Research article

Microwave oven as a clean technology for the eradication of fusariosis in melon


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Abstract – The causal agent of Fusarium wilt in melon (Cucumis melo) is the fungus Fusarium oxysporum f. sp. melonis. One of the main ways in which this fungus is transferred is the re-use of propagation trays in nursery greenhouses. This work shows that, during the commercial production of melon plantlets, subjecting these trays to microwave energy in a prototype oven can eradicate this fungus. The low energy performance of this oven would increase the price of seedlings by only $6.25 \times 10^{-3}$ Eurocents over the current market price of 19 Eurocents per plant. Since this method obviates the need for pesticides, it also affords environmental advantages.

Fusarium oxysporum f. sp. melonis / high frequency microwaves / artificial substrate / propagation trays / disinfestation

1. INTRODUCTION

Fusarium wilt, caused by the fungus Fusarium oxysporum Schlechtend f. sp. melonis Snyder and Hansen, is one of the most destructive diseases of melon (Cucumis melo L.) crops worldwide (Leach and Currence, 1938) (Fig. 1). Losses of up to 90% or more have been documented (Leach and Currence, 1938; Miller, 1955; Palohdi and Sen, 1981; González et al., 1988; Champaco et al., 1993).

In Castilla-La Mancha, one of the largest melon-producing areas in the world, most growers establish their crops by transplanting plantlets raised in controlled environment chambers at specialised nurseries rather than by the ultimately more expensive technique of direct seeding. This process requires the use of expanded polyurethane trays with cells containing a peat substrate in which seeds can germinate and develop individually.

The best way to protect against fusarium wilt is to prevent the introduction of the pathogen into the growing substrate. However, the re-use of seed trays does not help in this respect. Indeed, it is one of the main causes of the rapid dispersion of this disease. The same is also true of Fusarium oxysporum f. sp. api which affects celery (Awuah and Lorbeer, 1991).

The fight against this disease in non-resistant cultivars requires the use of pathogen-free plantlets. This demands the use of healthy seed and substrate, and the eradication of the fungus from seed trays (Evil and Yalcin, 1977). To achieve these aims, chemical methods can be used. Disinfectants such as sodium hypochlorite or formol, however, can cause serious toxicity problems for young plants as well as pose risks to handlers. Physical methods such as heat treatment are not viable when dealing with seed trays and are also difficult to undertake, and solarisation, although it reduces the viability of the pathogen, requires long exposure times and cannot guarantee eradication (Awuah and Lorbeer, 1991). In addition, these chemical and physical control methods can cause considerable environmental pollution.

It has been shown that microwave energy can inhibit a broad spectrum of soil parasites and weed seeds (Bhaskara et al., 1998; Calvacante and Muchovej, 1993; Ferriss, 1984; Lozano et al., 1986; Mavrogianopoulos et al., 2000; Reddy et al., 1999; Soriano et al., 1998, 2004a). Some authors indicate that, although it is a clean technology, a large amount of energy is required, and that this is a great drawback (Bhaskara et al., 1998; Lal and Reed, 1988; Lozano et al., 1986; Nelson, 1996; Rice and Putham, 1977). However, in the disinfection of the material used in melon propagation, only small quantities of substrate need to be treated and the materials used are microwave-transparent. Therefore, only small energy levels are required. To demonstrate this in conditions similar to those of...
commercial melon plantlet production, a prototype microwave oven was built and tested. This apparatus was designed to provide microwaves at a frequency of 2.45 GHz. The amount of energy required to eradicate the fungus from the production process was determined; this represented an almost negligible increase in the final cost of plantlets to growers.

2. MATERIALS AND METHODS

2.1. Plant material

Melon cv. Amarillo Canario seeds, which are susceptible to all Fusarium oxysporum f. sp. melonis races, were used in all experiments. All seeds were disinfected by immersion for 2 min in fresh 10% sodium hypochlorite prior to use.

