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Microwave Soil Heating Promotes Strawberry Runner Production and Progeny Performance

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Abstract: Strawberry runners (transplants) in many regions of the world are produced in soils treated with chemical fumigants to control pathogens and weeds and meet phytosanitary requirements. Many fumigants, however, are under threat of withdrawal because of concerns over their impact on the environment (e.g., methyl bromide). The current study considered the use of microwaves for heat disinfestation of soil for field-grown runners for the first time. Results from two field experiments showed that microwave treatment reduced the survival of buried inoculum of the strawberry pathogens *Fusarium oxysporum* (by up to 93%) and *Sclerotium rolfsii* (by up to 100%) compared with untreated soil. Furthermore, the treatment reduced the subsequent growth of these pathogens in the laboratory by up to 82% and 100%, respectively. Microwave treatment also reduced the natural DNA concentration of *Pythium* spp. (clades I & F) in soil by up to 94% compared with untreated soil. The effect of microwave against soilborne pathogens reduced as soil depth increased. Microwave treatment reduced the emergence of weeds in field soils by up to 65% and increased runner yields by 10–37%. The effect of microwave treatment on runner yield was greater when all soil was treated, rather than when strips of soil around the mother plants were treated. Results from complimentary pot experiments showed that early strawberry growth in the glasshouse was equivalent in soils treated with microwave or the fumigant methyl bromide/chloropicrin. Furthermore, the early performance of runners sourced from field soils treated with microwave or methyl bromide/chloropicrin was equivalent. Results from the pot experiments also showed that steam treatment required 10 times more energy per mass of soil to disinfest than microwave. The limitations of microwave in the current experiments are discussed, but the capacity for the technology to disinfest field soils in an energy-efficient manner demonstrates its potential for further development as an alternative to soil disinfestation with chemical fumigants.

Keywords: soil disinfestation; steam; methyl bromide; chloropicrin; *Fragaria* × *ananassa*

Citation: Brodie, G.I.; McFarlane, D.J.; Khan, M.J.; Phung, V.B.G.; Mattner, S.W. Microwave Soil Heating Promotes Strawberry Runner Production and Progeny Performance. *Energies* **2022**, *15*, 3508. https://doi.org/10.3390/en15103508

Academic Editor: Attilio Converti

Received: 1 April 2022
Accepted: 3 May 2022
Published: 11 May 2022

1. Introduction

Strawberry (*Fragaria* × *ananassa*) nurseries produce runners (transplants) under phytosanitary controls or 'certification' that often mandate the use of soil disinfestation with chemical fumigants [1]. In the past, mixtures of methyl bromide and chloropicrin were the most common and effective fumigants for disinfesting soils against pathogens, weeds, and pests, and ensuring the high health of strawberry runners [2,3]. For example, Wilhelm and Paulus [4] showed that soil fumigation with mixtures of methyl bromide and chloropicrin controlled fungi that cause disease in strawberry, especially Verticillium Wilt. Other benefits of soil fumigation include increased growth response, reduction in N fertilizer requirements (by approximately 50% in some cases), improved nutrient uptake, and control of weed infestation [4]. However, methyl bromide is implicated in the degradation

of stratospheric ozone and non-quarantine uses in soils are being phased out under the 1992 Montreal Protocol [5]. There are some exceptions to this phase out due to quarantine and pre-shipment requirements and critical use exemptions. In Australia, few alternative fumigants have disinfested soil to equivalent levels as methyl bromide/chloropicrin in strawberry nurseries [3,6]. Therefore, the strawberry industry is seeking alternative technologies to achieve soil disinfestation without methyl bromide or other fumigants. This paper examines microwave heating for soil disinfestation for the first time in strawberry production. Therefore, the theoretical background to soil disinfestation with heat and microwave is necessary to establish the principals of the development of this technology.

The combined effect of heat and time on biological organisms has been studied for over a century. Lepeschkin [7] presented an empirical formula relating temperature and holding time on the mortality of plant materials, while other authors have developed similar responses in pathogenic fungi [8], bacteria [9], and nematodes [10]. For example, Noling [10] demonstrated that holding time and lethal temperature for southern root knot nematode (*Meloidogyne incognita*) is described by:

$$\text{Time} = 10^{(8.05225 - 0.14325 \cdot T_c)}$$

where Tc is the soil temperature (°C), and Time is the required holding time (minutes). Models consistently showed that higher temperatures require shorter holding times to achieve high mortality rates. Figure 1 presents a summary of some of these relationships.

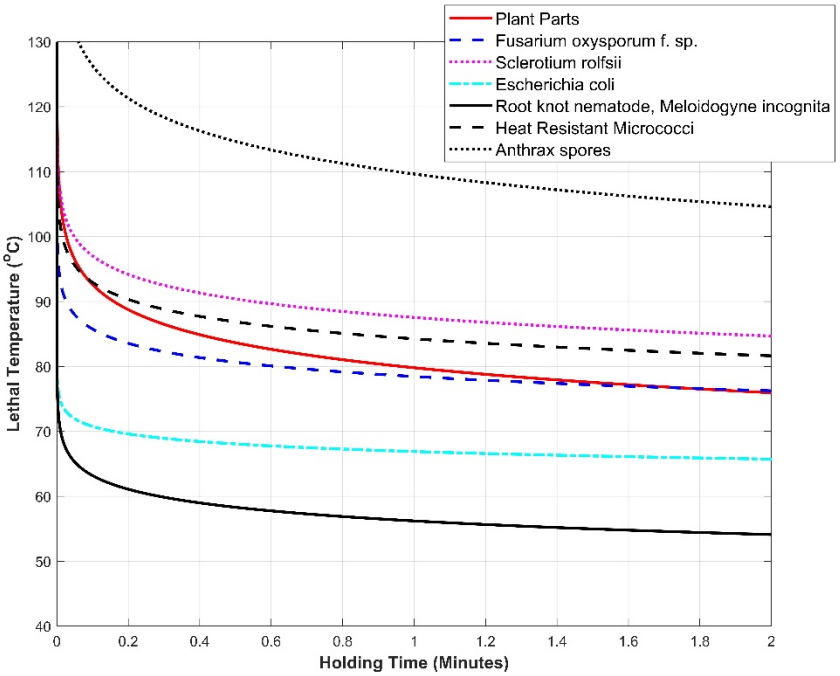

Figure 1. Lethal temperature as a function of holding time for several test species, based on empirical relationships developed in multiple studies [7–10].

