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Microwave energy supplied by a prototype oven prevents the spread of Fusarium wilt during the propagation of melon plantlets by seed

M. L. Soriano-Martín*, A. Porras-Piedra, I. Marcilla Goldaracena, A. Porras-Soriano, D. Martín-Sánchez, M. León-Egido, M. L. Porras-Soriano and R. Porras-Soriano

EUITA. Ronda de Calatrava, 7. 13071 Ciudad Real. Spain

Abstract

The re-use of propagation trays in nursery greenhouses is one of the main ways in which fusarium wilt is spread in melon crops (*Cucumis melo*). The causal agent of the disease is the fungus *Fusarium oxysporum* f. sp. *melonis*. This paper reports that exposing these seed trays to the energy produced by a prototype microwave oven during the commercial production of melon plantlets can prevent the spread of this pathogen with only a very small increase in production costs.

Additional key words: artificial substrate, *Fusarium oxysporum* f. sp. *melonis*, microwaves, seed trays.

Resumen

Prototipo de horno microondas para la aplicación de energía limpia para evitar la dispersión de la fusariosis durante la propagación de semillas de melón

La reutilización de las bandejas de alvéolos empleadas por los productores de plántulas en invernaderos especializados es una de las principales causas responsables de la dispersión de la fusariosis vascular del melón (*Cucumis melo*), causada por el agente patógeno *Fusarium oxysporum* f. sp. *melonis*. En este trabajo se ha demostrado que, durante la producción comercial de plántulas de melón, aplicando a dichas bandejas la energía producida por un prototipo de horno microondas, es posible, con una reducida incidencia en el coste de producción de las plántulas, evitar la dispersión de la fusariosis en el proceso comercial de producción de plántulas de melón.

Palabras clave adicionales: bandejas de propagación, *Fusarium oxysporum* f. sp. *melonis,* ondas de alta frecuencia, substrato artificial.

Introduction

Fusarium wilt, caused by the fungus *Fusarium oxysporum* Schlecht f. sp. *melonis* Snaider and Hansen (Fom), is a destructive disease of melons that can reduce production by up to 90% (González Torres *et al.,* 1988; Champaco *et al.,* 1993).

In Castilla-La Mancha, the foremost melon-producing region of Spain, transplanted melon plantlets are grown under plastic. These plantlets, produced by nurseries, are raised in controlled environment chambers in polyurethane propagation trays containing a number of cells filled with substrate; one melon seed is planted in each. The plantlets thus produced cost something over 0.19 € each, which gives an idea of the importance of this industry.

The re-use of propagation trays is one of the main ways in which fusarium wilt is spread in melon crops. *Fusarium oxysporum*. f. sp. *apii* (Awuah and Lorbeer, 1991), which affects celery, is spread in the same way.

The fight against this disease in non-resistant cultivars requires the use of pathogen-free seed and the eradication of the fungus from the propagation trays and the substrate they contain (Evcil and Yalcin, 1977). Chemical methods can be used to achieve this, but the compounds used (sodium hypochlorite and formol) can be toxic to young plants and their use

* Corresponding author: luisa.soriano@uclm.es
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A. Porras-Piedra is member of SEA.

entails risks for both handlers and the environment. Conventional thermal methods are inappropriate too since they are also harmful to the environment and cannot be used to disinfect the propagation trays. The final option, solarization, cannot guarantee eradication and in any event would require leaving the trays exposed to the sun for excessively long periods (Awuah and Lorbeer, 1991).

Investigations into the use of microwaves have shown that high frequency energy (2.4 GHz) can destroy a wide range of soil parasites. However, most researchers in the field indicate that large amount of energy are required – a major disadvantage (Lozano *et al*., 1986; Martyn and Amador, 1987; Lal and Reed, 1988; Bhaskara *et al.*, 1998; Mavrogianopoulos *et al*., 2000). The present authors, however, reported that only very low amounts of energy and short exposure times are necessary to prevent the spread of *Fusarium oxysporum* Schlecht f. sp. *melonis* during the commercial propagation of melon by seeds (Soriano-Martín *et al.,* 2006). To scale-up these earlier experiments, which involved the use of a kitchen microwave oven (Soriano-Martín *et al*., 2006) to a level more similar to commercial melon plantlet production, a prototype microwave oven was designed that can irradiate the polyurethane propagation trays used in nurseries with high frequency microwaves (2.45 GHz). This paper investigated the amount of energy required to prevent the spread of *Fusarium oxysporum* f. sp. *melonis* during the commercial propagation of melon plantlets, and shows that the effect of this on the final cost of these plantlets is virtually negligible.