2.2. Experimental pathogen

The pathogen used in all experiments was monotrichous isolate CR 6801 of the fungus Fusarium oxysporum f. sp. melonis race 1.2. This was obtained during 2001 from the diseased plants of a commercial melon crop in the province of Ciudad Real (Spain). This isolate was stored in sterile sand at 5 ± 1 °C and cultivated on potato dextrose agar for the preparation of a conidial suspension.

2.3. Microwave oven prototype

The prototype microwave oven was constructed from 2-mm-thick galvanised steel sheeting, fastened in place by aluminium rivets. The apparatus had two vertically-opening doors, one on either side, whose movements were controlled by a hydrostatic power transmission system (Roquet, model 9315). These doors, which are opposite one another, allow the oven to be installed in an automatic sowing line; the seed trays transported by the conveyer belt are introduced into the oven through one door, and leave via the other after irradiation. The capacity of the oven is some 122 L, allowing it to disinfect three 70 × 46 × 7.5 cm seed trays at any one time. The oven was provided with four 1000 W theoretical power magnetrons (Fagor MV3-254 B) that produce microwaves at 2.45 GHz. These magnetrons and their electrical circuits are mounted in pairs on the fixed walls of the oven, perpendicular to the doors. Both the hydrostatic apparatus and the oven proper run on 220 V AC. In all assays, the current was interrupted manually (Fig. 2).

Prior to performing the assays, the true power developed by the prototype was determined. Thin, 800-mL expanded polyurethane (microwave-transparent) trays were filled with water and placed in the oven. The temperature of the water was recorded before and after irradiation using a low inertia digital thermometer (Checktemp). One by one these trays were irradiated for either 30, 60, 120, 180 or 240 s (all shorter than the time required for the water to boil). The heating power of the apparatus was determined using the expression N = 3333.33 (ΔT/Δt) W, where N is the true power developed by the apparatus, ΔT the increase in the temperature of the water, and t the irradiation time.

2.4. Experimental assays

Table I shows the irradiation times (energy inputs) used with the conidial suspensions and trays with contaminated substrate. These values were chosen based on work performed by Soriano-Martín et al. (2004b, 2005).

![Figure 1. Melon plantlets affected by Fusarium wilt: (a) healthy, (b) affected, (c) dead.](image)

![Figure 2. The prototype microwave oven.](image)
Microwave oven as a clean technology for the eradication of fusariosis in melon

2.4.1. Effect of irradiation time on Fusarium oxysporum f. sp. melonis race 1.2 Conidial suspensions

This assay was performed to determine the minimum energy required to eliminate conidia in aqueous suspension (5 × 10^6 conidia/mL).

The pathogen was cultivated on potato dextrose agar. Three 5-mm-diameter explants of a 7-day-old colony were placed in an Erlenmeyer flask containing 100 mL potato dextrose agar broth. This was incubated at 25 ± 2 ºC for 7 days in an orbital agitator (150 rpm) and the content then filtered through four layers of sterile gauze. Conidia were enumerated using a haemocytometer, and the concentration of the suspension adjusted to 5 × 10^6 conidia/mL with sterile, distilled water.

Groups of six test tubes containing 10 mL of this suspension were placed inside the prototype microwave oven at points equidistant from the centre. They were then irradiated for either 0, 5, 10, 15, 20, 25 or 30 s.

The viability of the irradiated suspension was determined by successively diluting 1 mL of each tube to a concentration of 10^{-2} using sterile agar–water (1%). One millilitre of this was plated on V8 agar broth (three replicates) and the number of colonies formed were counted after three days of incubation in the dark at 25 ± 1 ºC. When the number of colony-forming units was > 300, enumeration was considered impossible. This experiment was performed twice.

2.4.2. Influence of irradiation time on the viability of the conidial suspension after contaminating the substrate employed in melon plantlet production

Ten of the 216 cells in each of seven 70 × 46 × 7.5 cm seed trays were randomly selected and filled with 1500 g of sterile substrate (Florasard, Kekila) plus 3 mL of the conidial suspension (5 × 10^6 conidia/mL) irradiated for either 0, 5, 10, 15, 20, 25 or 30 s.

