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Soil compaction enhances the impact of microwave heating on seedling emergence

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Abstract

An innovative microwave-based process to control invasive species in the field is currently under development. We investigated the effect of soil compaction levels under three microwave treatment pairings of power and duration (2kW4min, 2kW8min and 4kW4min), on seedling emergence of a diverse seed bank and three major invasive species in Europe (bohemian knotweed, giant goldenrod and jimsonweed). When soil was compacted, the number of seedling emergences of dicotyledons was significantly reduced by the 2kW4min treatment. Furthermore, while some dicotyledon and giant goldenrod's seedling emergences were observed respectively in plain soil in the 4kW4min and 2kW4min treatments, no seedling was recorded in compacted soil for the same treatments. Our results suggest that compacting soil increases the efficiency of microwave heating on seeds. Soil compaction level should therefore be considered in the design of the final microwave-based process.

Keywords: invasive species control; seed-bank; microwave heating

1. Introduction

Successful eradication of an invasive species requires an effective depletion of the seeds and vegetative propagules, e.g. rhizomes (Myers et al., 1998; Regan et al., 2006; Saito and Tsuyuzaki, 2012). Complete destruction of invasive seed banks is rarely achieved by herbicide application (Christoffoleti et al., 2007), thus alternative methods have been developed. Biological control using natural enemies can be effective in reducing seed production or accumulation in the soil (Adair, 2005; Impson et al., 2004; Zimmermann et al., 2004), but the release of exotic biological agents has been shown to raise ecological issues like negative impacts on native biota (Strong and Pemberton, 2000). Heating methods are of interest since they provide chemical-residue-free weed control. The use of fire is appropriate to reduce upper seed banks in fire-adapted ecosystems (Holmes, 1989; Richardson and Kluge, 2008), but is not suitable for some ecosystems (i.e. wetlands and dunes) and can sterilize soil for years (Campbell et al., 1999). Soil solarization, based on solar heating of soil by mulching it with plastic sheets during the hot seasons, raises temperatures usually up to 40 to 55°C in the upper 5 cm of the soil (Cohen, 2008), enabling the reduction of invasive species seed banks (Cohen et al., 2018). This method however showed certain limitations, such as length of treatment (i.e. several months), climate-dependency, and variable effectiveness (Cohen and Rubin, 2007). Microwave soil heating has been considered for weed control for several decades (Barker and Craker, 1991; Davis et al., 1971; Sartorato et al., 2006). Microwave radiation induces dielectric heating of moist materials, enabling soil to reach temperatures required for seed viability loss (i.e. 60°C-90°C; Brodie et al., 2011; Sahin, 2014; Thompson et al., 1997). Exposure to high temperatures can impact seed mortality directly *via* thermal killing, or indirectly *via* increased susceptibility to toxic compounds and microorganisms, i.e. “weakening effects” (Katan et al., 2012). In some cases, heat can break dormancy and stimulate germination (i.e. seeds displaying physical dormancy; Baskin et al., 1998; Herranz

et al., 1998; Tieu et al., 2001). Several studies revealed however a great potential for microwave heating to control germination of many weed species (Bebawi et al., 2007; Brodie et al., 2009; Nelson, 1996 ; Sahin, 2014), but the microwave technology used at the time was not developed enough to draw a cost effective and marketable application (Brodie et al., 2011). This study is part of a set of studies undertaken to help determine the technical characteristics of a continuous conveying tunnel with microwave equipment, designed for the treatment of soil infested by a wide range of invasive species (seeds and adult stages) collected *in situ*. Infested soil, once passed through the microwave oven on a conveyor belt, can be put back in place. First studies hold promising results for seedling emergence reduction, with high effectiveness when soil temperature reached 85°C (De Wilde et al., 2017; Hess et al., 2018). Before this process can be implemented full scale, however, more information is needed on the parameters influencing the effectiveness of microwave treatments. Soil characteristics that mostly alter the dielectric properties of soil, such as initial temperature, texture and moisture content, have been shown to strongly influence the temperature achieved after microwave exposure (Barker and Craker, 1991; Brodie, 2011; Brodie et al., 2011; Davis et al., 1971; Davis et al., 1973; De Wilde et al., 2017). Soil compaction has so far been neglected, although compaction directly influences the dielectric properties of the soil (De Vries, 1952). Increasing soil compaction leads to higher bulk density and lower porosity, thereby enhancing thermal conductivity (Abu-Hamdeh, 2000; Yadav and Saxena, 1973). Soil compaction may consequently impact the effectiveness of microwave heating in inhibiting seedling emergence.