Soil can be heated in several ways including solarization, steam, and microwave energy. Solarization utilizes radiant energy from the sun to heat soil. Soil solarization is initiated by covering the soil with clear or dark polyethylene film for four to six weeks, which increases soil temperatures well above ambient levels [11]. Solarization does not heat soil to high temperatures (e.g., 30–55 °C [12]) compared to other methods (i.e., steam and microwave) and therefore requires a relatively long time to disinfest soil. Data presented by Samtani, Gilbert, Ben Weber, Subbarao, Goodhue and Fennimore [11] in strawberry showed that solarization had no impact on *Verticillium dahliae* microsclerotia and reduced weed biomass by 44–55%, which was considerably less effective than methyl bromide/chloropicrin (89–100%).

Heating with steam is the oldest method of soil disinfestation, dating back to 1893, and is well accepted for its ability to kill soil pestsJohnson [13]. One advantage of steam treatment of soil is that it can be injected into the soil through hollow tines [14]. This heats soils to high and consistent temperatures and reduces the treatment time needed to disinfest soil compared with solarization. Steam treatment of soil is often used in the Netherlands for soil disinfestation in protected horticulture [15]. In strawberry, several studies have shown that steam treatment can be as equally effective as methyl bromide/chloropicrin and other fumigants for controlling weeds and stimulating strawberry fruit yields [11,16–18]. However, steam did not control the strawberry pathogen *V. dahliae* as effectively as methyl bromide/chloropicrin, or other fumigants, particularly at greater soil depths [11,19].

The effects of microwave energy on living systems occur at atomic, molecular, cellular, and subcellular level [20]. Microwaves are non-ionizing electromagnetic waves with a frequency of about 300 MHz to 300 GHz [21]. Biological and agricultural systems are a mixture of organic and dipole molecules [20] (i.e., H_2O) arranged in different geometries [22,23]. Electromagnetic heating has major advantages over conventional heating techniques including, rapid volumetric heating, precise control, rapid start up and shut down [24], and in the case of soil, having a lighter apparatus than a steam generator can avoid soil compaction issues.

Many of the early experiments that explored the effect of electromagnetic heating in plant material focused on the effect of radio frequencies [25] on seeds. In many cases, exposure to low energy densities resulted in increased germination and vigor of the emerging seedlings [26,27]; however, exposure to higher energy densities usually resulted in seed death [28–30]. Davis et al. [31,32] were among the first to study the lethal effects of microwave heating on weed seeds. They showed that seed damage was mostly influenced by a combination of seed moisture content and the energy absorbed in each seed.

Ferriss [33] conducted the first experiments on soil samples with moisture contents between seven and 37 percent (wet/dry weight). He showed that microwave treatment for 150 s eradicated populations of *Pythium*, *Fusarium*, and all nematode species, except *Heterodera glycines* in the soil samples. Compared with autoclaving or methyl bromide/chloropicrin (98:2) fumigation, Ferriss found that microwave treatments resulted in less nutrient release into the soil but had less effect on beneficial soil prokaryotes. The study found that microwave soil treatment resulted in less recolonization of the soil by *Fusarium* spp. and other fungi after treatment.

Khan, et al. [34] examined the impact of a single microwave heating event on the total bacteria and ammonia oxidizer abundance and their recovery over time using 16S rRNA amplicon sequencing and qPCR for the first time. They found no effect at low heating intensities (17–45 °C) and strong effects at high heating intensities (65–78 °C). Immediately after high heating treatments with microwave, the abundance of Firmicutes increased and that of Proteobacteria decreased significantly. The relative abundances of beneficial soil microbes (Micromonosporaceae, *Kaistobacter*, and *Bacillus*) were significantly higher in soils recovered from high heating intensities compared with untreated soils.

Gibson, et al. [35] demonstrated that shoot and root growth of birch (*Betula pendula*) significantly increased in microwave heated soil compared with plants grown in untreated soil. Their experiment evaluated the effect of microwave treatment of soil supplemented with two mycorrhizas on birch seedlings. Shoot growth increased with irradiation duration, with the highest dry shoot weight coinciding with the highest irradiation duration compared with non-irradiated soil. Other plant species such as *Medicago truncatula* have also showed increased biomass responses when grown in soil treated with microwave energy [36].

Microwave soil heating has not been used either in the strawberry runner or strawberry fruit production industries for soil disinfestation. The objectives of this study were to determine whether microwave soil treatment could reduce pathogen load, increase strawberry runner production, and improve the ongoing production performance of runners

grown in disinfested soils. The pot experiments in the study compared soil disinfestation with microwaves to steam and fumigation with methyl bromide:chloropicrin (50:50).

2. Materials and Methods

A series of sequential experiments were conducted to assess the impact of microwave soil treatment on soilborne pathogens, weeds, strawberry runner production, and the subsequent fruit productivity of runners. To determine whether microwave soil heating provided other benefits beyond thermal pathogen control, some of the pot experiments included steam treated soil as an example of an alternative soil heating technology.

2.1. Statistical Analyses

Data from the experiments were subjected to analyses of variance, according to the experimental designs imposed on each experiment. Least significant differences were calculated and used to determine differences between treatment means.

2.2. Experiments 1 & 2—Field Experiments

Two field experiments were conducted on a commercial strawberry runner farm at Toolangi, Victoria, Australia (37°32′05.0″ S, 145°27′27.7″ E). The soil type at the site was a red ferrosol with a clay texture. The site for the trial was prepared by rotary hoeing the soil to a depth of 20 cm. In Experiment 1 (2016/17), microwave soil heating was applied in narrow rows (200 cm long by 5 cm wide) between 19 and 21 December 2016. Treatments were imposed using a prototype microwave trailer with four 2-kW fixed output microwave generators, operating at 2.45 GHz, and projecting microwave energy to the ground through a WR340 wave guide and a horn antenna with an aperture of 11 cm by 5.5 cm (Figure 2). In Experiment 2 (2019/20), all soil in the plots (2 m × 2 m) was treated with microwave (see above) on 4 November 2019.