According to previous work performed by our group (Soriano-Martín et al., 2004a), the seed trays were previously sterilised using the prototype oven, irradiating them individually for 90 s. To demonstrate the effectiveness of this measure, fragments of the trays were cut, crumbled and placed on Petri dishes containing either potato dextrose agar or V8 agar broth. These plates were maintained in an incubator at 25 ± 1 ºC for 14 days, making daily checks for fungal and bacterial growth. In V8 agar broth, fragments of non-sterilised trays were associated with the growth of Macor spp, Aspergillus spp and Cladosporium spp. In potato dextrose agar, fungi of the same genera grew, as well as bacterial colonies. In contrast, no fungal or bacterial colonies grew when the fragments came from seed trays that had been irradiated for 90 s.

The conidial suspension was irradiated for 0, 20, 40, 60, 80, 100 and 120 s. The post-treatment viability of the inoculum was then determined by successively diluting 1 g of substrate to a concentration of 10^{-3} using sterile agar–water (1%). One millilitre of this was then plated on V8 agar broth (three replicates) and the number of colonies recorded after three days of incubation in the dark at 25 ± 1 ºC.

2.4.3. Influence of different irradiation times on the pathogenicity of the conidial suspension

Seventy melon seeds were placed in Petri dishes (containing sterile vermiculite) between two layers of absorbent paper moistened with sterile water. These plates were maintained for 5 days in a controlled environment chamber (16 h light; 450 µmol E m^{-2} s^{-1}, 25 ± 2 ºC, relative humidity 75%), wetting them every 48 h with sterile water. The germinated seeds were then individually placed in plastic containers (diameter 8 cm, height 12 cm) filled with sterile substrate. These were then arranged in groups of 40 on polystyrene trays with a metallic grill at the bottom, watered to saturation, and introduced into a controlled environment chamber for 13 days in the conditions described above. All containers were watered every day. When each plant developed its first true leaf, it was inoculated by immersing the roots for 2 min in 60 mL of conidial suspension (5 × 10^6 conidia/mL) irradiated in the prototype microwave oven for 5–30 s (with 5-s intervals) as described in Assay 1. For each of the seven irradiation times, four blocks of 10 plants were inoculated. As a positive control, four blocks of ten plants were inoculated by submerging their roots in a non-irradiated conidial suspension (control +). As a negative control, a further four blocks of 10 plants were subjected to the immersion of their roots in sterile, distilled water (control −).

After inoculation, the plants were replaced in their plastic containers with sterile substrate. After placing them on the trays they were introduced into the controlled environment chamber where they remained for 22 days in the conditions described above for 22 days. All were watered to field capacity every 48 h.

Disease levels were determined every two days, noting the incidence (percentage of plants infected) and the severity of symptoms on a 0–4 scale indicating the percentage of plants with yellowing or necrosis (0 = 0%; 1 = 1–33%; 2 = 34–66%; 3 = 67–100%; 4 = all dead). The disease severity data were first used to calculate a Disease Severity Index (DSI) using the expression

\[
\text{DSI} = \sum_{i=1}^{4} n_i \cdot s_i / \left(4 \cdot N\right) \cdot 100
\]

where \( n_i \) is the number of plants affected at each severity level, \( s_i \) the degree of severity (0–4) and \( N \) the number of plants used in the assay. The same data were also used to calculate the area under the disease progress curve over time (days). The area under the disease