We investigated the influence of soil compaction on microwave heating effectiveness in inhibiting seedling emergence by comparing the seedling emergence response in plain or compacted soil during exposure to three microwave treatment pairings of power and duration. We chose to investigate the seedling emergence response of a grassland seed bank extracted

in situ not for its invasion status (non-invaded), but because it contained a great diversity of species. We also monitored the seedling emergence response of three emblematic invasive species in Europe (i.e. *Datura stramonium* L., *Reynoutria × bohemica* Chrtek and Chrtková and *Solidago gigantea* Aiton) already used in previous studies (De Wilde et al., 2017; Hess et al., 2018) by placing a fixed number of seeds in the soil before treatments were applied. Reported as highly aggressive in some European countries (Kleunen et al., 2007; Weber, 2003; Weber and Jakobs, 2004), these species were selected because they reproduce by seeds (Gillies et al., 2016; Saayman, 2000; Weber and Jakobs, 2004) and more importantly because their seeds differ in shape and size. Indeed, these characteristics are known to strongly influence seed sensitivity to microwave heating (Bebawi et al., 2007; Brodie et al., 2011). Furthermore, none of the three species' seeds displays physical dormancy (Bochenek et al., 2016; Engler et al., 2011; Loddo et al., 2013).

2. Material and methods

The experiment was designed to test the impact of soil compaction ('plain' vs 'compacted') during three microwave treatment pairings of power and duration (2kW4min, 2kW8min and 4kW4min) on seedling emergence of three invasive species in Europe (*Datura stramonium*, *Reynoutria × bohemica* and *Solidago gigantea*) and a seed bank contained in soil extracted *in situ*.

2.1 Soil sampling

Topsoil, characterized as a silty loam (60% silt, 15% clay), was collected from uncultivated species-rich grassland on an abandoned organic hemp growing field (production halted in 2013) located near two rivers, La Cèze and La Tave (Laudun-L'Ardoise, France). The 25 soil samples collected ($1.35\text{m} \pm 0.15\text{m}$ length $\times$ $0.5\text{m} \pm 0.1\text{m}$ width $\times$ 0.1m depth) were mixed to ensure relative homogeneity of soil and seed bank. Blended soil cleared of

stones and organic residues was distributed in 1kg batches just before microwave treatments were applied.

2.2 Seed sampling of the three invasive species

Achenes of *Reynoutria × bohemica* and *Solidago gigantea* were collected in March 2016 along the Isère River in Savoie (France). Seeds of *Datura stramonium* were collected in October 2014 from stands growing along the Rhône River in Drôme (France). The achenes and seeds were collected randomly from ten ramets of the same population (to reduce variability) and then pooled. They were stored under dry conditions at room temperature until used for experiments.

2.3 Microwave system

An AMW200 batch microwave system (SAIREM SAS, Neyron, France) was used in this study. The 304 L microwave stainless steel chamber can contain a block of 0.6m × 0.4m × 0.25m maximum size and 30kg maximum weight. The microwave system has a polyethylene sliding table with a usable surface of 0.84m × 0.62m and a pneumatically driven sliding door, both making block loading/unloading easier. The microwave system operates at 915MHz, which allows greater penetration and treatment of thicker materials (up to 0.25m) than the frequently used 2450MHz microwave frequency (approximately a threefold increase). Coupling microwave heating from above and below and rotating the turntable enhance heating homogeneity. The output power of 10kW maximum is produced by 2 × 5kW generators (magnetron), adjustable from 1kW to 10kW. The system is water-cooled.

2.4 Experimental design

We applied three microwave treatments using the following power and duration pairings: 2kW4min, 2kW8min and 4kW4min. The effectiveness of these treatments was tested in previous experiments (De Wilde et al., 2017; Hess et al., 2018). Microwave treatments were performed on the premises of SAIREM (Neyron, France), each treatment being applied on

1kg soil replicates placed in 0.22m high $\times$ 0.09m diameter glass cylinders. Because the presence of water in soil is essential to creating heat by microwave exposure (Metaxas and Meredith, 1988), soil samples were not dried before compaction. Bulk density (i.e. mass of dry soil / volume of bulk soil), which is a common measure of compaction, could therefore not be calculated. Soil humidity before treatment was $7.3\% \pm 1.2$ (mean $\pm$ SD).

