with necrosis occurring at 47.5°C [32]. This may suggest an increased sensitivity for these cells compared to the cells above the surface. It has been suggested that higher lignin and cellulose may provide increased resistance to thermal effects [3], providing a possible explanation for this difference. The soybean meristematic cells also displayed reduced damage when held at a sub-lethal temperature (40°C) for two hours before being exposed to previously lethal temperature (47.5°C). This increased thermal resistance was observed to be due to physiological adaptations the plants make to temperature, although this was not verified.

The electrical weeding literature investigating the effects of the continuous contact approach suggests that the form of plant damage is resistive heating that volatises cell liquids and ruptures cell walls. To volatise cell liquids the temperature would need to be near or above the boiling point of water (100°C), so requires a much higher temperature than the thermal processes identified in other literature (50-60°C). Figure 7 shows a microscope image where this effect is shown. The author observes the chloroplasts are grouped around the cell wall in the image before treatment, and after treatment that the contents have collapsed into the center of the cell [10]. As the maximum temperature is not given in this research it does not substantiate the conclusion that the observed effect is caused by volatile liquids.

Figure 7 Thermal damage to pea plant cells before (a) and after (b) treatment [10]

Further detail on the effect of thermal stress caused by flaming on chloroplasts has been observed using an electron microscope, using a conveyor belt system to expose corn leaf tissue to 482°C for 125ms [31]. In Figure 8, the author observes that the mesophyll chloroplast has a range of differences to the untreated sample including a disrupted envelope and tonoplast (arrow). The severe effect of this short-duration thermal exposure reinforces that thermal impacts of electrical weeding – even without causing membrane rupture – could have a significant effect on transpiration and therefore plant growth.

Key:

cy: cell wall

cy: cytoplasm

en: chloroplast envelope

g: grana

is: intercellular space

Figure 8 Electron microscope images of corn leaf tissue chloroplast. (a) Untreated mesophyll chloroplast (29,000 X). (c) Flamed mesophyll chloroplast (20,000 X) [31]

### *3.4.2 Cell Permeabilisation (Electroporation)*

As discussed previously, subjecting tissue with a bilayer membrane to an electric field of sufficient strength causes pores to form in the membrane. This depends on the magnitude of the localised electric field across membranes, the duration of exposure to that field, and the characteristics of the tissue being affected. In general, a higher voltage combined with a longer pulse will cause increased pore formation and beyond a certain threshold will cause rupture of the cell membrane and necrosis (cell death). A pulse below the threshold will typically cause reversible electrical breakdown, where the pores close over again, and the cell recovers after a certain period of time. It seems that exposure to a very strong electric field for a very short (nanosecond) duration can affect the membranes of the cells internal organelles and may initiate alternative cell death mechanisms to lower voltage and longer duration electroporation approaches [33].

Electroporation is caused by the relatively very high resistivity of membranes compared to the electrolytic intracellular and intercellular liquids, forming a local capacitive element where charge can be stored in an electric field across the membrane. When an external field is applied to plant tissue, the capacitance across the many cell membranes charges initially, meaning a high initial current. After the local electric fields reach equilibrium, the electric field across membranes is a maximum. To cause membrane breakdown a critical transmembrane voltage must be exceeded. For most plant cells the critical voltage is in the range of 0.5-1.5 V [29]. The localised electric field on a cell membrane with DC voltage application and in steady state conditions – with charged plasma membrane capacitance – can be approximated by the relationship shown in Equation 5 [33]:

$$\Delta U_m = 1.5 E_f r_{cell} \cos\theta \tag{5}$$

Where $U_m$ is the localised transmembrane voltage, $E_f$ is the electric field strength in V/cm, $r_{cell}$ is the radius of the cell, and $\theta$ is the angle of the membrane relative to the electric field. This relationship is a widely accepted approximation but does not take into account variation of membrane conductivity that can cause significant inconsistencies in membrane voltage throughout tissue. However, it is possible to observe the impact of cell size and orientation through this equation. Smaller cells, or non-spherical cells that have the axis of their longest dimension in parallel with the electric field need an electric field of greater magnitude to induce the same localised effect on cell membranes.

This effect of the relationship shown in Equation 2 can be observed in Figure 9 for onion cells, where red dye is used to observe the effects of electroporation [34]. The neutral dye appears dark red when ionised in the acidic vacuoles of viable cells. In the larger and more spherical parenchyma onion cells, the exposure to 100 pulses of 333 V/cm is sufficient to cause almost total cell membrane rupture. However, with the same treatment the cells around the vascular bundles have survived intact.

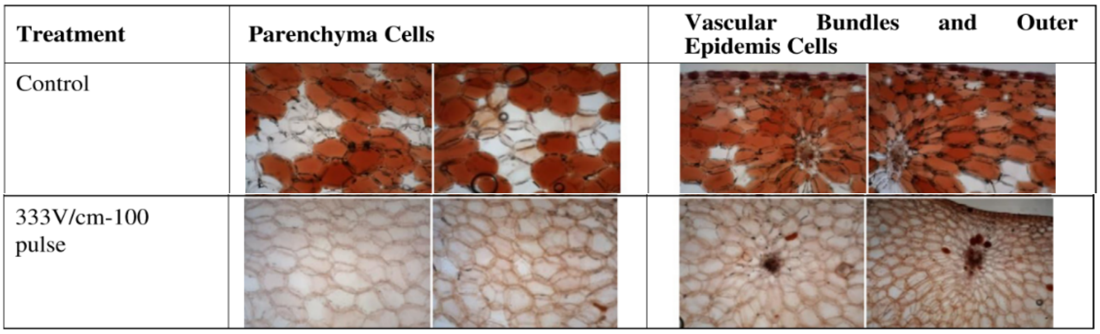


FIGURE 9 ONION CELLS EXPOSED TO NEUTRAL RED DYE [34]

The mechanism causing cell necrosis after exposure to an electric pulse has not yet been fully defined [33]. When pores are created in membranes an aqueous pathway for electrolytic liquids is provided, allowing ions and other molecules to travel across. This induced movement of molecules across cell membranes causes cellular stress and is believed to be the primary cause of necrosis. In electrical weeding literature, the formation of pores is suggested to cause mixing of the intra and inter-cellular solutions, causing denaturation of proteins [12]. As well as this effect, a localised thermal impact on cell membranes has been proposed [29]. When pores begin to form, conductive ionic liquid fills the pore and provides a path of much higher conductivity through the membrane, reducing the overall resistivity of the tissue and concentrating current (heat generation) in the very small volume of the pore. Given this very low pore volume the specific energy is very high, in the range of MJ/kg, potentially causing significant localised temperature rise and affecting the viability of the membrane.

Pore formation and consequent damage to plant tissue is dependent on the magnitude of the electric field along with the time exposed to it. The relationship between pulse time and energy is shown in Equation 6 [35]:

$$Q = \int_0^{\tau} \sigma(t) E_f(t)^2 dt \tag{6}$$

Where Q is the energy volume density (in J/cm$^3$), $\sigma(t)$ is the function of conductivity through time (in S/cm), $E_f(t)$ is the electric field strength (in V/cm), and $\tau$ is the time required to cause a characteristic damage degree. The characteristic damage degree P is representative of damage to cell membranes. The relationship between the measured tissue conductivity ($\sigma$) and P has been investigated for apple, carrot and potato tissue [35]. They suggest that the relationship between normalised conductivity Z, and P can be approximated by the empirical Archie's equation [36] as shown in Equation 7:

$$Z \approx P^m \tag{7}$$

Where m is a quantity that can be approximated for a type of plant tissue. Experimental results validated some usefulness for this relationship and they suggest m can be estimated as m=1.5 for spherical cells, m=1 for needle shaped spheroids oriented in parallel with the electric field, and m=2 for needle-shaped spheroids oriented perpendicular to the electric field. The same relationship has been proposed in work investigating electric pulse treatment, where they determine m=1.5 for sunflower stems and m=1.26 for tobacco stems [20].

The total energy required to achieve characteristic damage to plant tissue relies on the simple relationship shown in Equation 8:

$$Energy = QV \tag{8}$$

Where Q is the energy volume density and V is the volume of plant tissue the pulse is applied to. For electrical weeding, this volume could be minimised by optimisation of the location where the electrode contacts the plant and targeting the locations most crucial to plant survival, such as meristematic cells. If it were proven that electroporation is a factor in the death of living plants exposed to a high-voltage pulse, defining relationships between conductivity, cell damage, time and energy would allow optimisation of the method. The simplified Archie's equation shows some usefulness in predicting experimental outcomes but is severely limited by the inconsistency in plant material behaviour [21].

Electroporation has had limited reference in available electrical weeding literature. However, an electrical pulse treatment using electroporation has been proposed to treat weed seeds in soil, possibly killing weeds before they emerge from the ground [18]. White mustard seeds were steeped in water, then exposed to exponential pulses of varying field strength and duration. There was an observed optimum value of both time and field strength, with destruction reduced above this level. The study fails to consider the important role of energy but does demonstrate a possible drawback of excessive field strength and time constant that hasn't been observed in other literature. The study also varied concentration of calcium ions in the surrounding liquid and found that a lower field strength could destroy seeds at higher calcium concentration, supporting the notion that the movement of ions is a mechanism of damage.

Other electrical weeding work has identified the formation of membrane pores and claimed that a high voltage impulse will cause at least two times more damage than a sinusoid of equal energy [20]. In Japanese work from 1990 it was claimed that 5cm high weeds could be killed with 200mJ of energy [4]. This system used low energy 15kV capacitor discharges at 30Hz, and repeated pulses until the specified total energy was discharged through the plant. This system is very similar to methods used in electroporation research and it seems possible that energy requirements observed – orders of magnitude below other published electric weeding work – may have resulted from an electroporation effect on plant tissue.

### *3.4.3 Reactive Oxygen Species Generation*

It is known that ozone can be formed when high voltage ionises air. This ozone can enter the stomata of plant leaves during normal gas exchange and, as a strong oxidant, cause damage to plant cells [37]. The damage caused depends on ozone concentration and plant species, with short duration exposure to high concentration – termed acute exposure [38] – generating reactive oxygen species (ROS) within the cell. Reactions involving ozone and the generated ROS have a range of effects on cells and can induce necrosis. Of particular interest is the known association between acute ozone exposure and programmed cell death [39], a process where local damage can cause signals to spread and kill cells elsewhere in a plant.

It is common for an electrical weeding method to cause generation of arcing between an electrode and plant tissue, particularly in Russian research [20]. This discharge causes generation of ozone in a process where the oxygen in air reacts with the electrons in the arc channel to form oxygen atoms that subsequently form ozone [40]. The quantity of ozone produced depends on the effective reduced field strength, with unit $Vcm^2$, and the duration of the discharge. Although electrical weeding literature hasn't yet identified a correlation between arcing and cell death, the high toxicity of ozone and the capability for damage to spread through tissue from a specific exposure point through a programmed cell death mechanism, mean it can't yet be discounted as a factor in plant death.

## 3.5 Root and Soil Impedance

The conductivity of soil is dependent on a range of properties including its constituents – ion and clay content, and mineralogy – moisture, bulk density and temperature [15]. There are also different pathways through soil that depend on these constituents, such as flow through ionic liquid, flow between solid and liquid parts, and flow through solid soil particles in direct contact with each other. These are marked with the arrows in Figure 10 below. The air gap is typically highly resistive, meaning soil with a higher percentage of air typically has a higher overall resistivity.