<table>
<thead>
<tr>
<th>Irradiation time (s)</th>
<th>Energy input (J)</th>
<th>Irradiation time (s)</th>
<th>Energy input (J)</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
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<tr>
<td>5</td>
<td>5 879.6</td>
<td>20</td>
<td>23 518.5</td>
</tr>
<tr>
<td>10</td>
<td>11 759.3</td>
<td>40</td>
<td>47 037.0</td>
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<tr>
<td>15</td>
<td>17 638.9</td>
<td>60</td>
<td>70 555.6</td>
</tr>
<tr>
<td>20</td>
<td>23 518.5</td>
<td>80</td>
<td>94 074.1</td>
</tr>
<tr>
<td>25</td>
<td>29 398.1</td>
<td>100</td>
<td>117 592.6</td>
</tr>
<tr>
<td>30</td>
<td>35 277.8</td>
<td>120</td>
<td>141 111.1</td>
</tr>
</tbody>
</table>

Table 1. Irradiation times and true energy input to aqueous suspensions of conidia and contaminated substrates.

<table>
<thead>
<tr>
<th>Aqueous suspensions of conidia</th>
<th>Trays with contaminated substrate</th>
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<tbody>
<tr>
<td>Irradiation time (s)</td>
<td>Energy input (J)</td>
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<tr>
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<tr>
<td>0</td>
<td>0.0</td>
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<td>5</td>
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<td>29 398.1</td>
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<td>30</td>
<td>35 277.8</td>
</tr>
</tbody>
</table>
progress curve provides a clear idea of the severity of disease since it represents the sum of the products of the means of the DSI and the number of days elapsed between the two readings of the severity indices. A larger area under the disease progress curve indicates a shorter incubation time and greater severity of disease.

2.4.4. Influence of irradiation time on fungal viability in seed trays containing artificially contaminated substrate, in conditions similar to those of a commercial nursery

Three millilitres of the conidial suspension (5 × 10^6 conidia/mL) were placed in 10 randomly selected commercial seed tray cells containing sterile substrate. The trays were introduced individually into the prototype oven and irradiated for 120 s to eradicate the pathogen. They were then sown with melon seeds, one to each cell, and placed in a controlled environment chamber at 25 ± 2 ºC in the dark. After the cotyledons emerged (5–6 days) they were kept for 22 days under a 16 h light / 8 h dark regimen (450 μmol E m⁻² s⁻¹) at 25 ± 2 ºC and at a relative humidity of 75%. All cells were watered every 48 h. After this period the plantlets were transplanted to 3 L plastic pots containing a 1:1 (v:v) mixture of peat and sand. The plants received fertiliser at the time of transplant and then 15 days later in the form of 100 mL of Hewitt’s solution, modified as follows: 20 mL MgSO₄·7H₂O/L (18.4 g/L), 10 mL EDTA-Fe/L (2.45 g/L), 1 mL MnSO₄·7H₂O/L (2.23 g/L), 0.1 mL CuSO₄·5H₂O/L (2.4 g/L), 0.1 mL ZnSO₄·2H₂O/L (2.9 g/L), 0.1 mL H₂BO₃·2H₂O/L (18.6 g/L), 0.1 mL Na₂MoO₄·2H₂O/L (0.35 g/L), 10 mL KNO₃/L (30.3 g/L), 20 mL Ca (NO₃)₂/L (70.8 g/L) and 1 mL NaPO₄·H₂O/L (20.8 g/L). The pots were kept for 30 days under the same conditions of light and temperature. Periodic inspections for symptoms of fusarium wilt were made in order to determine the Disease Severity Index and area under the disease progress curve, as described above.

2.5. Statistical analysis

The least squares fits of different curve models were compared by calculating their correlation coefficients using the expression that $r = 1$. The linear and sigmoid MMF (Morgan-Mercer-Flodin) models were chosen for their adaptation to biological processes and high correlation coefficients.

Microsoft Excel 2000 and the CurveFinder routine of the CurveExpert v.1.3 programs were used to determine and represent the functions of the regressions between the energy input and the number of colony-forming units in the conidial suspension, the number of colony-forming units in the contaminated substrate, and the final disease severity.

The Pearson correlation coefficient was calculated to study the correlation between the energy input and the area under the disease progress curve.