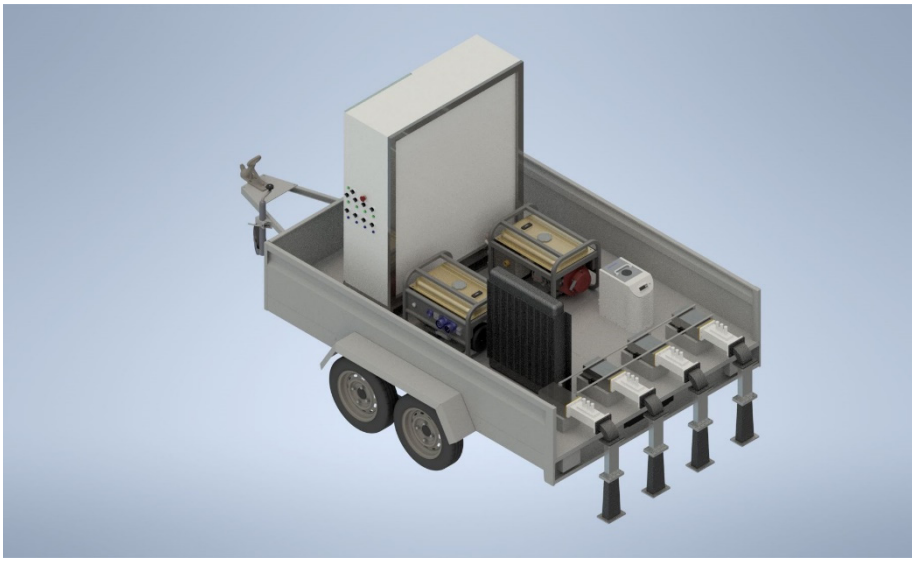

Figure 2. Rendering of experimental microwave trailer with four 2-kW microwave generators on board.

Microwave treatment was applied for 60 s to each section of soil to achieve a surface temperature above 80 °C. Measurements of soil temperature at a depth of 5 cm confirmed that the temperature at this depth was consistently at or above 70 °C after treatment. Treatments were set out in a randomized block design, with two treatments (control and microwave treated) and four or five blocks (replicates) in Experiments 1 and 2, respectively.

The fungal pathogen of strawberry, *Fusarium oxysporum*, was grown on sterile barley grain in the laboratory and infested grains (c. 30) were packed into muslin bags with

cords attached for easier location and retrieval from the soil. Similarly, sclerotia (c. 30) of the fungal pathogen of strawberry, *Sclerotium rolfsii*, were harvested from plates of potato dextrose agar (PDA) grown in the laboratory and packed into separate muslin bags. Pathogen bags were buried in the soil along the treatment rows at depths of 2.5 cm, 5 cm, and 10 cm, prior to microwave treatment. The pathogen bags were retrieved from the soil, one day after treatment. Grains and sclerotia within the bags were removed and surface sterilized (1% NaOCl, 2 min). Five surface sterilized grains or sclerotia were plated onto PDA and incubated at 22 °C; there were five replicate plates per bag (plot) (i.e., 25 grains/sclerotia per plot in total). After five days of incubation, the viability of *F. oxypsorum* and *S. rolfsii* were assessed, based on the percentage of fungal growth. The radial growth of one colony of *F. oxysporum* and *S. rolfsii* from the propagule in each plate was measured with a caliper (mm) to determine fungal growth rate.

A single row of bare-rooted strawberry transplants (cv. Monterey, 50 cm apart, i.e., five plants per plot) was planted through the trial site on 28 December 2016 (Experiment 1) and 11 November 2019 (Experiment 2). Plants were regularly watered with overhead irrigation using the growers' standard schedule. Runners were treated with foliar fungicides and insect predators (including prochloraz for leaf blotch, azoxystrobin + difenoconazole for leaf blotch and powdery mildew, cyflufenamid for powdery mildew, and *Persimilis* predators for two-spotted mite) as required. Daughter plants were pinned into the soil at monthly intervals and all runner plants were dug and harvested at the end of each experiment. No fertilizers were applied to the soil in the experiments, so that the effect of the direct effect of microwave treatment on strawberry yield were clearly apparent.

At planting and harvest, five soil samples were taken with a trowel from each plot from 0–2.5 cm, 2.5–5 cm, and 5–10 cm, and 500 g subsamples were submitted for analysis to the South Australian Research and Development Institute (SARDI). DNA was extracted from the soil subsample, and quantitative polymerase chain reaction for *Pythium* spp. clades I and F [37] performed using the general procedures described by Ophel-Keller, et al. [38]. The efficiency and consistency of SARDI's method has been confirmed in comparison with commercial extraction kits [39]. *Pythium* spp. clades I and F contain species that are pathogenic to strawberry and were detected at moderate concentrations in soil at the site prior to the experiments.

The emergence of weeds was measured on 16 January, 17 March, and 16 May 2017 in Experiment 1, and 8 January and 29 March 2020 in Experiment 2. In this procedure, the identity and number of weeds in four random quadrats (0.25 m^2) per plot were recorded. Results were expressed as total weeds m^{-2}. In Experiment 2, the dry weights of weeds (dried at 80 °C for four days) were also determined and recorded from one quadrat per plot. Results were expressed in grams m^{-2}.

Runners (rooted nodes on stolons from the mother plant) were harvested on the 16 May 2017 in Experiment 1 and 1 June 2020 in Experiment 2. Three replicate sections from a row (0.5 m width) were dug out per plot and the number of commercial-grade runners (crown diameter > 5mm and well-developed roots) were counted. Ten harvested runners were randomly selected from each plot and the diameter of the crown was measured on each individual runner (mm). Roots of the same ten runners were washed and rated for the severity of black roots using a modified score described by Wing et al. (0 = 100% white, healthy roots to 5 = 100% black roots) [40]. Fungal isolations were made from a selection of black lesions on roots in the laboratory using cultural methods.

2.3. Experiment 3—Pot Experiment to Assess Impacts of Different Soil Treatments

Two volumes of topsoil were taken from the same site on a strawberry runner farm at Toolangi where the field experiments were conducted (see above) during August 2019. One of the soil volumes was taken from a section of the site that had its soil fumigated with methyl bromide (methyl bromide/chloropicrin 50:50, 500 kg/ha) by a commercial contractor (R&R Fumigation Services, Bayswater, Australia). The other volume of soil was

taken from a section of the site that had not been fumigated for three years and could be regarded as untreated.

A 140 kg portion of the unfumigated soil was treated in a steam disinfestation system at the University of Melbourne, which had a nameplate electrical rating of 28.8 kW, for 90 min, until the soil reached a uniform 90 °C. Therefore, this steam treatment required 1.1 MJ kg^{-1} of soil. All the soils (fumigated, steamed, and untreated) were placed into forty 10 cm width pots.