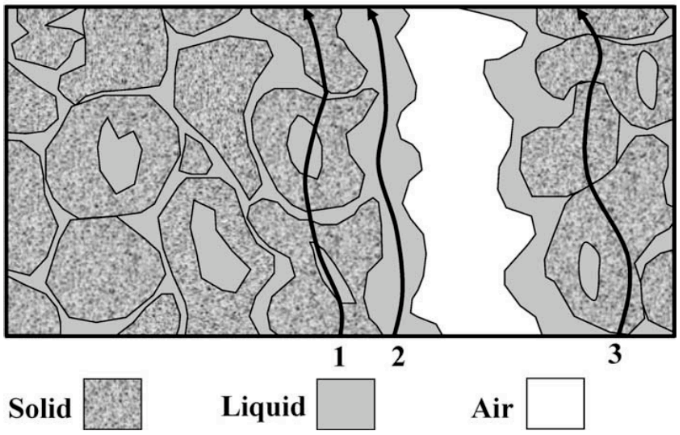


FIGURE 10 PATHWAYS OF ELECTRICAL CONDUCTANCE [15]

It has been observed that very high voltages in soil can cause air gaps to ionise and become much more conductive. It has been estimated for a spherical conductor that the region of soil within one radius may account for half of the total soil resistance [41]. This suggests a much higher electric field around roots, so soil breakdown may occur as the air pockets within the soil ionise and small arcs can develop. This effect is known to occur with an electric field of around 400 kV/m in soil, varying from 30 to 4000 kV/m depending on soil properties [42]. Ionisation in the soil around roots could have some effect on soil biological function or create ozone that damages root tissue. Some acidification in the region of soil near plant roots has been observed for electrical weeding [4].

Under most conditions soil has very poor conductivity per volume. However, as this volume of soil is relatively high, there are a large number of paths for current to travel through so the effective resistance is relatively low [11]. This is also why the resistance and electric field is higher near the roots, as soil close to roots has a relatively much lower cross-sectional area than the main volume of the soil. Root-soil conductivity will combine with the distance of the soil electrode from the plant to define the total resistance of the soil.

The other aspect of impedance for the root-soil system – that has been isolated in the method proposed in this work – is the capacitance of the interface between the roots and soil. It has been suggested in literature that the surface area of roots can be estimated by the capacitance of the root-soil interface [43]. In this work, it was found there was a relationship, but that the soil moisture also has a significant impact. This capacitance occurs at the surface of roots, and a range of factors including the area of roots in contact with solid soil particles or liquid, as well as the conductivity of the soil, would affect its magnitude.

## 3.6 SUMMARY

Although there has been research into electric weeding for many decades, the depth of understanding is still quite limited and this means there is a gap in knowledge with the underlying mechanisms involved. Other fields have investigated similar problems and phenomena such as electroporation – and the research conducted to understand it – provide a crossover of knowledge that can be applied to electric weeding. This and other knowledge, such as electrical characterisations of plants, has be used to help define the problem for the method proposed in this work, as well as providing a theoretical basis for the method design. Incorporation of this theoretical basis into a practical and reliable method is intended to allow bridging of the gap in knowledge that exists for electrical weeding, allowing the technology to develop into a viable and useful solution for farmers.

# 4 METHOD DEVELOPMENT

## 4.1 OVERVIEW OF DEVELOPMENT PROCESS

The project was instigated as a result of a problem identified in an electric weeding research project where a plant scientist wasn't able to measure the electrical parameters during treatment, meaning developing understanding was difficult. This set out the broad problem of measuring electrical parameters during treatment, but given the range of options and configurations that could be used, an investigation was conducted to understand the highest priority variables to measure. It was also important to understand the way data might be interpreted to inform selection of a physical configuration for measurement. This research resulted in the problem definition of accurate and reliable measurement of localised electrical parameters during an electric weeding discharge, and clarified what these electrical parameters could be.

From this point several concepts were developed for how the measurement device could work. Options are detailed in Section 4.3, and include a range of possible configurations and measurement devices. Based on the requirements identified in the background research as well as feedback from the plant scientist who posed the original question, a final approach was selected and developed. The method was then tested in an initial experiment with a lower pulse voltage of 400 V. Based on the results of this experiment several changes were made and a final experiment was conducted at 3000 V to prove the efficacy of the final developed approach.

## 4.2 CRITERIA FOR SELECTED METHOD

The method was selected based on three main criteria, which were decided based on conversations with a plant scientist and on the background research:

1) Simplicity, practicality and reliability

   The method needed to fit the intended purpose of use by plant science researchers to understand electric weeding. To achieve this, the physical setup, parts used and theoretical basis benefited from a simple and practical approach. The simplicity also helps improve the reliability of the system, which is critical to the usefulness of the method.

2) Possibilities for interpreting results

   The different possible options for the method would allow different interpretations of the results. For example, thermal imaging would focus on the energy generation in tissue, while physical voltage probing would allow derivation of electric field strength. It was important the chosen method allowed derivation of a range of the most important electrical parameters to developing understanding of electric weeding.

3) Accuracy and precision

   Whatever method was chosen needed to measure parameters with accuracy and precision. This is what allows small differences in plant responses to be observed and correlated with outcomes such as survival rates.

## 4.3 OPTIONS CONSIDERED

The possible options to solve the problem presented in this work range in terms of the technology that could be used for measurement and the physical configuration. Electrical parameters could be measured indirectly through thermal or hyperspectral imaging, or directly with current transformers or voltage probes. Some of the critical questions for selection of a physical configuration included what scale of measurement would add the most value, with options extending from microscopic imaging to the near single-cell level, to accurate measurement of the whole combined system. Another important question was how the system should be set up. Would it add more value to separate plant tissue – or even sub-regions of tissue such as root systems – and soil, or was there value in combining these for measurement. It was also an option to do field tests with the plant in situ, which provides the most realistic representation of the system but presents issues with random variability in the environment and in the practicality for measurement.

As accuracy, reliability, and practicality were the top priorities for the final method, the possible options were narrowed down. Although current transformers can measure current through separate regions of plant tissue, there are limited options for accurate devices that can isolate a voltage up to several thousands of volts. It would also be physical difficult to clip the bulky probes around the tissue of young plants – important for electric weeding from a weed management perspective – when they have small and sometimes complex shapes. Use of thermal or hyperspectral imaging was another possible option to observe thermal or physical changes to tissue. For example, thermal imaging would be able to pick up heat generated by current flow, while hyperspectral may have been able to pick up changes to the exterior of the plant due to leakage of cell liquid if electroporation was occurring. In the end the potential value of information gathered by imaging could not justify the sacrifice to practicality that it would incur, with expensive cameras and careful control of variables such as temperature and light required to gather accurate data.

In terms of setup, the microscopic imaging of a small region of tissue during and after treatment would have the potential to add significant value to the understanding of the effect on cells. Use of dyes such as have been used for detection of electroporation – as shown in Section 3.4.2 – could indicate the extent of permeabilisation. The issue with this is the practicality of setting up a high-voltage discharge in very close proximity to an optical microscope that will typically have many conductive metal parts. If reliable and safe isolation could be achieved this may be a useful future extension of the work. After deciding that microscopic observations were impractical for the resource and time available for this work, the next decision to make was whether to treat an isolated cut region of tissue or whether to attempt to measure the whole plant-soil system. An isolated region of tissue would allow a more controlled environment with set dimensions, allowing more simple derivation of electrical parameters. However, measuring the whole plant in soil provides these measurements in the application context and allows insight into complexities such as the interface between the roots and soil. This means the results can be interpreted more directly and also, crucially, means the plant survival after a discharge can be observed, which is not possible for a cut length of tissue.

## 4.4 INITIAL PROPOSED METHOD

Figure 11 shows a diagram of the measurement method that was selected. The whole plant and soil system is used for measurement, with a shunt-type current resistance and a voltage probe that can be placed at a chosen point on the plant. In the figure, 'voltage application' is the electrode applying the high voltage to tissue at point A. The 'voltage measurement' point is where a high-voltage oscilloscope probe is attached. The plant soil system is then broken into two measurable impedances. Impedance 1 represents the impedance of the plant tissue between the electrode and the measurement point, B. Impedance 2 represents the combined resistance of the plant root system and soil between point B and the grounding electrode, C. The shunt-type current resistance is connected between the grounding electrode at C and the 'voltage return' point D, which is the ground return for the high voltage applicator device.

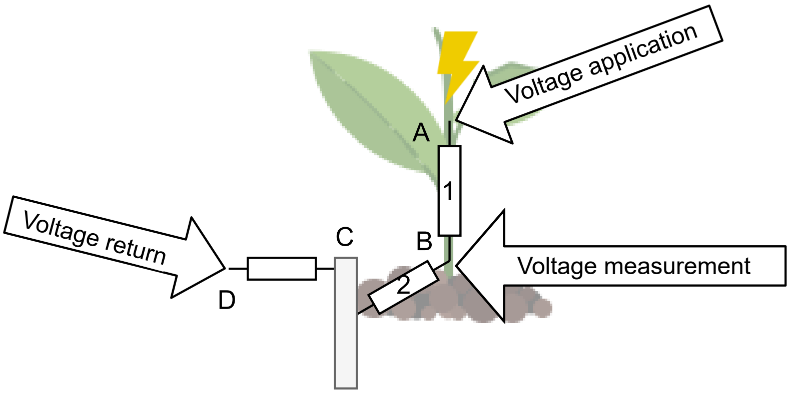


FIGURE 11 MEASUREMENT DIAGRAM

Based on the current through the whole system and the voltage at point B, a number of electrical parameters can be derived for the two separate parts of the system, including electric field strength, current density, and energy volume density. To derive each of these, the physical dimensions must also be measured as part of the process of the method. To maximise ease of use it was decided that the final method should be as automated as possible, with the electrical measurement system incorporated into a computer program.

## 4.5 ELECTRICAL THEORY

The voltage at the base of the plant is a division of the total voltage, and this value is defined by resistive and capacitive voltage divider theory. A resistive voltage divider is shown in Figure 12, where the resistance $R_1$ is the resistance between the voltage input ($V_{dd}$) and the measurement point ($V_o$) and $R_2$ is the resistance between the measurement point and ground.

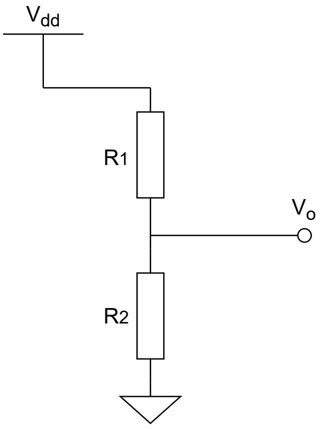


FIGURE 12 RESISTIVE VOLTAGE DIVIDER

The voltage $V_o$ is defined by the equation:

$$V_o = V_{dd}\frac{R_2}{R_1 + R_2} \tag{9}$$

So the voltage out for a theoretical circuit with only resistive impedance (no capacitance) is equal to the resistance being measured $R_2$ (the oscilloscope is connected between the measurement point $V_o$ and ground) divided by the total resistance.

The circuit for a theoretical circuit with only capacitive impedance (no resistance) is shown in Figure 13, where $C_1$ is the capacitance between the input voltage and $V_o$, and $C_2$ is the capacitance between $V_o$ and ground.