For each microwave treatment, two compaction levels were tested: 'plain soil' consisted of 1kg soil filling a volume of $725 \pm 19 \text{ cm}^3$ and 'compacted soil' consisted of soil compacted directly in the cylinder using a metal weight until soil height was reduced by $15 \pm 2\%$ ($616 \pm 15 \text{ cm}^3$) compared with plain soil. For each treatment (microwave treatment $\times$ compaction level), we used six replicates of soil containing (1) only the natural seed bank, (2) ten seeds of each tested invasive species. Controls without microwave treatment were replicates of plain soil containing (1) only the natural seed bank, and (2) ten seeds of each tested invasive species. After microwave treatments, soil temperature was immediately measured in each replicate, using mercury thermometers inserted in the center and at the periphery of the cylinder (2cm below soil surface). Before treatment, soil temperature was $18.6 \pm 0.6^\circ\text{C}$ (mean $\pm$ SD). After treatment, compacted soil samples were decompacted and all samples (control and treated soil) were stored for 10 days at 30°C .

After microwave treatment, each sample was spread in a 1 to 1.5cm thick layer over a 19cm diameter perforated plastic saucer containing from bottom to top: (1) a 21cm diameter Diatex filter (500-600 μm mesh size to prevent loss of coarse sand), (2) 1.5cm of sterilized and desalted sand and (3) a 23cm diameter Diatex filter (50-70 μm mesh size to avoid loss of silt and seeds). The plastic saucers were randomly placed in containers where a 1cm water layer was constantly adjusted so as to maintain soil moisture at a level conducive to germination and prevent submersion. The experiment was conducted in an outdoor, pollen-free greenhouse reducing the risk of granivory and contamination by seeds coming from the

outside (Institute Tour du Valat, Arles, France). Seedling emergences were recorded weekly for 6 weeks from June 23 to July 28, 2016. Identified individuals were regularly removed to reduce competition.

2.5 Statistical Analyses.

We analyzed variations in soil temperature depending on the explanatory variable ‘microwave treatment’ (‘control’: absence of microwave treatment and three power and duration microwave treatment pairings: ‘2kW4min’, ‘2kW8min’ and ‘4kW4min’) cross-checked against the explanatory variables ‘compaction level’ (‘plain’ or ‘compacted’) and ‘measurement position’ (‘center’ or ‘periphery’), using permutation tests. The p-values for the multiple comparison tests were adjusted with the BH adjustment (Benjamini and Hochberg, 1995). The tests were performed using the ‘coin’ R package (Hothorn et al., 2008; R Core Team, 2015).

We analyzed variations in the number of seedling emergences of monocotyledons and dicotyledons (seed bank) depending on the explanatory variable ‘microwave treatment’ in interaction with ‘compaction level’ using generalized linear models (GLM) with Poisson error distribution (Crawley, 2007). Tests were performed with function ‘glm’ in R (R Core Team, 2015). We also analyzed variations in the percentage of seedling emergences of *Datura stramonium*, *Reynoutria × bohemica* and *Solidago gigantea* depending on the explanatory variable ‘microwave treatment’ cross-checked against the variable ‘compaction level’ using Kruskal-Wallis or Wilcoxon rank sum tests.

All statistical tests were performed using R.3.3.2 software (R Core Team, 2015). P-values lower than 0.05 were considered as statistically significant.

3. Results and discussion

The three tested microwave treatments significantly raised soil temperature (close to 90°C in the 2kW8min and 4kW4min treatments and above 60°C in the 2kW4min treatment; Figure 1) and decreased seedling emergence of species from the seed bank and the invasive species tested (Figure 2 and 3; $p < 0.05$). These results are consistent with the notion that soil microwave treatment affects seed viability *via* heat diffusion from surrounding soil (Barker and Craker, 1991; Nelson, 1996). The 2kW4min treatment led to a ca. 50% decrease in seedling emergence from the seed bank (Figure 2a,b) and an absence of differences in seedling emergence of *D. stramonium* and *R. × bohemica* compared with the control, which is consistent with previous studies (Figure 3a,b; De Wilde et al. 2017). This low effectiveness may have been reinforced by the 20°C soil temperature differences occurring between the center and the periphery of the cylinder (Figure 1). Indeed, microwave heating commonly results in uneven temperature distribution patterns (Brodie, 2008; Vadivambal and Jayas, 2010), with more intensive heating around the central axis and evaporative cooling at the surface of a small cylinder (Remmen et al., 1996; Hess et al., 2018). Seeds located at the periphery may have therefore been exposed to substantially lower temperature, which could explain the low effectiveness of the treatment as well as the high variability between replicates.

