Reinvasion of Fumigated Soil by *Fusarium oxysporum* f. sp. *melonis*

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ABSTRACT

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A field with a history of Fusarium wilt of muskmelon was fumigated with Vorlex® (1,2-dichloropropane, 1,3-dichloropropene, and other related compounds plus methyl isothiocyanate) at 280 L/ha in 72-cm-wide beds and mulched with clear polyethylene. The pathogen was not detected 6 days after fumigation; however, by 32 days after fumigation, the pathogen was isolated consistently from the outer edges of the bed. Seventy-four days after fumigation there was a distinct pathogen population density gradient from the bottom 25 cm of soil to the top 5 cm of soil. By 102 days, the pathogen was distributed throughout the bed, similar to the nonfumigated beds. An experimental computer model was developed, which simulated the distribution of the pathogen propagules at weekly intervals after fumigation. The model indicated that the pathogen would spread more rapidly at depths of 15–20 cm than at 0–5 cm, and that an increase in depth and width of fumigated soil would be more effective in controlling pathogen recolonization than an increase in depth or width alone.

The combined use of broad spectrum biocides as soil fumigants and clear or black polyethylene mulch is standard practice in many agricultural production systems and results in earlier and increased yields of numerous vegetable and fruit crops. The soil fumigants control many plant pathogens, nematodes, and weeds. The polyethylene mulch maintains optimum soil temperature, reduces leaching of fertilizers, increases the efficiency of irrigation systems, and reduces diseases by preventing direct contact of fruit with soil. An excellent example of the success of fumigant-polyethylene mulch combination is the control of Verticillium wilt of strawberry in California (13).

Contrary to the many successes of integrated application of soil fumigants and polyethylene mulch, there are instances in which soilborne pathogens are not controlled. This is particularly true when a pathogen can grow as a saprophyte and is therefore able to reinvade fumigated soil, a phenomenon frequently observed when broad-spectrum biocides are applied to soils in an attempt to control diseases caused by *Fusarium oxysporum* (2,3,5,11).

Although *F. oxysporum* does not compete well as a soil saprophyte in unfumigated soils (9), it is a highly effective colonizer of fumigated soils, due to decreased competition and increased nutrient sources in freshly treated soils. The reinfestation of fumigated soils by a pathogen depends on its passive or active distribution through the soil. Passive distribution of fungal propagules results from the movement of water, soil fauna, or soil particles. Active distribution is movement by zoospores or growth of mycelium. Growth of mycelium depends on the ability of the fungus to compete for available substrates and to grow outward from a colonized food base. Although a pathogen may be able to grow actively through a freshly fumigated soil by utilizing available substrates, its ability to do so may diminish as competition becomes more severe with time after fumigation (8).

Fusarium oxysporum Schl. f. sp. *melonis* Snyd. and Hans., the causal agent of Fusarium wilt of muskmelon (*Cucumis melo* L. var. *reticulatus* Navd.), was investigated because the crop is grown on polyethylene mulched beds, broad-spectrum biocides are used to control insects, weeds, and pathogens, and the disease is severe in pathogen-infested fields even after fumigation.

The objectives of this study were to determine the effectiveness of the fumigants in reducing the population of the pathogen and to determine the potential of the pathogen to reinvade the fumigated soils. An experimental computer model was developed to identify important growth parameters involved in the reinvasion of soil by the pathogen. Portions of this work have been reported previously (7).

MATERIALS AND METHODS

A field at the University of Maryland Vegetable Research Farm, Salisbury, MD, with Sassafras sandy loam (pH 6.5), was fumigated with Vorlex® (80% chlorinated C_3 hydrocarbons [1,2-dichloropropane, 1,3-dichloropropene, and other related compounds] and 20% methyl isothiocyanate) (Nor-Am, Chicago, IL 60606) at 280 L/ha by injecting the fumigant to a depth of 15 cm with four chisels 20 cm apart. The 72-cm-wide raised bed was covered immediately with clear (2-mil) polyethylene 0.051-mm, which was left on the soil for the entire growing season. Two weeks after fumigation, the field was planted with muskmelon, cultivar Goldstar, as it had been for the past several years. The field had a history of high disease incidence, often with 100% of the plants killed before harvest. The experiment was conducted in 1981 and repeated in 1982.