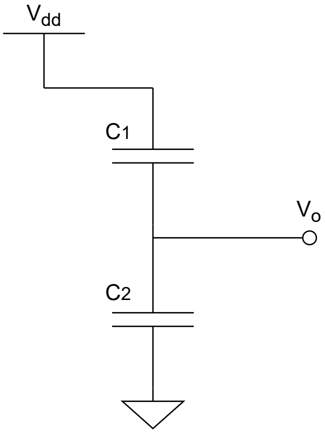


Figure 13 Capacitive voltage divider

For capacitors, the impedance to the flow of charge is low when a voltage is first applied. When there is no charge stored in the capacitor electric field there is nothing slowing the flow of charge onto the capacitor plates. While charge continues to flow the charge already on the plates begins to impede the flow of further charge. The impedance of a capacitor is defined by the equation:

$$X_c = \frac{1}{2\pi f C} \tag{10}$$

Where $X_c$ is the capacitance reactance (impedance to the flow of charge), f is the frequency of a sinusoidal input signal and C is the capacitance. A higher frequency reduces the build-up of charge on the capacitor plates so the reactance is reduced. The fact that a greater capacitance reduces the effective impedance results in the capacitive voltage divider equation:

$$V_o = V_{dd}\frac{C_1}{C_1 + C_2} \tag{11}$$

Which is the reverse of the resistive divider, where the fraction of the capacitance $C_1$ not measured to the total capacitance defines the measured voltage, $V_o$. When a square pulse is applied to a capacitive load, the initial leading edge of the voltage is affected by the capacitance, but after a period of time the capacitance will fill with charge and the measured impedance will be purely resistive. The total impedance Z is a combination of resistive and capacitive elements as shown in Section 3.3.2 and is dependent on the rate of change of voltage with time. Using the simplest model given in Figure 3 the impedance of plant tissue would be:

$$Z = \frac{R_2(R_1 + R_3) + 2\pi f C R_1 R_2 R_3}{R_1 + R_2 + R_3 + 2\pi f C R_3(R_1 + R_2)} \tag{12}$$

At very high frequency the impedance can be approximated to:

$$Z = \frac{R_1 R_2 R_3}{R_3(R_1 + R_2)} \tag{13}$$

And at very low frequency (DC voltage) the impedance can be approximated to:

$$Z = \frac{R_2(R_1 + R_3)}{R_1 + R_2 + R_3} \tag{14}$$

The voltage at the measurement point is then the division of the impedance of the plant tissue above the connection point and the root-soil system below the connection point. In real terms, this means the transient response of the voltage and current would be affected by the capacitive voltage divider, but after a period of time the signals will stabilise and the voltage and current will be purely defined by the resistive voltage divider. It was observed in initial testing – a typical waveform is shown in Figure 18 – that a transient response will last around 5 to 10 microseconds. Therefore, for a pulse width of 100 microseconds, the division of the resistance will be the sole influence for over 90 percent of the time, controlling parameters such as electric field strength, current density, and energy volume density. To simplify this work, it was decided to measure the stable resistive voltage and current during each pulse rather than trying to interpret the initial transient, which adds complexity while only reducing error slightly, and which could potentially introduce measurement error as described in Section 4.6 below.

## 4.6 CONSIDERING SURFACE IMPEDANCE

An important consideration when directly measuring the voltage of plant tissue is the location of contact with the tissue and the effect this has on the measurement. It has been established in literature that the drier surface tissue of a plant has a higher resistance than the moist internal tissue [26]. Therefore, when measuring the voltage at an external point on the plant, the surface impedance will be a factor. Figure 14 demonstrates the system to consider, where the plant tissue impedance is represented by one of the models shown in Section 3.3.2.

The measurement device is connected to the surface, but the objective is to measure the voltage in the center of the plant. To achieve this, the surface resistance R must be much smaller than the input resistance of the measurement device to maximise the proportion of the voltage divider that is across the measurement input. To measure transient response, the capacitance C must be much larger than the input capacitance of the measurement device to have the same effect. The oscilloscope probes used in this work have an input impedance of 10 MΩ and capacitance of 10-12 pF, which is typical for many commercial probes. For the method proposed in this work, the transient response is not measured, so the surface capacitance will not have any influence. An assumption is made that the surface resistance is much smaller than 10 MΩ, so can be ignored.

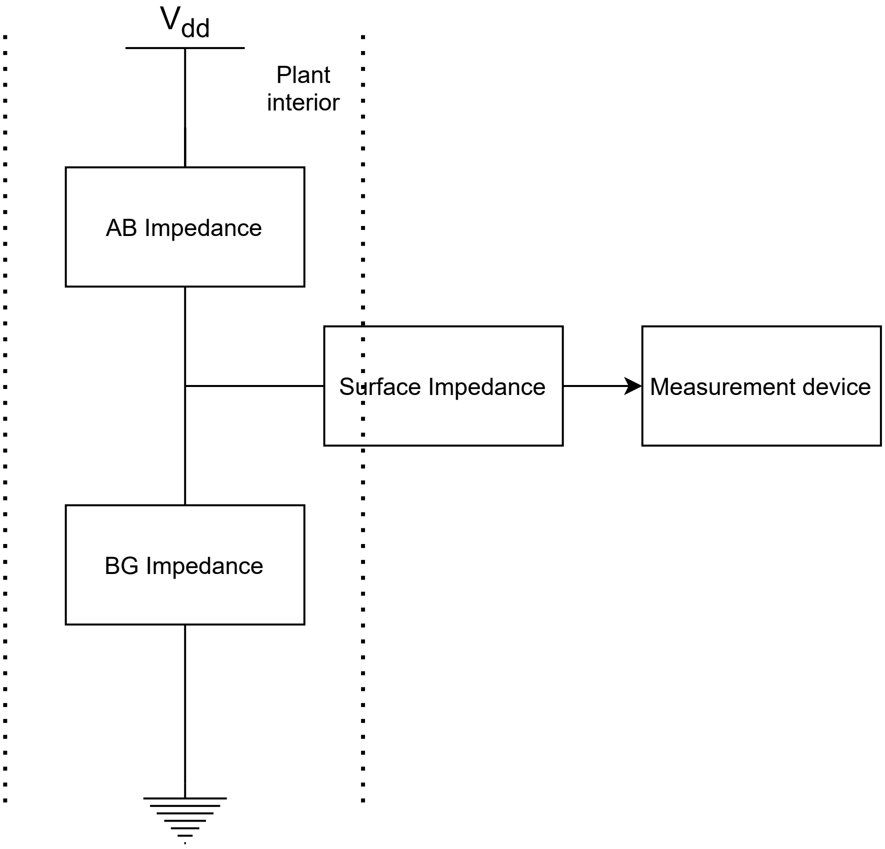


FIGURE 14 PLANT SURFACE TISSUE IMPEDANCE CONSIDERATION. A IS POINT OF INTEREST, B IS POINT OF CONNECTION TO MEASUREMENT DEVICE, R IS SURFACE TISSUE RESISTANCE AND C IS SURFACE TISSUE CAPACITANCE

The validity of this can be checked by calculating the error based on a maximum realistic estimate for the surface resistance of the plant, with the percentage error introduced defined by Equation 15.

$$\%\, error = 100 \times \left(1 - \frac{10 \times 10^6}{R_s + 10 \times 10^6}\right) \tag{15}$$

Where $R_s$ is the surface resistance. We can estimate the surface resistance to have a value comparable to or lower than the combined plant resistance. In the validation experiment described in Section 5, the maximum value found for the total combined resistance of the plant, roots and soil was just less than 200 kΩ. Assuming a surface resistance equal to this, the calculated error would be:

$$\%\, error = 100 \times \left(1 - \frac{10 \times 10^6}{200 \times 10^3 + 10 \times 10^6}\right) = 1.96\% \tag{16}$$

So the introduced error would be just under 2 percent. Although this does have some impact on the measurement, the assumption that surface resistance is negligible is valid and it is ignored in the calculations used as part of the method.

## 4.7 SYSTEM SPECIFICATIONS

Based on the type of measurement method chosen a system would need to be developed to meet the following specifications:

1. A maximum voltage rating for the voltage probe of 7000 V
2. A maximum current rating for the shunt resistor measurement device of 1 A
3. Minimum sampling rate for measurement system of 200 ns
4. Capability to record pulse width up to 100 μs
5. Capable of working within a semi-automated system
6. Possibility of physical attachment to both monocots and dicots of two to four weeks old

## 4.8 PART SELECTION

To build the system to the desired specifications parts would need to be selected or manufactured that met the requirements. It was decided that if possible the parts would be readily available commercial products. This means the method is accessible and repeatable for researchers who do not have access to the equipment used in this work. It was found that oscilloscopes and probes produced by Pico Technology (UK) would be fit for purpose. They produce a range of USB oscilloscopes with an associated software development kit (SDK) that would support the need for automation as well as an active differential voltage probe with a rating up to 7000 V. These parts also feature a generic spring-actuated hook end that is suitable for attaching to the required plants.

## 4.9 CALCULATIONS

The following calculations can be used to derive parameters based on the voltage and current measured during a pulse, along with the plant stem diameter, and the distance of plant stem between the voltage application and the voltage measurement point.

### *4.9.1 RESISTANCE*

The resistance of the root-soil system is calculated from:

$$R_{root-soil} = \frac{V_o}{I} \tag{17}$$

Where $V_o$ is the voltage at the base of the plant and I is the current. The resistance of the plant above the surface of the soil is then:

$$R_{plant} = \frac{V_{dd} - V_o}{I} \tag{18}$$

Where $V_{dd}$ is the input voltage at the high-voltage electrode (selected in the high voltage generator setup).

### *4.9.2 ENERGY*

The energy lost in the root-soil is calculated through:

$$E = IV_o t \tag{19}$$

While the energy in the plant above the surface of the soil is calculated through:

$$E = I(V_{dd} - V_o)t \tag{20}$$

Where E is the energy and t is the duration of the pulse.

### 4.9.3 *ELECTRIC FIELD STRENGTH*

The electric field strength is derived from the voltage and distance through the equation:

$$E_f = \frac{V}{d} \tag{21}$$

Where $E_f$ is the electric field strength in V/cm, V is the voltage across the region of interest and d is the distance in cm between the voltage application and the voltage measurement point.

### 4.9.4 *CURRENT DENSITY*

The current density can be estimated from the known current value and the cross-sectional area of the region of interest through the equation:

$$J = \frac{I}{A} \tag{22}$$

Where J is the current density in V/cm$^2$, I is the current and A is the cross sectional area in cm$^2$.

### 4.9.5 *ENERGY VOLUME DENSITY*

Energy is the multiple of the power over time, and can be derived for each region from the average power over time. The energy volume density is:

$$E_{per\ unit\ volume} = \frac{VIt}{v} \tag{23}$$

Where $E_{\text{per unit volume}}$ is in J/cm$^3$, V is the average voltage, I is the average current, t is the duration of the pulse in seconds and v is the volume of tissue in cm$^3$.

# 5 VALIDATION EXPERIMENT

## 5.1 ELECTRIC APPLICATION DEVICE

The high voltage pulse used in this experiment was generated by a device supplied by Weda Tech Ltd. The specifications of the machine are:

- Adjustable output voltage between 400-7000 V +/-20 V
- Adjustable DC pulse width from 1-1000 μs

In the validation experiment the device is set up to provide a 400 V rectangular pulse with 100 μs duration. The voltage was selected as it is the maximum voltage that can be directly measured by the 10:1 Keysight probe used for the experiment, as well as the minimum voltage that can be output by the Weda Tech pulse generation device. The pulse width was chosen as initial testing showed this was enough time for any voltage or current transients to settle and to get a consistent reading.

## 5.2 PARTS USED

The initial experiment was conducted manually using a benchtop oscilloscope. The full parts list includes:

- Keysight (US) InfiniiVision MSOX2012A mixed signal oscilloscope
- Keysight (US) N2862B 10:1 passive probe
- Pico Technology (UK) TA375 passive probe
- Weda Tech (NZ) 7 kV adjustable pulse generator
- Aluminium rod electrodes 9.7 mm diameter
- 1% 0.5 W metal film 100 Ω shunt resistor
- Ryegrass (Winter Star variety)

## 5.3 SETUP

The experiment was conducted with 22 bags with dimensions 100 mm height and 80 mm diameter. Each bag had six seeds of Winter Star ryegrass planted on the 4th of December 2020 in a greenhouse in Hawke's Bay and used sifted silt loam soil from a nearby paddock. After initial growth (two leaf stage), plants were selectively removed to leave three in each bag with size across bags as consistent as possible. The bags were watered regularly for fourteen days (up to the 18th of December) and then for five days eleven randomly selected bags were not watered to reduce the water content in the soil of these bags down to around 50%. Bag mass was measured on the 21st and 22nd of December and also during the experiment on the 23rd.