Ten of the untreated pots were heated in a microwave oven (rated at 900 W of microwave power) with a nameplate electrical power rating of 2.25 kW. Each pot of soil weighed 2.9 kg and each pot required 150 s of heating to achieve 90 °C, based on earlier crop responses to microwave soil heating [41]. Therefore, this microwave treatment required 0.12 MJ kg^{-1} of soil. The experiment consisted of four treatments (untreated control, methyl bromide fumigated soil, steam treated soil, and microwave treated soil) with 10 replications. Each pot had a single, bare-rooted strawberry runner transplanted into it (cv. Albion) and the pots were placed in growth cabinets at Dookie, Victoria, Australia (36°23′ S; 145°43′ E), which were programmed to provide good conditions for strawberry growth (temperature: 20 °C to 29 °C; 14-h photoperiod; soil maintained at field capacity). The experiment was established on 23 August 2019.

Plants were monitored for stolon growth (vegetative reproductive structures), daughter plants, fruit number, and fruit fresh weight until 29 February 2020. It is important to note that fruit production was not expected to be optimal under the experimental period (e.g., short time period, absence of pollinators); therefore, the recorded fruit production data could not be used to infer commercial production outputs.

2.4. Experiment 4—On-Growing of Runners from the Second Field Experiment

A further pot trial was established to explore the productivity of runners grown in the microwave treated and control soils from the second field trial. A third source of runner plants, from soil that was treated with methyl bromide/chloropicrin (50:50, 500 kg/ha) at the Toolangi trial site, was also included in this experiment. Including the runners from fumigated soil allowed the runners, which were grown in microwave treated and untreated soils, to be compared with runners, which were grown in soil that was subjected to an industry fumigation treatment.

Approximately 500 kg of soil from the Toolangi experimental site (see above), which had not been subjected to fumigation or microwave disinfestation for four years, was collected and subjected to the following treatments: an untreated control, steam treatment in the same facility that was used in Experiment 3, microwave treatment in a 30-liter experimental chamber (Figure 3), and fumigation with methyl bromide/chloropicrin in the field, as described previously.

The experimental microwave chamber was designed and fabricated to allow for microwave treatment of moderate sized samples (up to 25-liters in volume) using industrial quality microwave generators. In this case, 3-kg samples of soil were placed in pots and individually treated using a variable power, 3-kW maximum output, solid state microwave generator, operating at 2.45 GHz. Unfumigated soil was heated using 2.5 kW of microwave power until it was over 90 °C and then allowed to cool naturally in the air, while other pots were treated.

The soil for all the pots was mixed with builders' sand, at a ratio of 2:1 by volume, prior to placing in the pots, in order to reduce the expected collapse and compaction of soil in the pots.

One hundred and twenty strawberry runners from three sources (untreated soil, microwave treated soil, and methyl bromide/chloropicrin-fumigated soil) were divided into four groups for each source. The runners were planted in the center of each pot, one runner per pot. The pots were transferred to a temperature-controlled glass house on 7 May 2021 at Dookie, with the temperature set to 20 °C. Soils in pots were maintained at field capacity with an automatic irrigation system with dripper outlets attached to each pot.

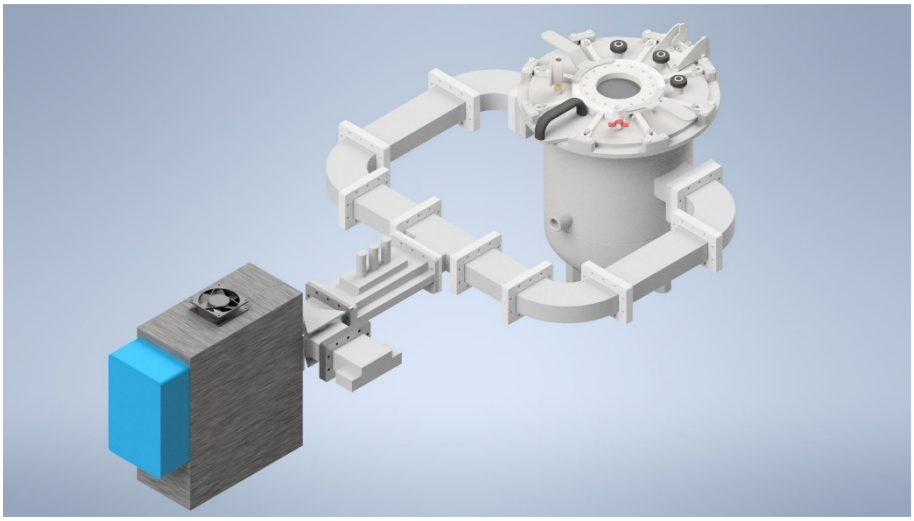

Figure 3. Experimental 30-liter microwave chamber, connected to a variable power 3-kW microwave generator.

Weeds were harvested from each pot on 28 July 2021 and again on 22 November 2021. Weeds were counted, dried in an oven at 65 °C for two days, and weighed on electronic scales with one hundredth of a gram precision. Stolons and daughter plants were harvested on 17 August 2021. The number and total length of stolons from each pot were determined and the number of daughter plants were counted. Both the stolons and daughter plants were dried in an oven at 65 °C for two days and weighed. Mature fruits were harvested throughout the experiment, counted, and weighed. The experiment was concluded earlier than planned, on 22 November, due to a lockdown associated with Severe Acute Respiratory Syndrome Coronavirus 2 or SARS-CoV-2, imposed under government mandates in the state of Victoria, Australia.

3. Results

3.1. Field Experiments

Microwave soil heating significantly reduced the viability (by up to 93% and 100%, respectively) and subsequent growth in the laboratory (by up to 82% and 100%, respectively) of buried inoculum of *F. oxysporum* and *S. rolfsii* (Tables 1 and 2). The effect of microwave heating against inoculum of buried pathogens decreased as soil depth increased.

Table 1. Viability (%) of inoculum of the strawberry pathogens *Fusarium oxysporum* and *Sclerotium rolfsii* buried at different soil depths following treatment with microwave in two field experiments.

Pathogen	Treatment	Experiment 1 (2016/17)			Experiment 2 (2019/20)		
		2.5 cm	5 cm	10 cm	2.5 cm	5 cm	10 cm
F. oxysporum	Untreated	98.0 ^{a}	100.0 ^{a}	98.0 ^{a}	100.0 ^{a}	96.7 ^{a}	100.0 ^{a}
	Microwave	7.0 ^{b}	57.0 ^{b}	87.0 ^{b}	23.3 ^{b}	56.7 ^{b}	90.0 ^{a}
	LSD (p = 0.05)		10.5			27.5	
S. rolfsii	Untreated	98.0 ^{a}	99.0 ^{a}	96.0 ^{a}	93.3 ^{a}	96.7 ^{a}	96.7 ^{a}
	Microwave	32.0 ^{b}	29.0 ^{b}	71.0 ^{b}	0.0 ^{b}	6.7 ^{b}	70.0 ^{b}
	LSD (p = 0.05)		9.2			14.8	

Note: Means with different superscripts in each of the columns are statistically different from one another.