After fumigation of the soil, the pathogen population density was monitored in 3-m-long, nonplanted areas of the beds. Every 14 days, nine soil cores 2.5 cm in diameter and 25 cm in depth were obtained at 8-cm intervals across the bed. Each core was divided into five 5-cm-segments, which resulted in cylindrical plugs 2.5 cm in diameter and 5 cm long. The 5×9 sampling pattern in a vertical plane provided 45 samples from each of four fumigated sites and two nonfumigated control sites arranged randomly in the field.

The population of *F. oxysporum* f. sp. *melonis* was determined in each soil sample by spreading 1 ml of a 1/20 dilution (5 g of soil per 100 ml of water) onto Komada's medium (6), which is selective for *F. oxysporum*. Plates were incubated in the dark at 23–26 C and examined for colony-forming units (*CFU*) after 10 days. Approximately 50 isolates from each sample date were transferred to potato-dextrose agar (PDA; Difco, Detroit, MI 48201). After growth for 2 wk at 23–26 C under continuous cool-white

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fluorescent light (3,000 lux), a conidial suspension (10^4 conidia per milliliter) was prepared from each isolate by washing the plates with distilled-autoclaved water. Seventy-five milliliters of the resulting conidial suspension were placed in three 1.5 × 20-cm test tubes, and one 2-wk-old seedling (cultivar Goldstar) grown in autoclaved peat-perlite was added to each test tube. After 2 wk in a greenhouse, the seedlings were compared to controls for symptom expression. If any of the three seedlings exhibited symptoms, the isolate was considered to be *F. oxysporum* f. sp. *melonis*. The test is a simplified version of that developed by Wensley and McKeen (12).

Population data were plotted on three dimensional graphs. An experimental computer model was used to test if data similar to results from the field could be generated. In the model, soil was represented as a 3-dimensional matrix (width × depth × length), which was composed of nine cells for width (representing the 72-cm-wide bed) and five cells for depth (representing a depth of 25 cm). Therefore, each cell represented an 8 × 5-cm^2 area, and any cell could be referred to by its horizontal and vertical coordinates (W,D). This two-dimensional plane across the bed thus consisted of 45 cells. The third dimension, length, was represented by the cells on either side of the cells described by the two-dimensional plane across the bed. A schematic view of a portion of this plane is shown in Fig. 1.

The model estimated the number of *CFU* in each cell by calculating the increase of the population within the cell using the logistic growth function and adding to it the population contributed by net immigration from the six adjacent cells (Fig. 1). The *CFU* was assumed to be directly correlated with the population density of the pathogen. The logarithmic growth curve was used because the formula fit the data from preliminary tests (J. J. Marois, *unpublished*). The following equation was developed:

$$CFU_{(W,D)t+1} = [K/\{1+((K-CFU_{(W,D)t})/(1+CFU_{(W,D)t}))e^{-r}\}] + (CFU_{(W-1,D)t} \cdot X_D) + (CFU_{(W+1,D)t} \cdot X_D) + (CFU_{(W,D+1)t} \cdot X_{D+1}) + (CFU_{(W,D-1)t} \cdot X_{D-1}) + 2(CFU_{(W,D)t} \cdot X_D)$$

in which: CFU = the number of colony-forming units in cell W,D; K = 1,200, the maximum asymptote; r = rate of increase of CFU due to multiplication within the cell; X = net rate of immigration from adjoining cells to cell W,D; t = time in weeks.

The first component of the formula is a modified logarithmic growth function, the middle components describe immigration from surrounding cells, and the last component of the formula ($2[CFU_{(W,D)t} \cdot X_D]$) allows for the third dimension, length, which is assumed constant (but important) in immigration. Using the *CFU* in each cell provided by the 32-day baseline data, this equation computed the population increase in each cell, which represented approximate growth during 1 wk. Thus, subsequent computations using this equation and the previously computed data provided a stepwise view of the population increase, which, after a simulated 7-wk period, approximated the 74-day level observed in the field.

The computer model was modified to simulate the effect of the injection of fumigants deeper into the soil by eliminating the pathogen population from the lower cells in the middle of the bed, in the 32-day baseline data. The model also was modified to simulate the fumigation of a 120-cm-wide bed by inserting cells for 48 cm of bed into the middle of the bed and assuming that the densities in the lower cells were consistent with those observed in the 32-day data. The model was then modified to simulate the combined effects of deeper fumigation and wider bed fumigation. After the above modifications, the model was used to simulate population increase over the next 42 days.