20 bags were used for the experiment, with one bag spare in each experiment as a backup. The bags were arranged in a grid in the greenhouse and the run order was randomised. The experiment was conducted inside the greenhouse in one continuous block commencing at 9.50am and finishing at 12.50pm on the 23rd of December.

Before commencing the experiment a ruler was taped to the high voltage electrode as shown in Figure 15 with the end of the ruler approximately 20 mm from the end of the electrode. This was used to improve consistency in the height above the soil of contact with the plant.

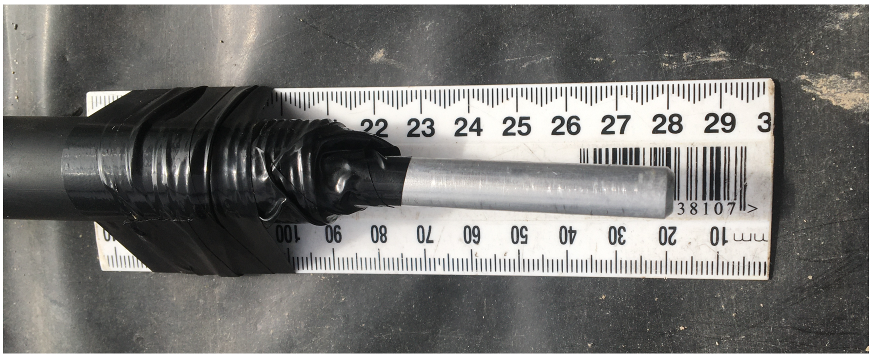


FIGURE 15 RULER ATTACHED TO ELECTRODE

The bags were tested in the run order shown with the process for each bag being:

1. Attach the positive end of the TA375 low voltage 1:1 probe to the shunt resistor and ground clip from this probe to the discharge device side of the shunt resistor
2. Weigh the bag and record manually
3. Place the grounding electrode down the side of the bag.
4. Measure the length of each leaf from the base of the plant.
5. Attach the voltage probe to the base of the plant.
6. Set the oscilloscope to single trigger mode.
7. Place the high voltage electrode on the stem of the plant with the end of the ruler sitting flat on the surface of the soil.
8. Trigger the high voltage discharge.
9. Record the voltage and current waveforms from the cursor function on the oscilloscope.
10. Repeat steps 4 to 8 for next two plants in bag
11. Repeat steps 2 to 10 for next bag

The bag after the first three steps were completed is shown in Figure 16. The grounding electrode is placed down the right hand side of the bag, with a shunt resistor between the electrode and the white return line for the pulse generator. The Pico Technology passive probe is set to 1:1 and attached across the shunt resistor. The voltage measured through the shunt resistor can be converted to current through Equation 24:

$$I = \frac{V}{R} \tag{24}$$

For the experiment conducted in this work the shunt resistor was 100 $\Omega$, meaning the current is:

$$I = \frac{V_I}{100} \tag{25}$$

Where $V_I$ is the voltage measured across the shunt resistor.

FIGURE 16 GROUNDING ELECTRODE AND SHUNT RESISTOR

An image of the setup during treatment is shown in Figure 17. The Keysight passive probe is attached to the base of the plants in line with the surface of the soil (this is point B from Figure 11).

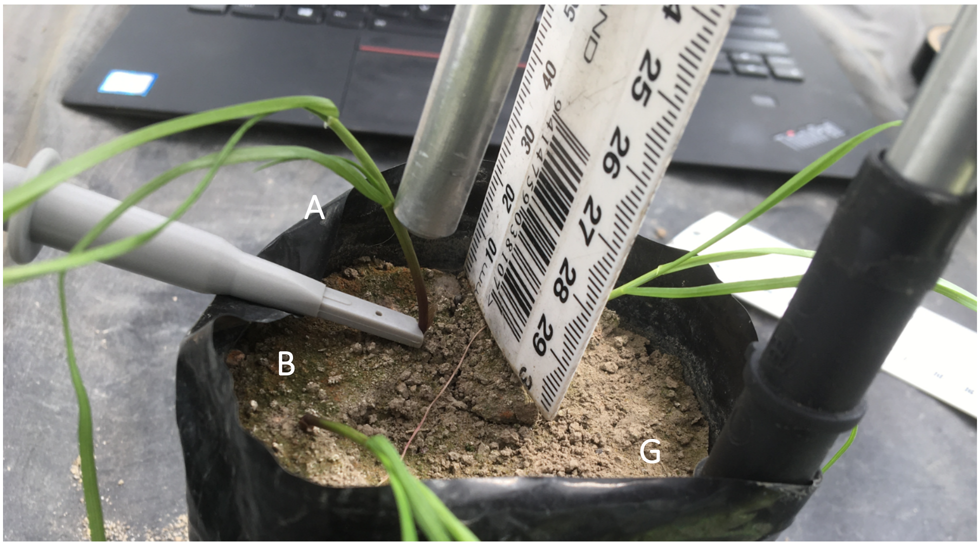


FIGURE 17 PHOTO OF MEASUREMENT SETUP

The leaf length measured in step three was added together to create a total leaf length metric that gives a representation of the total size of the plant. Total leaf length has been shown to have a close correlation with plant dry weight (Dan Bloomer, pers. comm.) – the best recognised metric for plant size. Total leaf length was chosen as it was much easier to measure.

The waveform on the oscilloscope after a pulse is shown in Figure 18. The voltage and current are measured at the steady state point shown in the figure, after the transient effects caused by capacitance charging at the start of the pulse have settled. This means the impedance measured by the method will include only the resistance of the tissue.

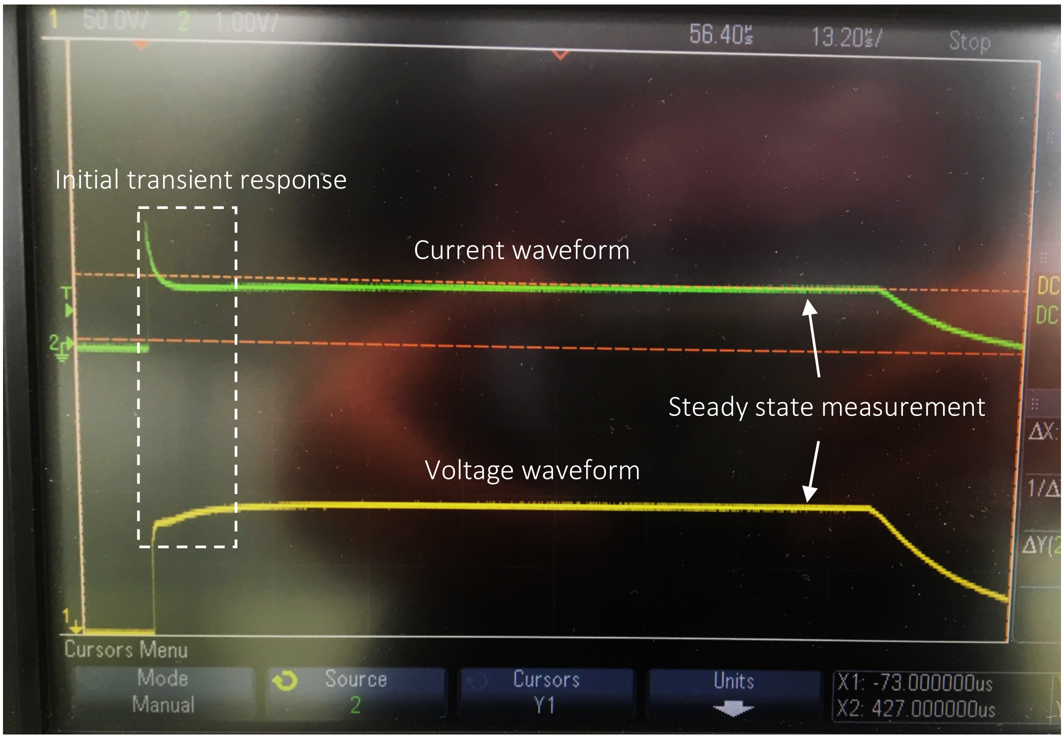


FIGURE 18 WAVEFORM SHOWN AFTER PULSE

After several weeks of drying the bags were weighed again on the 25th of January to determine the mass of the soil with approximately zero moisture. This was used to calculate the gravimetric soil water content (called soil moisture) during the experiment through the equation:

$$Soil\ moisture\ \% = 100 * \frac{Experiment\ weight - Dry\ weight}{Saturated\ weight - Dry\ weight} \qquad (26)$$

In the results below the bags that were dried to a soil moisture of around 50% are called ‘dry’ bags, while the bags that were continually watered up to the experiment day are called ‘wet’ bags.

## 5.4 RESULTS AND DISCUSSION

The results of the experiment are presented in Table 1. The values found for the three plants in each of the ten bags have been averaged to account for natural variation in both plants and the distance between the plant and the electrode through soil. The p-value is the result of a two-tail t-test on the two treatments. Each value is rounded to 4.s.f.

TABLE 1 SUMMARY OF VALIDATION EXPERIMENT RESULTS

| Parameter | Moisture | Mean | SEM | p-value |
|---|---|---|---|---|
| Soil moisture (%) | Wet | 84.86 | 1.946 | 1.013E-07 |
| | Dry | 46.50 | 1.020 | |
| Leaf length (cm) | Wet | 54.57 | 1.686 | 7.642E-04 |
| | Dry | 46.67 | 1.145 | |
| Plant voltage (V) | Wet | 296.9 | 4.860 | 1.062E-05 |
| | Dry | 178.6 | 7.006 | |
| Soil voltage (V) | Wet | 89.11 | 4.860 | 1.062E-05 |
| | Dry | 178.6 | 7.006 | |
| Current (mA) | Wet | 7.000 | 0.3850 | 1.341E-06 |
| | Dry | 2.716 | 0.1276 | |
| Plant resistance (Ω) | Wet | 45560 | 2374 | 8.088E-06 |
| | Dry | 80160 | 3704 | |
| Soil resistance (Ω) | Wet | 13660 | 1317 | 3.636E-06 |
| | Dry | 69220 | 5074 | |

These show the relative difference between soil moisture in the 'wet' and 'dry' groups, with an average of 46.5% for the dry group and 84.9% for the 'wet' group. The data from each individual plant combined with stem diameter (if measured) could be used to derive the electric field strength, current density and energy volume density as shown in Equations 25-29. The relationship between soil moisture, plant size and root-soil resistance is shown in Figure 19. These results, combined with a statistical analysis, can be used to prove that the method can reliably record results that align with the underlying theory.