Table 2. Growth (colony diameter, mm) of inoculum of the strawberry pathogens *Fusarium oxysporum* and *Sclerotium rolfsii* buried at different soil depths and subsequently grown in the laboratory for five days following treatment with microwave in two field experiments.

Pathogen	Treatment	Experiment 1 (2016/17)			Experiment 2 (2019/20)		
		2.5 cm	5 cm	10 cm	2.5 cm	5 cm	10 cm
F. oxysporum	Untreated	30.12 ^{a}	32.45 ^{a}	32.07 ^{a}	29.23 ^{a}	28.54 ^{a}	28.23 ^{a}
	Microwave	5.28 ^{b}	16.54 ^{b}	22.45 ^{b}	5.27 ^{b}	9.26 ^{b}	18.46 ^{b}
	LSD (p = 0.05)		4.11			3.07	
S. rolfsii	Untreated	33.23 ^{a}	33.66 ^{a}	33.13 ^{a}	34.17 ^{a}	35.86 ^{a}	34.58 ^{a}
	Microwave	13.64 ^{b}	17.34 ^{b}	27.41 ^{b}	0.00 ^{b}	1.12 ^{b}	14.32 ^{b}
	LSD (p = 0.05)		4.82			3.91	

Note: Means with different superscripts in each of the columns are statistically different from one another.

At planting, treatment with microwave significantly reduced DNA concentrations of *Pythium* spp. in soil by up to 94% (Table 3). By harvest in Experiment 1 (2016/17), there was no significant difference in DNA concentrations of *Pythium* spp. in soil between treatments. In Experiment 2 (2019/20), however, concentrations of *Pythium* spp. were significantly lower at soil depths of 0–10 cm (by up to 56%), but not at 10–15 cm, in the microwave treatment compared with the control.

Table 3. Concentrations of DNA of *Pythium* spp. (clades I and F) in soil (Log_{10} pg DNA g^{-1} soil) at different depths following treatment with microwave in two field experiments.

Assessment Time	Treatment	Experiment 1 (2016/17)			Experiment 2 (2019/20)		
		2.5–5 cm	5–10 cm	10–15 cm	2.5–5 cm	5–10 cm	10–15 cm
Planting	Untreated	2.31 ^{a}	2.45 ^{a}	2.70 ^{a}	2.25 ^{a}	2.40 ^{a}	2.41 ^{a}
	Microwave	1.76 ^{b}	2.13 ^{a}	2.37 ^{a}	1.96 ^{b}	2.05 ^{b}	2.16 ^{b}
	LSD (p = 0.05)		0.34			0.03	
Harvest	Untreated	2.11 ^{a}	2.03 ^{a}	2.50 ^{a}	2.18 ^{a}	2.23 ^{a}	2.27 ^{b}
	Microwave	1.94 ^{a}	1.83 ^{a}	2.40 ^{a}	1.89 ^{b}	1.97 ^{b}	2.38 ^{a}
	LSD (p = 0.05)		0.37			0.16	

Note: Means with different superscripts in each of the columns are statistically different from one another.

Microwave heating significantly reduced total weed emergence and the dry weight of weeds in the field experiments compared to the control by up to 65 and 51%, respectively (Figures 4 and 5). Microwave treatment also reduced the diversity of emergent weed species.

Strawberry runner production, from mother plants that were grown in the microwave treated soil, was significantly higher by 10–37% than from the mother plants grown in the untreated soil (Table 4). There was no significant difference in the crown diameter or black root scores of harvested runners. Fungal assessment from the lesions on the plant roots, which were associated with black roots, were identified as *Pythium irregulare* and *Pythium sylvaticum.*