Seeds of *S. gigantea*, however, appeared vulnerable to soil microwave heating, with seedling emergence reducing almost five-fold in the 2kW4min treatment (plain soil) compared with the control (Figure 3c). This sensitivity, already revealed in previous experiments (De Wilde et al., 2017; Hess et al., 2018), could be linked to *S. gigantea*'s small seed size (Diprose et al., 1984). Testing the influence of seed size on a higher number of samples and species, but also on seeds from one species having different sizes, would be useful to assess the role of seed size in the sensitivity to microwave heating. The thickness of seeds tegument, dormancy type, or the nature of seed reserves could also be impacting.

The two most intensive treatments (i.e. 2kW8min and 4kW4min) resulted in more homogeneous soil temperatures within the cylinders than the 2kW4min treatment (Figure 1), and induced temperatures superior to 90°C, considered as lethal for many types of seeds (>80°C during 30s; Barker and Craker, 1991), whatever soil compaction level. This enhanced temperature homogeneity could be due to the increased exposure time or, more likely, output power, resulting in changes in temperature spatial distribution by thermal diffusion from the zones where the microwave energy is being absorbed to the rest of the sample (Brodie, 2011). These more intensive treatments led to the total inhibition of invasive species seedling emergence (Figure 3) and a drastic decrease in seed bank seedling emergence (Figure 2). With the 2kW8min treatment, seedling emergence still occurred in the seed bank for both levels of soil compaction but was reduced by 97% on average (Figure 1). Microwave heating induces a rapid dehydration of plant tissues, causing plant death in few seconds of microwave treatment (Brodie et al., 2011). Above-ground and belowground plant tissues would therefore be easily destroyed by microwave treatments used to control seedling emergence, but their sensitivity will need to be assessed.

Increasing soil compaction appears to enhance microwave treatment effectiveness. In the 2kW4min treatment, the number of seedling emergences of dicotyledons was significantly reduced when soil was compacted (Figure 2b). Furthermore, while some dicotyledons and *S. gigantea* seedlings emerged in plain soil respectively in the 4kW4min and 2kW4min treatments, no seedling emergence was observed in compacted soil for these treatments (Figure 2b, 3c). The higher effectiveness observed in compacted soil is most probably due to the higher bulk density and lower porosity obtained when compacting soil, resulting in higher thermal conductivity during microwave treatment (Abu-Hamdeh, 2000; Yadav and Saxena, 1973). Such assumption corroborate with our results, showing that soil compaction tended to increase soil temperature (Figure 1).

Our results suggest that soil compaction, at the level tested in our experiment, does not diminish but rather enhances the effectiveness of the microwave-based process (i.e. inhibition of seedling emergence). This implies that microwave heating will be effective at reducing unwanted species emergence in compacted soil, prevalent in agricultural areas (Drewry et al. 2008). However, deliberately compacting soil to enhance microwave effectiveness may be detrimental to crop germination, growth, and water infiltration (Bassett et al., 2005; Benvenuti and Mazzoncini, 2019; Skinner et al., 2009), so that soil might have to be decompacted before sowing. These results also point out that soil compaction should be considered in the design of the final microwave-based process: adjusting power and duration pairings to soil compaction level may help saving energy or increasing treatment effectiveness.

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400

401 **Figures**

402 Figure 1. Effect of microwave treatment (control: without microwave treatment and three
403 pairings of power and duration: 2kW4min, 2kW8min 4kW4min), position in the cylinder
404 ('center' or 'periphery') and soil compaction level ('plain' or 'compacted') on soil
405 temperature (°C). Values are expressed as means $\pm$ SE of the six replicates. Letters
406 (^{a, b, c, d, e, f, g, h}) refer to results of permutations tests : values with no letter in common are
407 significantly different (multiple comparison tests adjusted with the BH adjustment; $p < 0.05$).
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Figure 2. Effect of microwave treatment (control: without microwave treatment and three pairings of power and duration: 2kW4min, 2kW8min 4kW4min) and soil compaction level ('plain' or 'compacted') on the number of seedling emergences of the seeds bank including (a) monocotyledons and (b) dicotyledons. Values are expressed as means $\pm$ SE of the six replicates. Letters (^a, ^b, ^c, ^d) refer to results of *post-hoc* Tukey's tests : values with no letter in common are significantly different ($p < 0.05$).

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Figure 3. Effect of microwave treatment (control: without microwave treatment and three pairings of power and duration: 2kW4min, 2kW8min 4kW4min) and soil compaction level ('plain' or 'compacted') on the percentage of seedling emergences of (a) *Datura stramonium* (b) *Reynoutria x bohemica* and (c) *Solidago gigantea*. Values are expressed as means $\pm$ SE of the six replicates. Letters (^a,^b) refer to the result of a Wilcoxon test : values with no letter in common are significantly different ($p < 0.05$). NS means "non-significant", when no statistical difference was found ($p < 0.05$).