3. RESULTS AND DISCUSSION

3.1. Power of the prototype microwave oven

Table II shows the true power developed by the oven, the irradiation times, the initial and final temperatures, the increase in temperature (ΔT) and the true energy input, determined by irradiating seed trays containing 800 mL of water.

Table II shows the true power developed by the prototype of a commercial nursery.

<table>
<thead>
<tr>
<th>Irradiation time (s)</th>
<th>Initial temperature (ºC)</th>
<th>Final temperature (ºC)</th>
<th>Increase in temperature (ΔT ºC)</th>
<th>Energy (J)</th>
<th>Power (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>24.0</td>
<td>32.5</td>
<td>8.5</td>
<td>28 333.3</td>
<td>1144.4</td>
</tr>
<tr>
<td>60</td>
<td>24.0</td>
<td>47.5</td>
<td>23.5</td>
<td>78 333.3</td>
<td>1165.6</td>
</tr>
<tr>
<td>120</td>
<td>24.0</td>
<td>76.0</td>
<td>52.0</td>
<td>173 333.3</td>
<td>1244.4</td>
</tr>
<tr>
<td>180</td>
<td>24.0</td>
<td>88.0</td>
<td>64.0</td>
<td>213 333.3</td>
<td>1185.2</td>
</tr>
<tr>
<td>240</td>
<td>24.0</td>
<td>96.0</td>
<td>72.0</td>
<td>240 000.0</td>
<td>1140.0</td>
</tr>
</tbody>
</table>

The mean true power developed by the prototype was 1175.9 W.

A notable difference was seen between the energy consumed by the magnetrons and their respective electronics, and the radiation energy output. This suggests that, although the method is efficient, the prototype makes relatively poor use of the energy it is supplied with (performance rating 0.3), an aspect that should be improved when attempting to make a commercial model.

3.2. Effect of irradiation time on Fusarium oxysporum f. sp. melonis race 1.2 conidial suspensions

The Morgan-Mercer-Flodin curve that best represented the change in the number of colony-forming units/mL of conidial suspension with respect to irradiation time was ($r = 0.99$)

$$y = \frac{0.109 + 39 \cdot 10^{-4} \cdot x^{-5.52}}{0.0107 + x^{-5.52}}$$

($x =$ energy in J; $y =$ final disease severity index) (Fig. 3).
When the energy input was < 5879.6 J (5-s irradiation), the number of colony-forming units/mL was uncountable. However, when the six test tubes containing the conidial suspension were irradiated with an energy input of 35 277.8 J (30-s irradiation), no viable colony-forming units remained. Below this level, the viability of the suspension decreased as the energy input increased. It is important to note that even when the energy applied was close to 11 759.3 J (10-s irradiation) a reduction in conidial viability was observed. This confirms the efficacy of microwaves for this task. The results of this first assay show that, in agreement with Mavrogianopoulos et al. (2000), microwave radiation can eradicate *Fusarium oxysporum* f. sp. *Melonis* and that the energy required is low. This should help nurseries protect their plantlets from this fungus, one of the most serious pathogens affecting melon plantlets derived from seeds.

### 3.3. Influence of irradiation time on the viability of the conidial suspension after contaminating the substrate employed in melon plantlet production

The line that best represented the change in the number of colony-forming units/g of substrate with respect to irradiation time \( r = 0.95 \) was \( y = 7.495 - 0.094 \cdot x \) (\( x = \) energy in J; \( y = \) final disease severity index) (Fig. 4).

When the energy input was <70 555.6 J (60-s irradiation) the reduction in pathogen viability was scant; however, when the energy input rose to 94 074.1–117 592.6 J (80- and 100-s irradiations), viability was greatly reduced. When the energy input was >141 111.1 J (120-s irradiation), total eradication of the pathogen was achieved. The number of colony-forming units/g remaining after such treatment was nil. Below this level, the number of colony-forming units/g of substrate decreased as the energy input increased.