RESULTS

Recolonization observed in the field. The population density of *F. oxysporum* f. sp. *melonis* varied from 200 to 400 *CFU*/g of air-dried soil in the nonfumigated control plots. The population density was relatively uniform across the bed in mulched nonfumigated soils (Fig. 2). Approximately 85 to 95% of the isolates of *F. oxysporum* from nonfumigated and fumigated soils were determined to be pathogenic to muskmelon.

The pathogen was not detected in treated soils 6 days after fumigation. Fourteen days after fumigation, the pathogen was isolated sporadically from the edges of the bed. However, by 32 days after fumigation, the pathogen was isolated consistently from the edges of the bed at all depths and occasionally from the 20–25 cm depth (Fig. 3). The average population density of the pathogen was 33.5 ± 41 *CFU*/g of soil at the outer edge of the bed, but the pathogen was not detected in the middle of the bed.

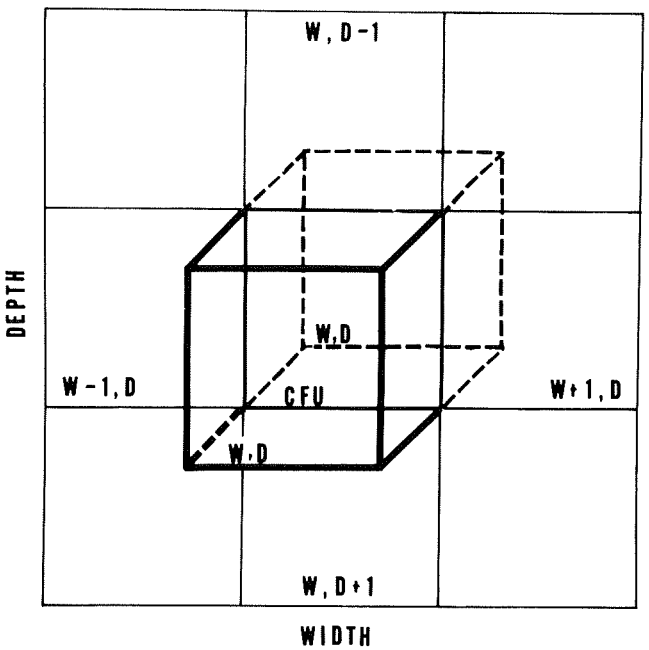

Fig. 1. Schematic representation of experimental simulation model of three dimensional spread of *Fusarium oxysporum* f. sp. *melonis* in fumigated soils. The colony-forming units (*CFU*) of the pathogen in the center square (W,D) depend on the amount of immigration from the six squares adjacent to it and the amount of increase due to population growth within the square.

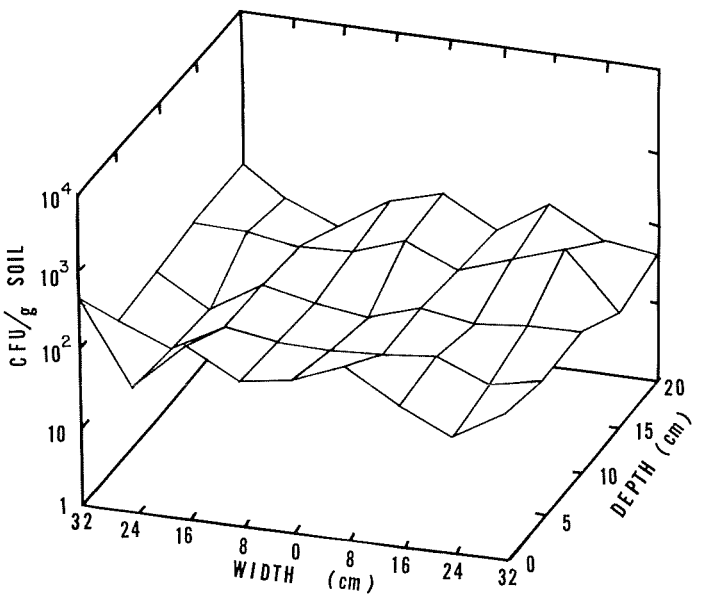

Fig. 2. Distribution and population density of *Fusarium oxysporum* f. sp. *melonis* in polyethylene mulched bed not fumigated.

Seventy-four days after fumigation, the pathogen population density was higher at a soil depth of 20–25 cm than at the 0–5 cm depth, and there was an even distribution of the pathogen across the bed at the 10–25 cm depths (Fig. 4). However, the higher populations at the edges of the bed were still evident at the 0–10 cm depths. The pathogen still was not detected in the center-top 5 cm of the bed, even 74 days after fumigation, although most of the soil in the remainder of the bed was recolonized with the pathogen attaining population densities as high or higher than those in the nonfumigated soils.