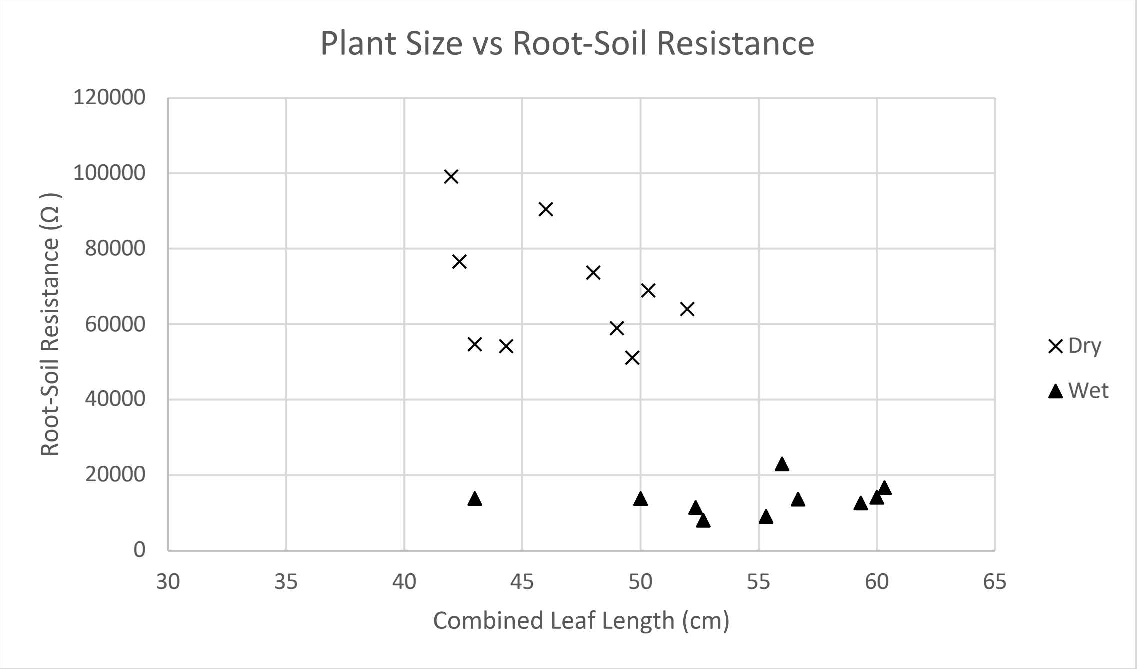


FIGURE 19 PLANT SIZE VS ROOT-SOIL RESISTANCE GRAPH

There is a clear difference in root-soil resistance between the 'wet' and 'dry' groups, with a p-value indicating a significant difference. The theory of soil resistance suggests this could be caused by a reduction in soil moisture decreasing the paths for current through the soil [15], or changes in root conductivity and surface area as a result of changes in plant size induced by moisture. The difference in size between plants from ‘dry’ bags and plants from ‘wet’ bags was also significant. It could be expected that a reduction in the combined leaf length for a plant would be reflected in changes such as a reduction in root size and surface area. These physiological changes, along with reduced contact area between roots and soil and a decrease in the number of conductive paths through soil would all theoretically combine to increase the resistance of this part of the system. An important consequence of this is that in the field an increased proportion of voltage and energy will be lost in the root-soil system as a result of the voltage divider effect. The results of the experiment clearly reflecting the theory with no obvious outliers indicates the method is reliable and accurate, and helps to prove validity.

It is established in horticulture that plants with more access to water grow faster, but it was possible that in the short timeframe of difference during this experiment this change would not be significant. Although it is interesting to observe the combined effect of moisture on the roots and soil, for the measurements of the plant resistance that are one of the objectives of the final method, it is preferable that the plant sizes are consistent across all bags. As a result, the method for drying plants was adjusted in the final experiment as described in Section 6.

The diameter of plant two in bag ten was estimated based on a later measurement of plants of a similar age to allow calculation of the parameters of interest for this experiment. The purpose of this estimate is to demonstrate the capability of the equations involving diameter to give reasonable answers, but besides proving this the results of the calculations using diameter (current density and energy volume density) cannot be used to draw further conclusions. After the experiment it was decided it was worth measuring the diameter of all plants, which was a change made for the final experiment as described in Section 6. The results for plant two in bag ten are shown in Table 2.

TABLE 2 RESULTS FOR PLANT TWO IN BAG TEN

| Wet | Soil moisture (%) | Leaf length (cm) | Total voltage (V) | Measured voltage (V) | Current (mA) | Plant resistance (Ω) | Soil resistance (Ω) |
|---|---|---|---|---|---|---|---|
| N | 46.04 | 46 | 386 | 152 | 2.250 | 104000 | 67560 |

The equations used for derivation of each of the parameters shown below are from Section 4.9. The following approximate dimensions are used to provide a basis for the example calculations:

TABLE 3 APPROXIMATE DIMENSIONS

| | |
|---|---|
| **Plant length above ground** | 20 mm |
| **Diameter of stem** | 1 mm |

$$Energy\ (root - soil) = IV_o = 0.00225 \times 152 \times 0.0001 = 0.0342\ mJ \qquad (27)$$

$$Energy\ (plant)\ = I(V_{dd} - V_o) = 0.00225 \times (386 - 152) \times 0.0001 = 0.0527\ mJ \qquad (28)$$

$$Electric\ field\ strength\ (E_f) = \frac{V}{d} = \frac{234}{2} = 117\ {}^{V}\!/_{cm} \tag{29}$$

$$Current\ density\ (J) = \frac{I}{A} = \frac{0.00225}{\pi \times (0.1 \times 0.5)^2} = 0.286\ {}^{A}\!/_{cm^2} \tag{30}$$

$$Energy\ volume\ density = \frac{E}{V} = \frac{0.0000527}{\pi \times (0.1 \times 0.5)^2 \times 2} = 3.35\ {}^{mJ}\!/_{cm^3} \tag{31}$$

It can be observed that the power (energy per second) in the plant is higher than the combined roots and soil for this treatment. Given this was a 'dry' bag where it is expected a higher proportion of energy would be lost to the drier and more resistive soil, this is an interesting observation that indicates even in 'dry' soil it can be possible to have the majority of energy treating the plant. However, in the field it will be impractical to provide consistent grounding within a few centimetres of every treated plant so the same soil moisture (and soil resistivity) would lead to more power loss in the root-soil part of the system. The other three parameters – electric field strength, current density and energy volume density – have been successfully calculated, again supporting the conclusion that the method is valid.

## 5.5 CONCLUSION AND IMPROVEMENTS

The ability to calculate these parameters – and for the results to make sense based on theory – combines with the clear significance of the root/soil resistance dependence on moisture to allow the conclusion that the method is valid, with some improvements required. One of the improvements is to change the method of creating a difference in soil moisture. In this validation experiment, all plants were watered up to five days before the experiment and then the 'dry' bags were not watered. It is expected that the time with difference between soil moistures was too long, causing the significant difference in plant size. In the final experiment a new approach will be used, where all bags will be dried for the last few days before the experiment, then the 'wet' bags will be watered twice the night before the experiment takes place to bring the soil moisture in these bags up to near 100%. The other significant changes to make include expanding the types of plants tested to include a dicot variety, operating at higher voltage, developing a semi-automated system for conducting the method, and measuring the diameter of all plants to allow calculation of the electrical parameters for all plants in the experiment.

# 6 FINAL PROPOSED METHOD

This section provides details of the final method that was developed to meet the objective of the project. Many aspects are similar to the validation experiment, but based on lessons learned there have been several improvements made, including the conversion of the measurement process from manual to semi-automated.

## 6.1 OVERVIEW OF CHANGES

The final method follows the same basic system as the validation experiment, with a voltage measurement device attaching to a plant in line with the surface of the soil, as well as a current measurement, to allow calculation of the division of the voltage, current, resistance and energy. The significant changes include:

1. Change in voltage probe and contact method

   For the final experiment a high-voltage 1000:1 oscilloscope probe from Pico Technology (UK) was used, which allows the experiment to be conducted at high voltage. The probe tips that are built into this part have a much stronger spring and sharper hook than the generic oscilloscope tips that were used for the validation experiment. To ensure that plants were not damaged in the process of attachment, the surface area in contact was maximised using the approach described in Section 6.3.

2. Change in shunt current-measurement resistor value

   Because the experiment was conducted at a higher voltage (3000 V) of nearly ten times the validation experiment voltage, the 100 Ω shunt resistor was changed to a 10 Ω resistor to capture a similar voltage range across the resistor (through the $voltage = current \times resistance$ relationship). The resistance was selected based on typical current observed in the initial experiment. A 10 Ω resistance for expected current in the range of 0.1 – 0.5 A would lead to voltages in the range of 1 – 5 V, which is well clear of the saturation voltage of the oscilloscope (10 V) but high enough to be measured with reasonable resolution.

3. Measuring stem diameter for all plants

   For the final method the diameter of the plant stem was measured for all plants, allowing calculation of current density ($A/m^2$) and energy volume density ($J/cm^3$) within all plants above the point of contact with the voltage electrode.

4. Using a semi-automated measurement system

   To streamline the process of plant measurement and data collection a semi-automated system was developed as described in Section 6.4. This was intended to reduce the complexity of measurement to help reliability and to allow an experiment to be completed in a reduced total time so any variance between samples with time is reduced as much as possible.

## 6.2 PARTS USED

TABLE 4 FINAL PARTS LIST

| Part type | Name | Company |
|---|---|---|
| **Oscilloscope** | 2406B 50MHz 4 channel oscilloscope | Pico Technology (UK) |
| **Oscilloscope probe tip** | N2862B 10:1 passive probe tip | Keysight (US) |
| **Oscilloscope probe** | TA375 passive probe | Pico Technology (UK) |
| **High voltage oscilloscope probe** | TA044 high voltage active differential probe | Pico Technology (UK) |
| **High voltage pulse generator** | 7 kV adjustable pulse generator | Weda Tech (NZ) |
| **Computer and software** | Laptop with Pico SDK [44]and a python IDE [45] installed | - |
| **Electrodes** | Aluminium rod electrodes 9.7 mm diameter | - |
| **Shunt resistor** | 1% 0.5 W metal film 10 Ω shunt resistor | element14 (USA) |
| **Calipers** | TD2082 digital vernier calipers 0.01 mm resolution, 0.02 mm accuracy | Protech (Aus) |
| **Ryegrass plants** | Ryegrass seeds var. Winter Star | - |
| **Bean plants** | Green bean seeds var. Clarion | - |

## 6.3 CONTACT METHOD

To achieve contact with the plant using the high voltage oscilloscope probe that, as mentioned in Section 6.1, has a strong enough spring and sharp enough hook to potentially damage plants, a new contact method was devised. This is shown in Figure 20, and involves hooking the tip of the Keysight passive probe (disconnected from the probe itself) and the tip of the high voltage probe. By orienting the two tips as shown in the figure – with the flat side of the metal hook of the high-voltage probe and the plastic end of the low voltage probe tip in contact with the plant – the plant stem can be connected with good contact area but with a surface area and force that was observed to not cause significant damage to the plants.