It should be noted that no significant differences (95% confidence level) were ever recorded between the four blocks of 10 plantlets inoculated with conidia irradiated at each energy level. This highlights the great uniformity of the efficacy of each microwave treatment.

### 3.4. Influence of different irradiation times on the pathogenicity of the conidial suspension

The change in the Disease Severity Index (percentage) over time (number of days elapsed since inoculation) after irradiation of the conidial suspension for different lengths of time was represented on Cartesian axes. This provided the disease progression curve (Fig. 5). From this the area under the disease progress curve was calculated. The line that best represented the area under the disease progress curve with respect to irradiation time \( r = 0.89 \) was \( y = 1763.47 - 71.78 \cdot x \) (\( x = \) energy in J; \( y = \) final disease severity index) (Fig. 6).

No signs of fusarium wilt appeared in the plants whose roots were either submerged in sterile water (control –) or in the conidial suspension following its irradiation with an energy input of 35 277.8 J (30-s irradiation). This shows that no contamination occurred and, more importantly, that microwave
treatment at a sufficiently high energy level can eradicate the fungus in aqueous suspension.

The disease progression curve shows when the energy input is below that required to eradicate the pathogen, diseased plants can still appear over time. This demonstrates that although lower levels of energy may not eliminate the fungus, they can reduce its aggressiveness, leading to an increase in its incubation time. This delay lengthens as the energy input increases towards the eradication level (35 277.8 J; 30-s irradiation). In the present assays, delays of up to 10 days were recorded.

Analysis of the area under the disease progress curve shows that as the energy input to the contaminated substrate increases, the area under the curve becomes smaller, and is eventually extinguished when eradication energy levels are used. Pearson correlation analysis showed a strong, negative correlation ($p = 0.0031; 99.69\%$ CI) between energy input and the area under the disease progress curve. These assays indicate that the energy necessary to prevent infection is very low, a finding that contrasts with the results of Lal and Reed (1988), Martyn et al. (1996), Rice and Puthan (1997) and Mavrogiannopoulos et al. (2000). This might be explained by the fact that, in the commercial production of melon plantlets, the mass of substrate used is very small.

### 3.5. Influence of irradiation time on fungal viability in seed trays containing artificially contaminated substrate, in conditions similar to those of a commercial nursery

An input of 141 111.1 J (120-s irradiation) to each of the three input trays containing substrate artificially contaminated with the fungus which, according to the earlier assays is sufficient to eradicate the pathogen, allowed the melon plantlets to develop normally both in the trays themselves and after their transplant to plastic pots. No signs of fusarium wilt were observed.

The influence of irradiation time on fungal viability in seed trays containing artificially contaminated substrate, in conditions similar to those of a commercial nursery, showed that microwave treatment at a sufficiently high energy level can eradicate the fungus not only from aqueous suspensions but also from growth substrate. Given the small amount of energy consumed by the oven, any increase in plantlet production costs would be minimal. At a production rate of 4 x 10^6 plantlets per year (quite a normal figure for nurseries in Castilla-La Mancha), and for a required 141 111.1 J (120-s irradiation) to eradicate the fungus from seed trays with 216 cells, an (electrical) energy consumption of 2500 KWh (90 000 x 10^3 J) is required (taking into account the performance of the prototype). Given that in Spain the price of 1 KWh is approximately € 0.10, the cost of introducing this prototype (even with its low energy performance) into the production system would only be € 250 per year. This would increase the final price of each plantlet to growers by only 6.25 x 10^-3 Euro cents over the current market price of 19 Euro cents per plant.

### 4. CONCLUSION

Although further work is necessary to determine the effect that microwave radiation has on the chlamydospores and other more resistant structures produced by this fungus, the microwave oven tested provides a rapid, economic, efficient, safe, non-destructive, non-contaminating and easy method for eradicating the transmission of fusarium wilt, even though its energy performance is relatively low. The process leaves no residues and poses no risks to operators or final handlers of the plants thus raised.

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