Material and Methods

'Amarillo Canario' melon (*Cucumis melo* L.) seeds were used in all assays. This cultivar is susceptible to all strains of *Fusarium oxysporum* f. sp. *melonis*. All seeds were surface-sterilised by submerging them for 2 min in 10% sodium hypochlorite.

The pathogen used was the monoconidial strain of *Fusarium oxysporum* f. sp. *melonis* race 1.2 (CR 6801), obtained from infected commercial crops in the Province of Ciudad Real (Spain). The pathogen was stored in sterile sand at 5 ± 1ºC and cultivated on potato dextrose agar for the preparation of the inoculum.

Figure 1 shows the prototype microwave oven. The apparatus was made with 2 mm galvanised steel sheeting (given shape by appropriate folding and cutting machinery) held together with aluminium rivets. It has two vertically opening doors, one on either side, whose movements are controlled by a hydrostatic power transmission system (Roquet, model 9315). Its 122 L volume allows three 70 × 46 × 7.5 cm propagation trays to be introduced at the same time. The oven has four 1,000 W theoretical power magnetrons (Fagor MV3-254 B) that produce microwaves at 2.45 GHz. These magnetrons are mounted in pairs on the fixed walls of the oven, perpendicular to the doors. Both the hydrostatic equipment and the oven itself work with 220 V AC.

The propagation trays were introduced manually into the oven in a horizontal fashion. After irradiation they were removed with the help of a ramp at the exit door. As the trays entered the oven they pushed out those that had already been irradiated; these slid down the exit ramp.

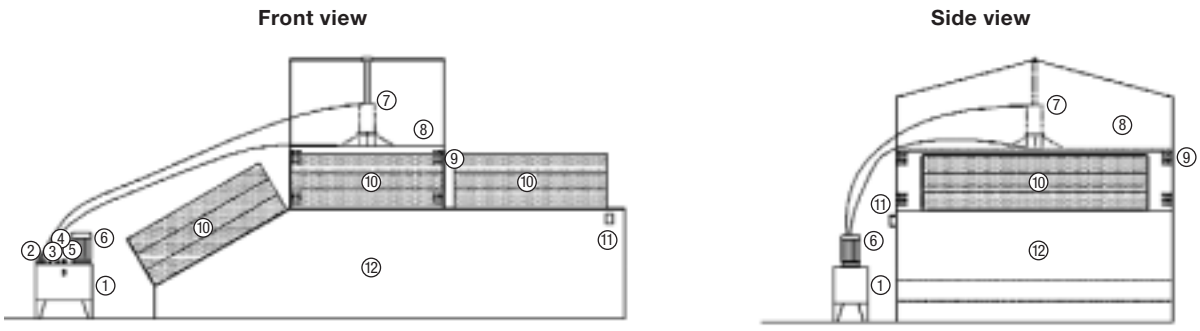

Figure 1. Diagram of the prototype microwave oven.

All trials were performed by switching the current manually (although the prototype now has a Cebex model I-25 high precision digital timer that switches the magnetrons on and off). The true power produced by the oven was measured using 800 ml trays made of thin expanded polyurethane (a microwave-transparent material) filled with water. These were introduced into the oven and the temperature of the water recorded before and after irradiation using a Checktemp low inertia digital thermometer. The irradiation times used were shorter than those required to boil the water. The true power delivered by the oven was calculated using the expression:

$$N = 3333.33\ (\Delta T / t)$$

where N is the true power delivered (in W), ΔT the change in water temperature, and t the irradiation time.

Two assays were performed, the first to determine the influence of microwave treatment on the pathogenicity of conidial suspensions of the fungus, and the second (under conditions similar to those of commercial melon plantlet production) to determine the influence of irradiation on the fungus in propagation trays containing experimentally contaminated substrate.