One hundred four days after fumigation, the entire bed was recolonized with the pathogen and no discernible population density gradients existed in either depth or width of the bed (Fig. 5). The pathogen reinvaded the bed at an average density of 270 *CFU*/g of soil, which was within the range of 200–400 *CFU*/g of soil observed in nonfumigated soils. Every soil sample contained the pathogen, including the sample from the center-top of the bed.

Recolonization observed with an experimental computer model. The experimental computer model was designed to simulate the population growth observed 74 days after fumigation, with the population distribution observed 32 days after fumigation used as the input into the model equation. The model simulated actual field results (Fig. 6) when the immigration rate was adjusted for cell depth. For the model to simulate field results, it was necessary for the rate of immigration (X) of the pathogen to be different at each depth (1–5) and to be higher at the lower soil depths ($X_1 = 0.0$, $X_2 = 0.04$, $X_3 = 0.06$, $X_4 = 0.18$, $X_5 = 0.20$). The rate of increase (r) within a cell due to proliferation was set at 0.2. The maximum asymptote (k) was set at 1,200.

To simulate injection of fumigant to a depth of 30 cm rather than 20 cm, the model was modified by setting *CFU* values in the 32-day baseline data to zero in the lower cells in the center of the bed. When

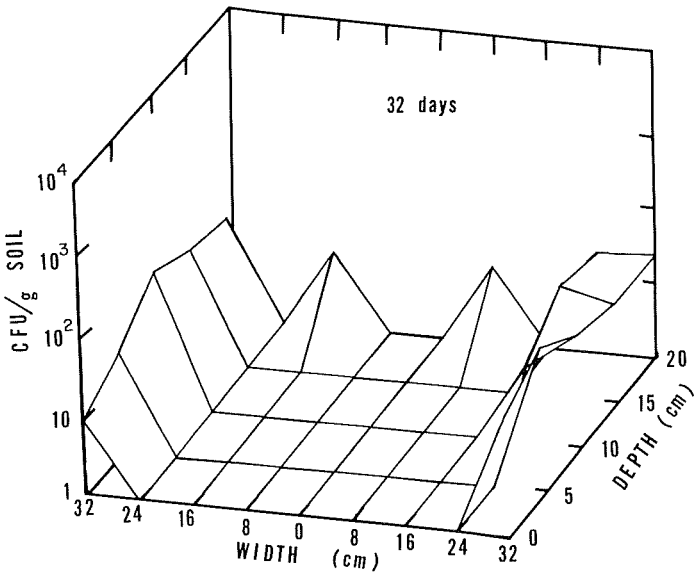

Fig. 3. Distribution and population density of *Fusarium oxysporum* f. sp. *melonis* in polyethylene mulched bed 32 days after fumigation with a mixture of 1,2-dichloropropane, 1,3-dichloropropene, and other related compounds plus methyl isothiocyanate at 280 L/ha.

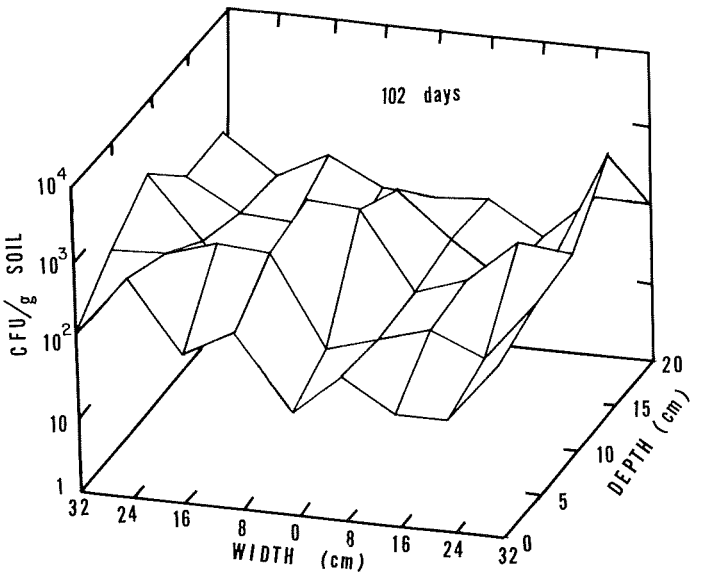

Fig. 5. Distribution and population density of *Fusarium oxysporum* f. sp. *melonis* in polyethylene mulched bed 120 days after fumigation with a mixture of 1,2-dichloropropane, 1,3-dichloropropene, and other related compounds plus methyl isothiocyanate at 280 L/ha.