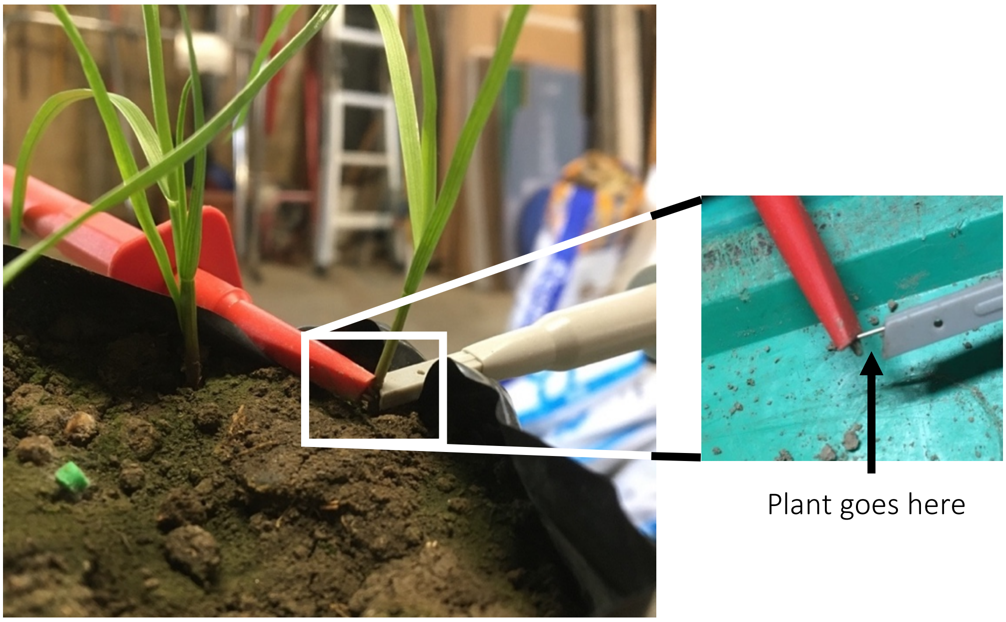
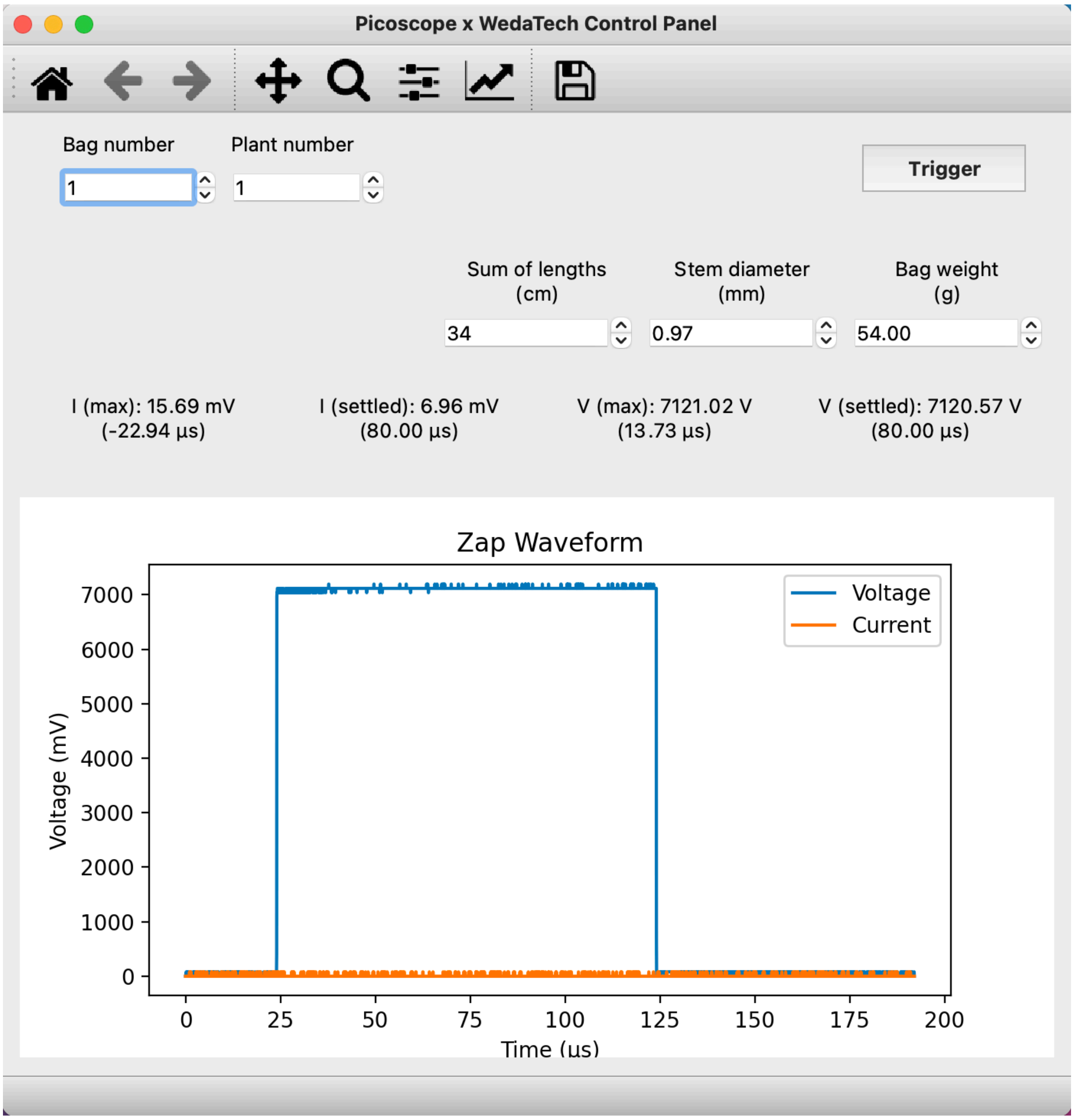


FIGURE 20 CONTACT METHOD DIAGRAM

## 6.4 SEMI-AUTOMATED PROGRAM

The semi-automated program was developed in Python, and Qt Designer was used to create a graphical user interface (GUI). The program makes use of a software development kit (SDK) made available by Pico Technology for the control of the USB oscilloscope using a variety of languages such as Python. The program also allows for recording of bag and plant measurements such as the sum of lengths, stem diameter and bag mass, and recording of these parameters along with the electrical measurements onto a csv file on the computer. The GUI is shown in Figure 21 below.



Bag number: 1

Plant number: 1

Sum of lengths (cm): 34

Stem diameter (mm): 0.97

Bag weight (g): 54.00

I (max): 15.69 mV (-22.94 μs)

I (settled): 6.96 mV (80.00 μs)

V (max): 7121.02 V (13.73 μs)

V (settled): 7120.57 V (80.00 μs)

FIGURE 21 GRAPHICAL USER INTERFACE

As shown in the figure, the program allows the user to set up a trigger on the oscilloscope (button at top right), and displays a graph of the waveform captured by the oscilloscope once complete.

## 6.5 MEASUREMENT PROCESS

The process for measurement in the final method has been updated from the initial experiment to reflect the changes outlined in Section 6.1.

1. Attach the positive end of the TA375 1:1 current probe to the shunt resistor and ground clip from this probe as well as the negative (black) end of the TA044 1000:1 voltage probe to the discharge device side of the shunt resistor.
2. Weigh the bag and input to GUI.
3. Place the grounding electrode down the side of the bag.
4. Measure any required plant dimensions including the diameter of the plant stem approximately 10 mm from the surface of the soil and input to GUI.
5. Attach the positive end of the TA044 voltage probe to the base of the plant using the contact method described in Section 6.3.
6. Place the high voltage electrode on the stem of the plant with the end of the ruler sitting flat on the surface of the soil.
7. Press trigger on the GUI to tell the USB oscilloscope to wait for a discharge.
8. Trigger output from high voltage pulse generator device.
9. Repeat steps 4 to 8 for next two plants in bag.
10. Repeat steps 2 to 9 for next bag.

## 6.6 SUMMARY

The final method proposed in this work has the same basic form as the validation experiment, but learns from some of the weaknesses observed during this experiment and adds a range of improvements. To test this final method an experiment that is conducted with both monocot and dicot plants at high voltage is conducted as described in Section 7. This experiment utilises the final method – including the semi-automated computer program that was developed – to provide proof of the capabilities of the method.

# 7 FINAL EXPERIMENT

## 7.1 SUMMARY

The final experiment was conducted using the method described in Section 6. 32 bags of the same dimensions as the validation experiment – 100 mm height and 80 mm diameter – were filled with sifted silt loam soil from a nearby paddock. 16 bags were planted with 6 seeds of ryegrass – the same batch from the validation experiment – and 16 were planted with 6 green bean seeds, all on the 4th of February 2021. Bean plants were chosen as the dicot plant as they were available and practical to grow in bags.

## 7.2 SETUP

The ryegrass and bean bags were placed in an alternating grid in a shade house, and on the 22nd February some plants were removed from each bag to leave three plants per bag with as consistent a size as possible across all bags. All bags were regularly watered up to the 25th of February, when all bags were left to dry. On the 1st of March, half of the bean bags and half of the ryegrass bags were watered twice to bring them back up to near 100% soil moisture. The experiment took place the next day on the 2nd of March in one continuous block from 1.30pm to 5pm, with the run order randomised. For each trial the process described in Section 6.5 was used, with the setup at the time of triggering high voltage discharge for a ryegrass plant shown in Figure 22. The contact method shown is as described in Section 6.3.

FIGURE 22 SETUP DURING TREATMENT

The gravimetric soil water content (soil moisture) was measured using the same equation shown in Section 5.3, but with the soil dried in ovens at the Plant and Food Research site in Hawkes Bay.

## 7.3 DATA INTERPRETATION

During the experiment 96 plants in total were treated – 3 each from 32 bags – but before analysing results five samples were discarded for a variety of reasons. Bag 2, plant 1 and bag 12, plant 2 were both ryegrass plants that were much smaller than the population and produced erroneous results. Bag 28, plant 2, bag 5, plant 1 and bag 10, plant 1 were treatments where the high voltage discharge generator was accidentally set to produce the wrong voltage. As these failed treatments all happened in different bags, it was decided that the average results for these bags would depend on the average of the two successful plants. The results from the average of the bag were used for measurements in the root-soil system as the distance through soil between the plant and the return electrode will be an influencing variable, so averaging the results for plants that are distributed around the bag reduces the difference between trials. There will still be a difference as bags had plants removed to keep plant size as even as possible and the remaining three plants could be in any of the six equally distributed positions that seeds were originally planted in. For plant interpretations, such as electric field strength, current density, and energy volume density in the plant stem, the distance through soil will not impact results so the results are interpreted based on the individual plants, rather than the average of a bag. Statistical analysis included calculation of the standard error of the mean (SEM) as well as a two-tail t-test to determine the p-value for the two different independent variables (‘dry vs wet’ and ‘bean vs grass’).

# 8 RESULTS AND DISCUSSION

This section covers the results from the final experiment and how these can be interpreted based on the background theory covered in Section 3, as well as how they prove the capabilities of the method against the aim of the project.

## 8.1 SUMMARY OF RESULTS

The results of the final experiment are shown in Table 5. The statistics are calculated based on the mean values for each bag, so each mean value includes the 8 bags that were part of that treatment. The p-value columns give the significance based on a two-tail t-test for means. The 'dry vs wet' column is a comparison of all the 'dry' bags for that parameter vs all of the 'wet' bags.

TABLE 5 SUMMARY OF FINAL EXPERIMENT RESULTS

| Parameter | Plant type | Moisture | Mean | SEM | p-value (dry vs wet) | p-value (bean vs grass) |
|---|---|---|---|---|---|---|
| **Soil moisture (%)** | Bean | Wet | 86.01 | 2.712 | 5.535E-11 | 6.820E-04 |
| | | Dry | 51.58 | 2.175 | | |
| | Grass | Wet | 92.26 | 1.241 | | |
| | | Dry | 62.29 | 1.361 | | |
| **Diameter (mm)** | Bean | Wet | 2.998 | 0.05933 | 4.427E-01 | 2.117E-11 |
| | | Dry | 2.840 | 0.07089 | | |
| | Grass | Wet | 1.452 | 0.1483 | | |
| | | Dry | 1.472 | 0.06242 | | |
| **Plant voltage (V)** | Bean | Wet | 1949 | 48.43 | 7.877E-09 | 6.135E-06 |
| | | Dry | 1401 | 60.25 | | |
| | Grass | Wet | 2377 | 44.98 | | |
| | | Dry | 1911 | 77.02 | | |
| **Soil voltage (V)** | Bean | Wet | 1051 | 48.43 | 7.877E-09 | 6.135E-06 |
| | | Dry | 1599 | 60.25 | | |
| | Grass | Wet | 622.7 | 44.98 | | |
| | | Dry | 1089 | 77.02 | | |
| **Current (mA)** | Bean | Wet | 478.1 | 22.34 | 5.523E-06 | 1.620E-03 |
| | | Dry | 191.5 | 8.948 | | |
| | Grass | Wet | 299.5 | 26.52 | | |
| | | Dry | 184.2 | 9.103 | | |
| **Plant resistance (Ω)** | Bean | Wet | 4118 | 158.8 | 1.095E-03 | 2.267E-10 |
| | | Dry | 7370 | 293.0 | | |
| | Grass | Wet | 8665 | 1217 | | |
| | | Dry | 10430 | 266.2 | | |
| **Soil resistance (Ω)** | Bean | Wet | 2250 | 170.1 | 3.524E-07 | 7.370E-02 |
| | | Dry | 8554 | 667.9 | | |
| | Grass | Wet | 2211 | 279 | | |
| | | Dry | 6151 | 739.2 | | |

The results indicate that the different treatment had significance at 0.05 for the t-test in all except the 'dry vs wet' test for diameter and the 'bean vs grass' test for soil resistance. Not having a significant difference in the diameter of plants in the 'dry' and 'wet' treatments indicates that the updated method of achieving the difference in soil moisture – drying both treatments then watering one – was successful in avoiding a significant change in plant size for the different treatments.