In the first assay, several hundreds of melon seeds were placed between two layers of absorbent paper, moistened with sterile water and placed in Petri dishes containing sterile vermiculite. These plates were kept for 5 days in a growth chamber under a 16 h light/8 h dark cycle (450 μmol E m^{-2} s^{-1}) at 25 ± 2ºC and at a relative humidity of 75%. The seeds were watered every 48 h with sterile water. Three hundred and twenty plastic pots (diameter 8 cm, height 12 cm) were then filled with sterile substrate and one germinated melon seed was planted in each. These pots were then divided into groups of 40 and placed on polyethylene trays with a metallic grill base. The pots were watered to saturation and the trays introduced into a controlled environment chamber where they remained for 13 days under the same conditions outlined above. The plantlets were watered every two days. When these had developed their first true leaf they were inoculated with the fungus by immersing their roots for 2 min in a conidial suspension (5×10^6 spores ml^{-1}) previously irradiated in the prototype oven for 5, 10, 15, 20, 25 or 30 s. Four blocks of 10 plantlets per block underwent each of these treatments. The control plants were inoculated by submerging their roots in a non-irradiated conidial suspension (control +). The roots of plantlets in another four blocks of 10 plantlets per block were submerged in sterile distilled water (control –). Table 1 summarises the treatments applied in the first assay.

Following inoculation the plantlets were again placed in their plastic pots containing sterile substrate, and these placed once again on the above-mentioned trays. These were then introduced into the controlled environment chamber where they remained under the above conditions.

Disease development was monitored every two days. The number of plants with disease and the severity of their symptoms was evaluated on a scale of 0-4 indicating the percentage of plants showing yellowing or necrosis (0 = 0%; 1 = 1-33%; 2 = 34-66%, 3 = 67-95%; 4 = all dead). These disease severity data were used to calculate a Disease Severity Index (*DSI*, represented as a percentage) as follows:

$$DSI\,(\%) = \frac{\sum_{i=1}^{i=4} n_i \cdot s_i}{4 \cdot N} \cdot 100$$

Table 1. Treatments to quantify the influence of microwave radiation on the pathogenicity of *Fusarium oxysporum* f. sp. *melonis* conidial suspensions (CS)

Group	Number of blocks/group	Number of plants/block	Treatment applied to the roots by immersion into:
1 (Control –)	4	10	Distilled water
2	4	10	CS irradiated for 5 s
3	4	10	CS irradiated for 10 s
4	4	10	CS irradiated for 15 s
5	4	10	CS irradiated for 20 s
6	4	10	CS irradiated for 25 s
7	4	10	CS irradiated for 30 s
8 (Control +)	4	10	Non-irradiated CS

where n_i = the number of plants affected at each severity level, s_i = degree of disease severity (0 to 4), and N = total number of plants in assay.

To study the influence of irradiating the propagation trays containing substrate experimentally contaminated with the pathogen, three commercial propagation trays were filled with sterile substrate. Ten cells of each tray were then selected at random and 3 ml of the conidial suspension (5×10^6 spores ml^{-1}) added to their substrate. These trays were then introduced individually into the microwave oven and irradiated for 120 s – a time which should have allowed the eradication of the pathogen given the quantity of material placed in the oven. Once irradiated, these trays were sown with one melon seed per cell. The trays were then introduced into a controlled environment chamber at 25 ± 2ºC and maintained in the dark. Once the cotyledons had emerged (5-6 days) the plantlets were kept for the next 22 days as above [16 h light/8 h darkness (450 µmol E m^{-2} s^{-1}), 25 ± 2ºC, 75% relative humidity, plants watered every 48 h]. They were then transplanted, one by one, into 3 L plastic pots containing a 1:1 v/v mixture of peat and sand. Hewitt's solution (100 ml) was provided as a fertilizer at transplant and again 15 days later. The plantlets were kept for 30 days under the same conditions of light and temperature described above. All were monitored for the appearance of symptoms of fusarium wilt in order to determine the *DSI*.

Results

The true mean power of the microwave oven was 1175.9 W. In assay one, the changes in *DSI* over time (days elapsed since inoculation) caused by the conidial suspensions that received different doses of radiation were determined by plotting the *DSI* against the number of days after inoculation; this provided the corresponding disease progression curves (Fig. 2). In the plants artificially contaminated with conidial suspensions that had received low doses of microwave radiation (≤ 15 s), the first disease symptoms appeared after just two days. After 12 days more than 80% of these plants were sick, and at the end of the experiment nearly all were dead. When irradiation was ≤ 20 s, however, 11 days went by before the first symptoms appeared, and in no case was the *DSI* in excess of 20% at the end of the experiment. When the irradiation time was 30 s, no plants showed any symptoms of disease.