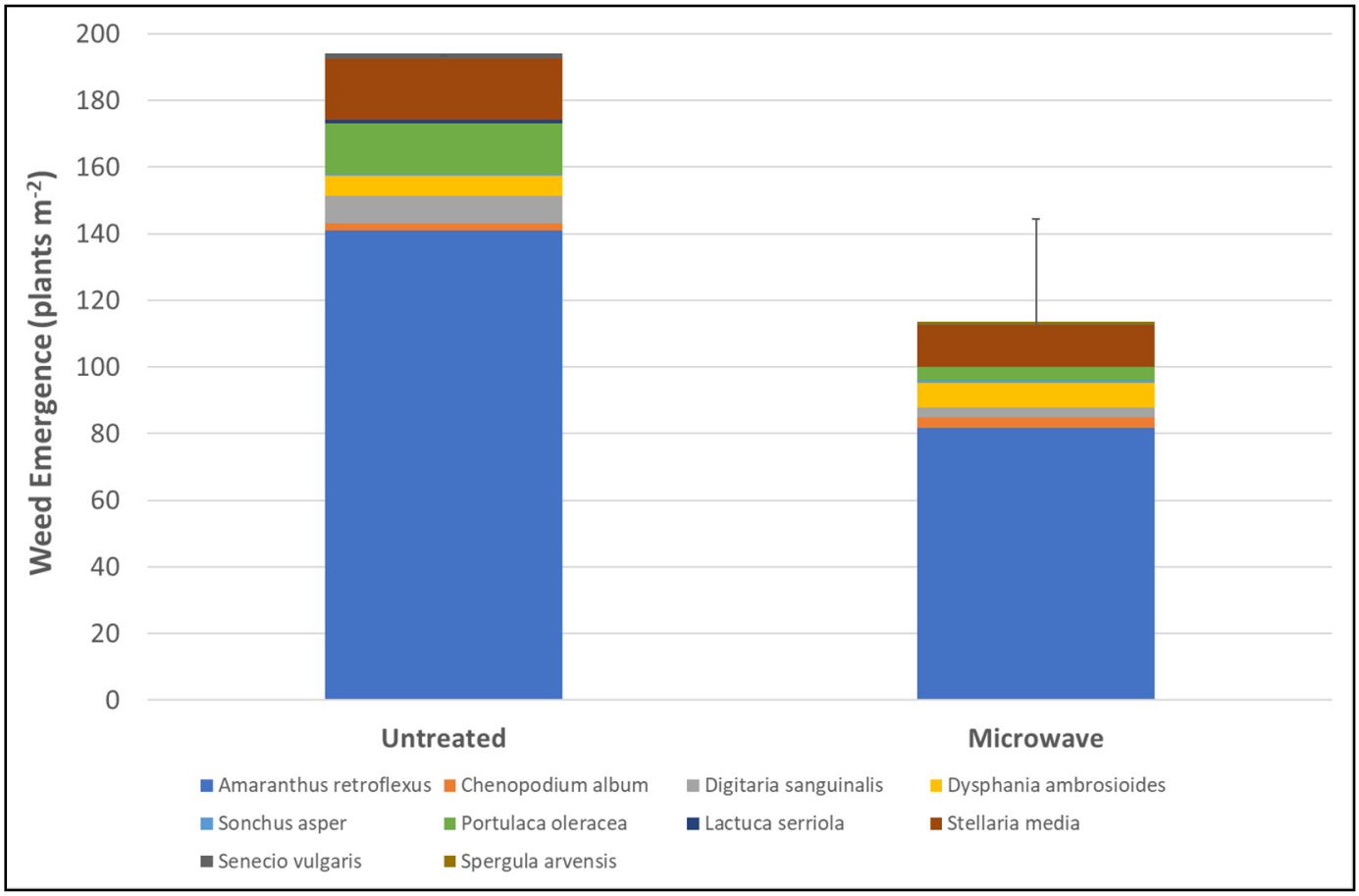

Figure 4. Weed emergence in soils treated with microwave or an untreated control in field Experiment 1 (16/17) in the strawberry runner sector. The bar is the LSD where $p = 0.05$.

Table 4. Concentrations of DNA of *Pythium* spp. (clades I and F) in soil (Log10 pg DNA g^{-1} soil) at different depths following treatment with microwave in two field experiments.

Year	Treatment	Runner Yield m^{-1} of row	Runner Crown Diameter (mm)	Black Root Score (0–5)
Experiment 1 (2016/17)	Untreated	83.5 ^{b}	10.35 ^{a}	0.72 ^{a}
	Microwave	91.9 ^{a}	11.13 ^{a}	0.45 ^{a}
	LSD ($p = 0.05$)	7.0	0.99	0.40
Experiment 2 (2019/20)	Untreated	53.8 ^{b}	8.33 ^{a}	2.03 ^{a}
	Microwave	74.0 ^{a}	7.84 ^{a}	2.34 ^{a}
	LSD ($p = 0.05$)	10.5	1.05	0.83

Note: Means with different superscripts in each of the columns are statistically different from one another.

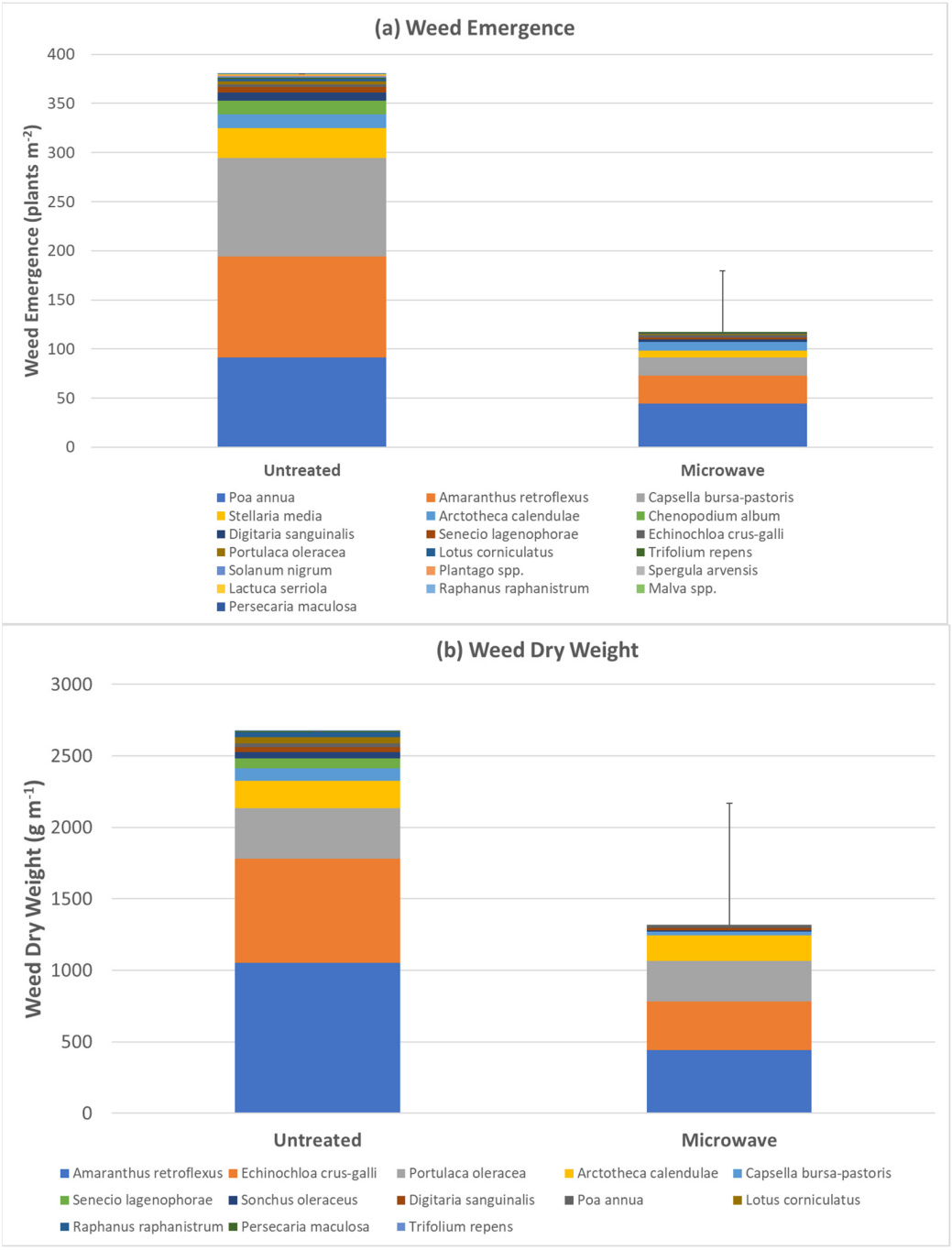

Figure 5. Weed (**a**) emergence and (**b**) total dry weight in soils treated with microwave or an untreated control in field Experiment 2 (19/20) in the strawberry runner sector. The bars are the LSDs where $p = 0.05$.

3.2. Experiment 3—Pot Experiment to Assess Impacts of Different Soil Treatments

There was considerable variability between plants within each treatment. In most production parameters, microwave soil treatment was significantly better than steam treatment and the untreated control (Table 5). In most parameters, microwave treatment was statistically similar to the fumigated treatment (Table 5). Observational data showed that several of the control pots grew weeds (Figure 6). Few weeds were evident in the other treatments.