## 8.2 SOIL MOISTURE EFFECT ON RESISTANCE

As with the first validation experiment, the final experiment demonstrated a significant difference between the resistance for the root-soil system in ‘dry’ soil with around 50% soil moisture versus ‘wet’ soil with around 100% soil moisture. The results for the average root-soil resistance in each bag versus the soil moisture percentage are shown in Figure 23.

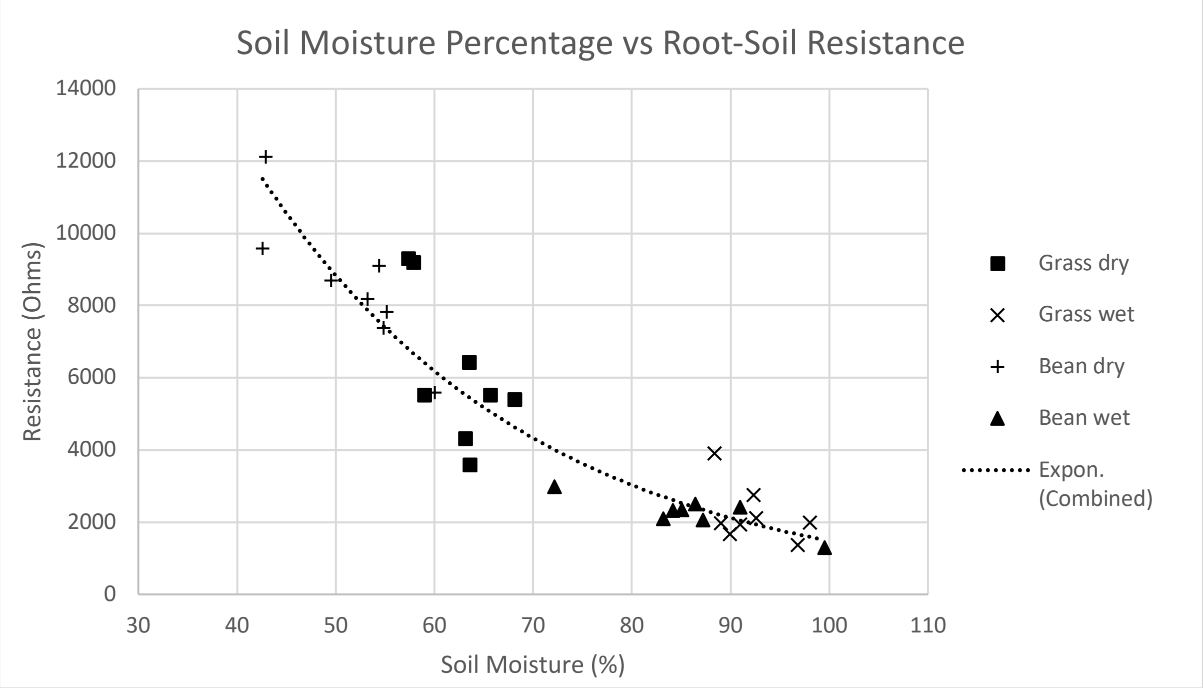


FIGURE 23 SOIL MOISTURE PERCENTAGE VS ROOT-SOIL RESISTANCE

The results of the two-tail t-test show that the ‘dry’ soil has a greater resistance with a significant p-value. As can be seen in Figure 23, the moisture of bags with beans grown appears to be lower than the bags with grass plants with the same watering regime. The two-tail t-test for the significance of the difference between the grass and bean bags gives a p-value of 0.002478 for the ‘dry’ bags and a p-value of 0.09010 for the ‘wet’ bags. The ‘dry’ bags are therefore significant, while the ‘wet’ bags are not. As the plants grew, the bean plants grew large flat leaves with a much higher surface area that the ryegrass plants at the same age. Plant transpiration causes water to evaporate from the leaves and is dependent on surface area, so it is logical that the bean plants would have evaporated more moisture than the grass plants that have had the same amount of watering. This effect was observed to have a significant effect on root-soil resistance for the ‘dry’ bags.

An interesting side-effect on the bean plants having a slightly lower soil moisture than the grass plants is that there is a more continuous range of soil moistures in the experiment. In Figure 23, an exponential line has been fitted to the data to plot the trend that occurs in the data. An R-squared for this line of 0.8964 indicates a good fit to the data. Although more data would be required to verify the equation that governs this relationship, a relationship between the percentage moisture in soil and the resistance in the root-soil system allows prediction of the best voltage waveform application based on how much voltage and energy would be expected to be lost in the soil. The high R-squared for this line also suggests that other factors – such as the different root systems for ryegrass and beans – may not have a large impact on the overall root-soil resistance. If this was confirmed in future experiments it would allow a simpler characterisation of the electrical parameters within the root-soil system, and help the prediction of how much voltage and energy would be lost in the soil for a planned treatment.

## 8.3 MEASURING DIAMETER

The diameter of all plants in the final experiment was measured, with calipers placed halfway between the electrode and the voltage measurement point as described in Section 6.5. This was successful for the bean plants where the stem is nearly cylindrical and is solid enough to allow the callipers to be attached with reasonable force but without flattening the tissue. However, for the ryegrass plants the operator noticed that the sheath-like nature of the plant stem, the fact that many plants had developed tillers (separate shoots) and the soft shoot tissue made it very difficult to get reliable stem diameter measurements. Although the relative difference between diameters will give an indication of relative plant size, where there were multiple shoots there was no guarantee that the current would travel through each evenly, so grass plants were not considered for the analysis in Section 8.4 and the error needs to be considered in the comparison plots in Section 8.5.

## 8.4 AREA AND RESISTANCE FOR BEANS

One of the tests for the method is how the results compare to theoretical physical relationships. One of the relationships that the data can be compared to is the relationship between resistivity and cross-sectional area for a conductor that is defined by Equation 32:

$$R = \rho \frac{L}{A} \tag{32}$$

Where R is the resistance, ρ is the resistivity in Ωmm, L is the length of the conductor and A is the cross sectional area. As mentioned in Section 8.3, the diameter of the grass plants did not seem to be reliable, particularly when analysing the current or resistance through this region, so this testing is completed for all of the bean plants in the experiment. In Equation 32 above, the length of the stem is constant at 20 mm and the resistivity would be expected to be dependent on the extracellular water quantity and ionic concentration based on theory outlined in Section 3.3.2. An inversely proportional relationship would therefore be expected between the cross-sectional area and resistance for plants grown in the same conditions. The graph of the data is shown in Figure 24. The cross-sectional area is calculated based on the stem being a cylinder with constant diameter and the length of 20 mm. For each of the trials the resistivity can be calculated based on Equation 32. This should be consistent for each of the sets of ‘dry’ and ‘wet’ trials and should be different between the two sets based on the underlying theory. The individual bean plant trials are shown in Figure 24, as the data from the individual plants appears to show this type of relationship. To statistically prove the resistivity relationship a further trial would be needed.

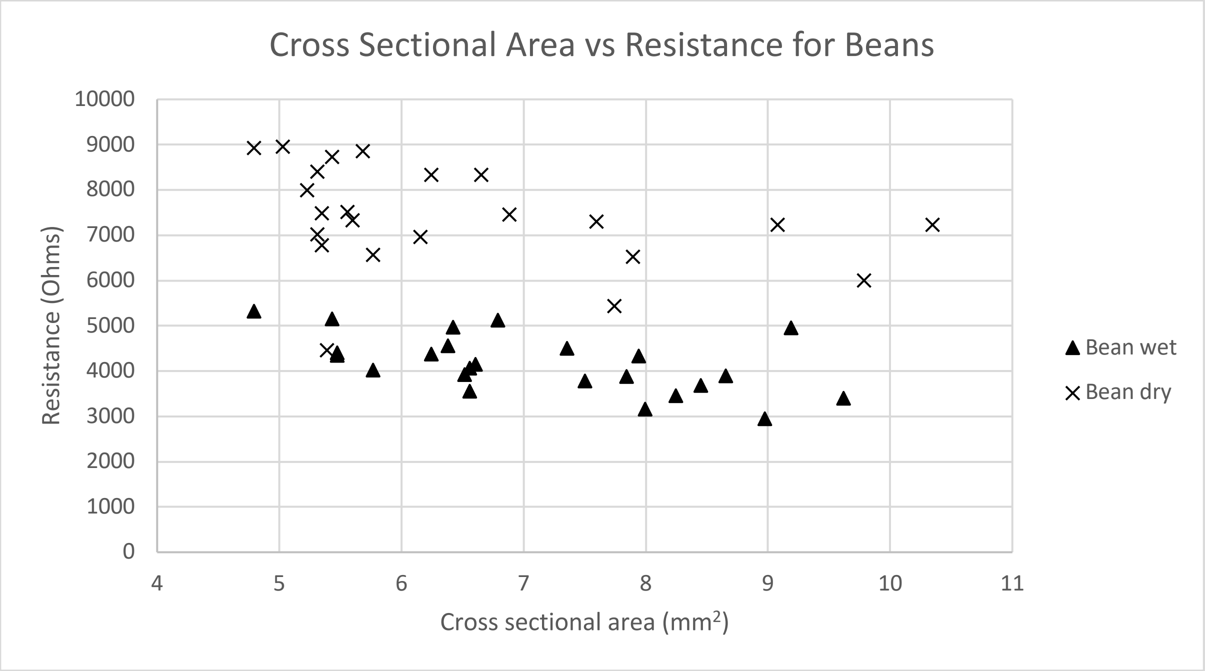


FIGURE 24 CROSS-SECTIONAL AREA VS RESISTANCE FOR BEANS

The p-value for the two-tail T-test of wet and dry bean trials is shown in Table 5 and is significant. Interpreting the resistivity is helped by considering the relationship shown in Equation 32, where resistivity defines the relationship between the resistance and the length divided by the cross-sectional area. The higher resistivity for the dry plant stem indicates that a region with particular dimensions will have a higher resistance. Based on the theory outlined in Section 3.3.2, this indicates an increase in the extracellular fluid resistance (the fluid between the cells). It has been established that plants with lower access to water will be more stressed, the quantity of extracellular fluid will reduce, and the resistance of tissue will increase [46]. The fact that the method could prove a relationship aligned with the existing theory helps to prove the overall validity. These results also have a potential value in electric weeding research as it shows that the soil moisture doesn't just impact the resistance of the root-soil system but also the plant itself, and that this along with the type and size of the plant will impact the resistance of the plant stem.

## 8.5 ELECTRICAL PARAMETER CALCULATIONS

A range of electrical parameters have been calculated and compared for the four different treatments, as shown in Figure 25. As can be observed, there are clear differences in the values for each treatment for each of the different parameters. For example, the energy loss in the plant is clearly dependent on the moisture of the soil as shown in Figure 25b. This would be influenced by the resistance of the plant changing with soil moisture as explained in Section 8.4, but also the increased resistance of the soil for 'dry' trials reducing the proportion of the voltage, and therefore energy, that is dispersed in the plant. This effect can also be observed in Figure 25c, where the electric field strength across the plant stem appears to be dependent on both the plant type and the soil moisture.