In assay two, the application of 141,111.1 J (120 s irradiation time; sufficient to eradicate the fungus

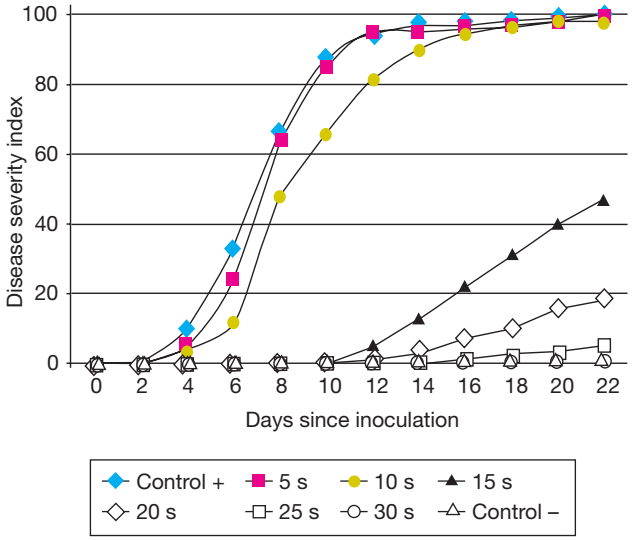

Figure 2. Disease progress curves for Fusarium wilt in 'Amarillo Canario' melon plantlets inoculated by submerging their roots in an aqueous suspension of *Fusarium oxysporum* f. sp. *melonis* race 1.2 conidia irradiated with different doses of microwave energy.

Table 2. Assay to determine the influence of microwave radiation on the propagation of *Fusarium oxysporum* f. sp. *melonis* in tray cells

Number of trays	Number of cells per tray	Number of cells artificially contaminated per tray	Treatment applied	Number of plants affected at the end of the second assay
3	216	10	Microwave irradiation for 120 s	0/648

according to preliminary assays) of microwave energy to the propagation trays containing the contaminated substrate rendered the pathogen completely non-viable. The plantlets that grew in these trays (216 plants per tray, 3 trays) showed no signs of Fusarium wilt, nor were any symptoms seen after their transplantation to pots containing peat and sand (10 cells artificially contaminated per tray before treatment). Table 2 summarises the results of treatments applied in the second assay.

Discussion

The prototype oven showed notable energy losses: a large difference was seen between the energy consumed by the magnetrons and the mean output of microwave energy (only 30% of the input energy became microwave energy). This low energy performance will require improvement before a commercial model is produced.

In the first assay, neither the plants whose roots were submerged in the distilled water (control –) nor those that were inoculated by immersion in the 5.10^6 spores ml^{-1} conidial suspension that received at least 35,277.8 J of microwave energy (30 s irradiation), showed any symptoms of disease. This indicates that no undesirable contamination had occurred, and more importantly that this level of irradiation was sufficient to eradicate the fungus in aqueous suspension.

The disease progress curves showed that when the energy applied was less than optimum for destroying the pathogen, diseased plants begin to appear over time. This suggests that the pathogen, although rendered less aggressive by such treatment, was not eliminated; sub-optimum irradiation only serves to lengthen the incubation period.

Unlike that reported by other authors (Lozano *et al*., 1986; Martyn and Amador, 1987; Lal and Reed, 1988; Bhaskara *et al.*, 1998; Mavrogianopoulos *et al*., 2000), the energy required to avoid the spread of *Fusarium oxysporum* f. sp. *melonis* race 1.2 during the commercial production of melon plants is very low. This can be explained by the fact that, in commercial melon production, the mass of substrate used is very small. If, as the results show, that 141111.1 J (120 s irradiation) are required to eradicate the pathogen from a 216-cell propagation tray, then for a nursery with a production rate of 4×10^6 plants per year, an energy consumption of just 2,500 kWh ($90{,}000 \times 10^5$ J – given the efficiency of the present oven) would be necessary to make sure all were free of Fusarium wilt. In Spain, 1 kWh of electricity costs about 0.10 €; therefore the cost of such an operation would be just 250 € per year. The effect of this on the cost of the plantlets to growers would be almost negligible compared to the price currently paid (0.19 €). It should be remembered that the increment for fixed and variable costs associated with the acquisition and running of the oven need to be added; however these are likely to be negligible given the length of time the oven should last and the low cost of repairs and maintenance associated with this technology.

In conclusion, this prototype microwave oven, although of low energy performance provides a rapid, efficient, non-destructive, non-contaminating and economic means of eradicating Fusarium wilt in commercial melon plantlet production. The apparatus is safe to use and poses no risk either to its operators or to the plants eventually produced.

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