Table 5. Strawberry reproductive and production performance per plant according to individual treatments in a pot experiment (Experiment 3).

Treatment	Parameter					
	No. of Stolons	Length of Stolons (cm)	Stolon Fresh Weight (g)	No. of Daughter Plants	No. of Fruit	Fruit Fresh Weight per Pot (g)
Control	1.2 ^{a}	48.0	7.5 ^{ab}	1.2 ^{a}	2.5 ^{a}	14.9 ^{b}
Steamed	0.7 ^{a}	36.1	4.0 ^{a}	0.9 ^{a}	1.6 ^{a}	5.8 ^{a}
Fumigated	2.2 ^{b}	82.4	17.6 ^{c}	3.0 ^{b}	2.6 ^{a}	14.6 ^{b}
Microwaved	2.1 ^{b}	43.8	10.2 ^{b}	2.3 ^{b}	5.0 ^{b}	21.5 ^{b}
LSD (p = 0.05):	0.7	35.0	5.2	1.0	1.6	7.4

Note: Means with different superscripts in each of the columns are statistically different from one another.

Figure 6. Subset of experimental pots illustrating differences between treatments, approximately six weeks after crop transplant. **Note:** The tall plant in the control pot on the left is a weed (*Amaranthus retroflexus*).

3.3. Experiment 4—On-Growing of Runners from the Second Field Experiment

Table 6 summarizes the key results for the experiment to understand the on-growing of runners from the second field experiment. Microwave heating of the soil in the pots significantly reduced the number and mass of weeds (3.7 g pot^{-1}) compared to the untreated soil (20.8 g pot^{-1}) and the steam treated soil (9.2 g pot^{-1}). There were no significant differences between the number and mass of weeds in the microwave treated soil and the fumigated soil (3.3 g pot^{-1}).

Plants that were sourced from microwave treated soil produced the longest mean stolon lengths (3432.9 mm), which were significantly longer ($p < 0.05$) than those produced by the plants sourced from untreated soil (2548.2 mm). The stolon lengths for plants sourced from the fumigated soils (3082.1 mm) were not significantly different from either the plants sourced from the untreated soils, or the plants sourced from the microwave treated soils. Plants growing in microwave treated soil produced significantly longer stolons (3673.0 mm) than plants growing in steam treated (2472.3 mm) or untreated soils (2648.1 mm). There was no significant difference in the stolon length of plants grown in the microwave treated and fumigated soil (3290.7 mm).

The number of daughter plants showed a similar outcome. Plants that were sourced from microwave treated soil produced significantly more daughter plants (6.55) compared with either the plants sourced from fumigated soils (6.0) or untreated soils (5.3). Plants that were growing in microwave treated soil (6.8) and fumigated soil (6.0) produced significantly more daughter plants than plants growing in steam treated soil (5.5) and untreated soil (5.5).

Table 6. Summary of results from a pot experiment on the on-growing of strawberry runners (Experiment 4).

Source of Mother Plants	Treatment Imposed on Pots	Parameter								
		Number of Weeds (pot^{-1})	Weight of Weeds (g pot^{-1})	Number of Stolons (pot^{-1})	Total Stolon Length (mm)	Number of Daughter Plants per Pot	Weight of Stolons and Daughter Plants (g pot^{-1})	Number of Mature Fruit (pot^{-1})	Fruit Weight (g pot^{-1})	Fruit Size (g)
Untreated Soil	Untreated Soil	11.1 ^{a}	16.5 ^{b}	2.8 ^{ab}	2638.5 ^{b}	5.0 ^{b}	10.6 ^{a}	6.6 ^{ab}	53.0 ^{b}	7.1 ^{b}
	Fumigated Soil	2.7 ^{c}	2.9 ^{bc}	2.4 ^{b}	2390.0 ^{b}	4.4 ^{b}	11.8 ^{a}	5.9 ^{b}	57.0 ^{b}	11.9 ^{a}
	Steamed Soil	4.6 ^{b}	8.7 ^{bc}	2.5 ^{b}	2330.4 ^{b}	5.0 ^{b}	10.7 ^{a}	6.9 ^{ab}	61.5 ^{ab}	8.2 ^{b}
	Microwave Heated Soil	2.7 ^{c}	3.2 ^{bc}	3.5 ^{a}	2834.0 ^{b}	6.6 ^{a}	12.4 ^{a}	4.6 ^{b}	44.1 ^{b}	8.0 ^{b}
Fumigated Soil	Untreated Soil	11.2 ^{a}	24.4 ^{a}	3.2 ^{ab}	2955.7 ^{b}	6.0 ^{ab}	8.6 ^{bc}	8.5 ^{a}	75.0 ^{a}	8.6 ^{b}
	Fumigated Soil	3.0 ^{c}	2.3 ^{c}	3.0 ^{ab}	3145.0 ^{b}	6.6 ^{a}	6.2 ^{c}	7.9 ^{a}	60.2 ^{ab}	7.2 ^{b}
	Steamed Soil	5.8 ^{b}	8.2 ^{c}	2.8 ^{ab}	2682.5 ^{b}	5.8 ^{b}	10.6 ^{a}	6.3 ^{ab}	47.8 ^{b}	6.4 ^{b}
	Microwave Heated Soil	1.6 ^{c}	2.0 ^{c}	2.4 ^{b}	3545.0 ^{ab}	5.7 ^{b}	13.1 ^{a}	5.2 ^{b}	49.5 ^{b}	8.3 ^{b}
Microwave Treated Soil	Untreated Soil	9.2 ^{a}	21.6 ^{a}	2.4 ^{b}	2350.2 ^{b}	5.4 ^{b}	13.1 ^{a}	9.0 ^{a}	87.9 ^{a}	8.7 ^{ab}
	Fumigated Soil	2.9 ^{c}	4.6 ^{bc}	3.0 ^{ab}	4337.0 ^{a}	7.0 ^{a}	9.6 ^{b}	7.9 ^{a}	62.9 ^{ab}	8.2 ^{b}
	Steamed Soil	6.5 ^{b}	10.9 ^{b}	2.9 ^{ab}	2404.0 ^{b}	5.8 ^{b}	10.0 ^{b}	7.4 ^{ab}	69.9 ^{ab}	9.1 ^{ab}
	Microwave Heated Soil	2.6 ^{c}	5.8 ^{bc}	3.5 ^{a}	4640.2 ^{a}	8.0 ^{a}	11.2 ^{a}	6.0 ^{b}	45.3 ^{b}	7.5 ^{b}
LSD (p = 0.05)		2.4	7.7	0.9	1363.6	2.0	2.5	2.9	28.5	3.2

Note: Means with different superscripts in each of the columns are statistically different from one another.