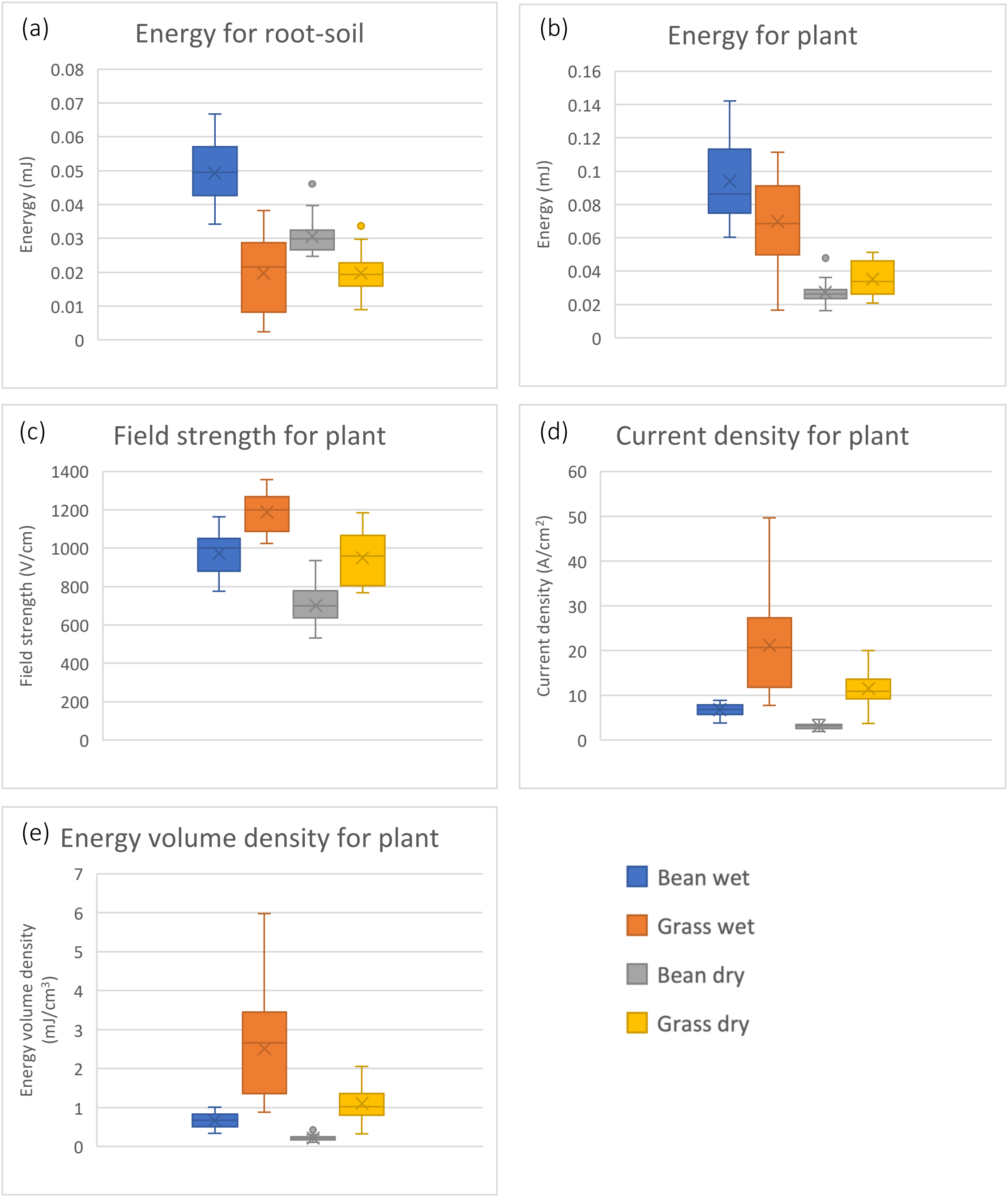


FIGURE 25 ELECTRICAL PARAMETERS BY TREATMENT

For the two parameters that rely on measurement of the diameter – current density for plant Figure 25d and energy volume density for plant Figure 25e – the values for the grass plants seem to have an extremely different range to the bean plants. Although some difference between the types of plants would be expected, the extreme difference indicates the measurement issues discussed in Section 8.3 may be introducing an error. A potential solution to this problem is discussed in Section 8.6.

## 8.6 SOLUTIONS TO GRASS DIAMETER MEASUREMENT PROBLEM

One of the main weaknesses identified with the method is the difficulty to measure the dimensions of grass stems. This means the derivations of stem electrical parameters are not reliable. There are alternative options for measuring this value, with a potential alternative being image analysis. If images of a plant stem were captured from multiple angles with a reference of known size placed next to the stem, it would be possible to estimate the dimensions of the stem. The reliability of this approach could be limited by occlusion due to leaves or from the complexity of the shape of some plants, such as ryegrass. If there were multiple tillers, as in this work, the size of the tillers could be derived but some additional observation and image processing could be required to find which parts of the stem were in contact with the electrode at the point of electrical discharge. Observing arcing using a high-speed camera or using thermal imaging to observe the path of current could be additional options to solve this problem.

## 8.7 MEASUREMENT OF ROOT-SOIL SYSTEM

Although the method can allow derivation of parameters such as electric field strength, current density and energy volume density for the plant stem, it is limited in the extent of analysis that can be conducted on the root-soil system. This is because there are a number of complex structures within this part of the whole system that have complex and potentially confounding electrical characteristics. The shape, size and surface area of the roots all impact the impedance of this root tissue as well as the electrical connection with the surrounding soil. Current will pass through any channel that is available and will pass from the roots to the soil at any point where there is physical contact. The resistance of a channel from the roots to soil will be affected by a number of factors including the moisture and ionic content in the local area.

Once current has passed into the main soil structure, the impedance of the soil also has complex characteristics. As explained in Section 3.5, soluble salt and clay content, mineralogy, water content, density, organic matter and temperature all have an impact on conductivity. In addition, ionisation and breakdown of the small air gaps within soil would reduce resistance. This means that the impedance of soil partly depends on voltage, and at higher voltage it may have reduced impedance. The combined complexity of the roots, the region of contact between the roots and soil, and the soil itself makes it difficult to draw more localised conclusions based on measurements made on the whole system using this method.

## 8.8 CONTACT BETWEEN ELECTRODE AND PLANT

The calculations in Section 8.5 rely on an assumption that along with a negligible surface resistance at the measurement point, there is also a negligible resistance between the high voltage electrode and the more conductive tissues within the plant stem. As opposed to the high-impedance measurement probe where there is very little current through the surface tissue, the point of contact between the high voltage electrode and the plant involves all the current that is measured through the system. This, along with the high initial voltage difference between the electrode and plant at the very start of the pulse, causes localised ionisation and heating and irreversibly changes the structure of the tissue at the point of contact. Heating and ionisation of ionic solutions are both known to reduce resistance [40], [47]. Therefore, the assumption that the electrode has a low impedance path to the central tissues of the plant stem was made.

## 8.9 MULTIPLE POINTS OF PLANT CONTACT WITH SOIL

The presented method has been designed for early stage ryegrass where there is typically only one point of contact with soil at the base of the stem. However, for larger ryegrass plants or many other types of plants such as broadleaf dicot plants that grow flat on the soil surface, there can be multiple points of contact with the soil through leaves. In this case, a proportion of the current would flow through these separate paths rather than the main stem, producing an error in the current measurement. There should be a negligible effect on voltage measurement as these are parallel paths, but resistance and the other calculated parameters that rely on current would be affected. The proportion of current in each path is defined by the current divider formula for two parallel conductive paths:

$$I_x = \frac{R_T}{R_x + R_T} I_T \tag{33}$$

Where $I_x$ is the current through each path, $R_x$ is the resistance of the path, $R_T$ is the total combined resistance and $I_T$ is the total combined current. The error therefore depends on the impedance of the alternative paths for current, which would be significantly influenced by the impedance of the contact point between the leaves and the soil. A waxy leaf surface and dry conditions would increase impedance at this point and reduce the error.

## 8.10 FUTURE USES OF METHOD

### *8.10.1 ASSOCIATION OF PARAMETERS WITH PLANT SURVIVAL*

The proposed method is designed to allow future development of a precision electric weeding approach that meets the requirements of modern agriculture. It is intended to provide additional information during experiments that can be used for derivation of electrical parameters and association of these parameters with plant survival. For example, the method may observe the distribution of voltage, current, or energy between the plant above the surface and the root-soil system and associate the values read with the soil type or moisture. Relationships could then be derived between soil type/moisture, the electrical parameters and the degree of damage to the plant. An example would be using the calculations of electric field strength for each plant and the energy volume density from each plant and comparing the values of each of these with plant survival. A more obvious threshold for electric field strength causing death of a particular plant – rather than energy volume density – would indicate that electroporation was having more of an impact than thermal effects, based on the theory in Section 3.4.

### *8.10.2 OPTIMISATION FOR TIME AND ENERGY EFFICIENCY*

One of the objectives for the development of a successful electric weeding system for agriculture is to maximise time and energy efficiency. The reduction in the time taken per plant or per hectare of crop is crucial to the labour cost for a farmer, while energy savings impact economics as well as compatibility with low-energy robotics systems that may be available in the future. To achieve this, the method allows optimisation of the electrical pulse shape and time based on the association with plant survival described in Section 8.10.1. An electric weeding system could then be designed to only apply the minimum energy required to kill weeds, and to achieve this in the minimum time possible.

### *8.10.3 DEFINING WHEN FLASHOVER OCCURS*

As well as optimising the voltage waveform applied to a plant, it is important to consider how surface flashover – as discussed in Section 3.1.3 – could affect what actually treats the plant. If arcing occurs across the surface of a plant to soil, it becomes a much lower resistance path than through the plant itself, due to ionisation of the air. This means the voltage, current and energy that the plant is exposed to is reduced and the effectiveness of the system would potentially be reduced, while the energy output is increased for the duration of arcing. The method proposed in this work allows observation of when flashover occurs – which is visibly and audibly clear to the operator – and also allows measurement of the electrical parameters in the plant and root-soil during this period. It could be improved by utilising image analysis to more accurately measure the location and intensity of any flashover events. Reducing the chance of flashover occurring and considering its effects is helpful to optimisation of the energy and speed of an electric weeding system, as outlined in Section 8.10.2 above.

# 9 CONCLUSION

The project achieved the aims of developing a practical and reliable method for measuring electrical parameters during an electric weeding discharge, and proving the method in an experiment. The measurements of electrical phenomena that can be made using the method will allow better understanding of the mechanism of plant death, and how an electrical treatment can be adjusted for different plants and conditions. This will allow development of improved electrical weeding systems that can be a useful non-herbicide weeding option for agriculture. The main flaw with the method was the difficulty in measuring the diameter of ryegrass, which would likely be an issue for some other types of weeds. Also, for flat weeds where there is no stem to measure, diameter measurement would not be possible. Either an advanced system for measuring the dimensions of the plant could be developed, or in these cases the method may only be able to measure the division of voltage and energy between the plant above the surface and the root-soil system. For plants that don't encounter these issues, such as many dicotyledon type weeds, the method allows calculation of the electric field strength, current density, and energy volume density within plant tissue. The final experiment indicated that these calculations were consistent and correlated with the underlying theory.

The main area for improvement in future work is the ability to accurately measure the dimensions of a wider range of plants, including grasses and flat dicot plants, where the shape or rigidity of the plant makes mechanical measurement impractical. If this were to be incorporated into a multiple-camera imaging system – which could incorporate observation of a current path and/or flashover effects – there would be a sacrifice to the practicality and cost of the system. Compared to the method proposed in this work – which is simple and practical enough to allow field trials – an imaging system could require artificial light and a more controlled environment to achieve accurate measurement, so field trials would be difficult. Other areas of future work include extending the method to measure a wider range of dicot and monocot plants, finding a safe way to make microscopic observations, and to incorporate field trials to measure electrical parameters in real conditions for electric weeding.

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