There was no significant difference in fruit production that was associated with where the plants were sourced; however, plants that were growing in microwave treated soil produced significantly less mature fruit (46.3 g pot^{-1}) than the steam treated soil (59.7 g pot^{-1}), fumigated soil (60.0 g pot^{-1}), and untreated soil (72.0 g pot^{-1}). The highest fruit production was from plants that were sourced from microwave treated soil but growing in untreated soil (Table 6).

4. Discussion

This study was the first to evaluate the use of microwave for soil disinfestation in the field in strawberry runners. Results showed that microwave has the capacity to disinfest field soil against pathogens and weeds and increase the yields of strawberry runners by 10–37% compared to untreated soil. The results concur with previous studies that showed that heating with steam disinfested soil against pathogens and weeds [11,16–18] and increased strawberry runner yields [19]. Furthermore, in a pot experiment in the current study, runners from soils treated with microwave performed as well as runners from soils treated with methyl bromide/chloropicrin.

It is well established that microwave heating of soil can significantly reduce weed emergence [28–32,42,43]. Results from the current study showed that microwave reduced the number, dry weight, and diversity of emerging weeds in the field. There was no evidence that any of the emerging weed species were tolerant of the microwave treatment. Rather, microwave reduced populations of all emerging weed species in the field experiments. Furthermore, weed control in a pot experiment with microwave was as effective as treatment with methyl bromide/chloropicrin, and significantly more effective than steam heating.

The results from the current study that microwave reduced the survival artificial and natural inoculum of pathogens of strawberry in soil in the field agrees with a previous study in the literature that examined similar genera of pathogens [33]. In the current study, microwave treatment also reduced the subsequent growth of surviving pathogens. It is possible that this may contribute to slower recolonization of disinfested soils by the pathogens or reduce the pathogenicity of the isolates. This hypothesis, however, requires further research to verify.

The effect of microwave in increasing strawberry runner yield in the field was greater when all the soil surrounding the mother plants was treated (i.e., Experiment 2) than when bands of soil were treated (i.e., Experiment 1). It is likely that reduced competition from weeds and lower pathogen pressure from *Pythium* spp. contributed to the higher runner yields in microwave treated soils. Despite this, microwave did not reduce the severity of black roots on harvested runners or the isolation of *Pythium* spp. from the lesions. In addition to controlling pathogens, weeds, and pests, soil disinfestation can induce an increased growth response in crops such as strawberry through changes in soil nutrition and biology that favor the plant [4,44]. Although soil nutrition was not thoroughly investigated in the current experiments, previous studies have shown that microwave can increase soil fertility [35,45], particularly nitrogen concentration, and this has been linked with increased plant growth [35]. In the current field experiments, a separately published study showed that microwave treatments altered the soil bacterial community in favor of species implicated in enhanced plant growth [34,46]. It is possible that changes in edaphic factors of nutrition and biology associated with the increased growth response also contributed to higher runner yields in microwave-treated soils in the current study.

Despite its promise, much more research is required to develop microwave for soil disinfestation and strawberry production in the field. For example, the levels of pathogen and weed control observed in the current experiments with microwave were much lower than previously reported in the same soil and location for methyl bromide/chloropicrin [1,3]. Phytosanitary controls on the production of strawberry runners in Australia and many countries mean that pathogen concentrations in soil must be close to zero following disinfestation to meet certification standards. Furthermore, it is vitally important that treatments

disinfest soils at depths from 0–30 cm. This is to prevent the risk of pathogens infecting runner plants or recolonizing soil from greater depths by harvest. In the current study, microwave disinfested soil against pathogens at shallow depths more effectively than greater soil depths. Furthermore, the effect of the pre-plant microwave treatment was much greater against *Pythium* spp. in soil at planting than at harvest. In a similar manner, studies showed that heat disinfestation with steam did not adequately control the strawberry *Verticillium dahliae* at greater soil depths [11,19]. Additionally, previous research found that *Fusarium* pathogens could recolonize soil disinfested with microwave but at slow rates [33]. To achieve a more consistent soil disinfestation effect, future research needs to consider different wavelengths and energy inputs of microwave to target soil at greater depths and its effect on pathogens though the soil profile.

The capacity for microwave to disinfest soil was demonstrated in pot trials in the current study, where it was possible to treat the soil more evenly. In these experiments, strawberry production was equivalent in soils treated with microwave and methyl bromide/chloropicrin. Steam treatment, however, was not as effective in reducing weed emergence or promoting plant growth and productivity of strawberry as fumigation or microwave treatment. Despite the statistical significance of effects in the pot trials, it is important to recognize that fruit yields in these experiments were approximately 5% of what would be achieved in the field. Therefore, it is important that field experiments are conducted in the future to validate the results from the pot trials. Nonetheless, steam treatment in the pot experiments in this study required almost ten times more energy per unit mass to achieve the necessary soil temperatures for effective disinfestation. This concurs with previous work that demonstrated that microwave heating was faster and possibly more thermally efficient than steam or flame heating [47]. Therefore, microwave treatment warrants further investigation as a method of heat disinfestation of soil and a possible alternative to steam and chemical fumigation for strawberry production.

5. Conclusions

Soil heating with microwave showed the capacity to disinfest soil in the field against pathogens and weeds for strawberry production. The results demonstrate its potential for further development as an alternative to steam and chemical disinfestation in strawberry and other crops.

Author Contributions: Conceptualization, G.I.B. and S.W.M.; Methodology, S.W.M., D.J.M., G.I.B., V.B.G.P. and M.J.K.; Formal analysis, S.W.M. and G.I.B.; Investigation, S.W.M., G.I.B. and D.J.M.; Resources, S.W.M. and G.I.B.; Data curation, S.W.M. and G.I.B.; Writing—original draft preparation, G.I.B.; Writing—review and editing, S.W.M., D.J.M. and G.I.B.; Supervision, G.I.B. and S.W.M.; Project administration, G.I.B. and S.W.M.; Funding acquisition, G.I.B. and S.W.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: There are no additional data for this paper. All results are presented within the paper.

Acknowledgments: The authors acknowledge the invaluable assistance of Ravneet Kaur Jhajj, the laboratory manager at Dookie campus of the University of Melbourne where the pot trials took place.

Conflicts of Interest: The authors declare no conflict of interest.

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