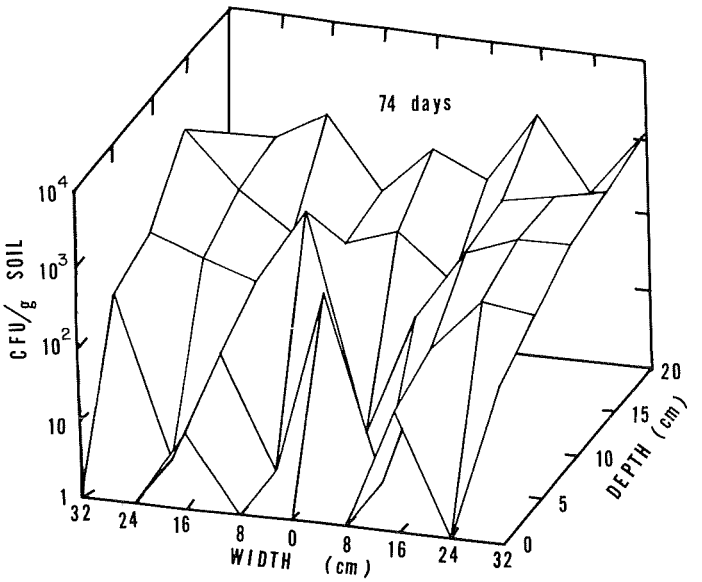

Fig. 4. Distribution and population density of *Fusarium oxysporum* f. sp. *melonis* in polyethylene mulched bed 74 days after fumigation with a mixture of 1,2-dichloropropane, 1,3-dichloropropene, and other related compounds plus methyl isothiocyanate at 280 L/ha.

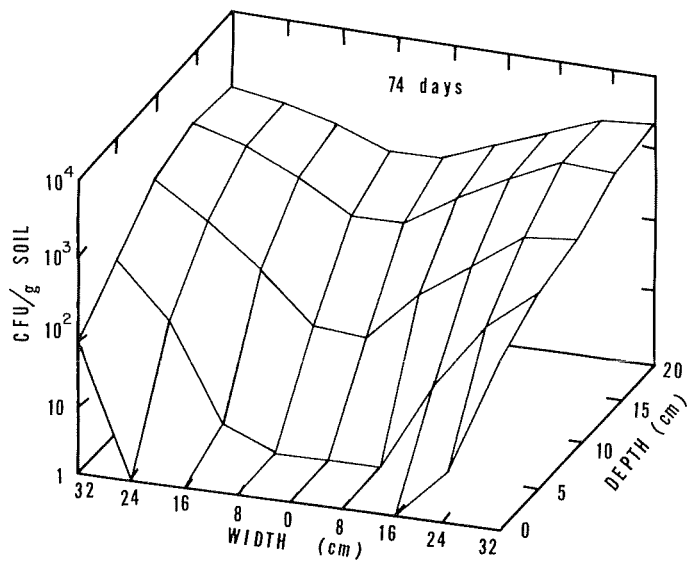

Fig. 6. Population density of *Fusarium oxysporum* f. sp. *melonis* from an experimental computer model 74 days after fumigation.

this was done, the simulated population distribution of the pathogen 74 days after fumigation was reduced in the upper center of the bed (Fig. 7).

In order to simulate a wider fumigation width (120-cm-wide, as opposed to the original 72-cm-wide bed), the model was modified by adding cells with comparable values in the center of the bed. The simulated pathogen population distribution was not altered except in the 0–5 cm soil depth and did not reinvade the middle of the bed, even at 74 days after fumigation (Fig. 8). A bed with deeper and wider fumigation procedures than the original was simulated by combining both of these modifications of the model, and the simulated pathogen population did not invade the center of the bed (Fig. 9).

DISCUSSION

The application of Vorlex® (1,2-dichloropropane, 1,3-dichloropropene, and other related compounds plus methyl isothiocyanate) reduced the population density of *F. oxysporum* f. sp. *melonis* to below detectable levels (20 *CFU*/g). The recolonization of the treated soils by the pathogen began at the outer edges of the polyethylene mulched bed and continued in an inward and upward direction. At first, populations at high densities were observed at the edges of the beds and low densities at the middle of the beds. The gradient eventually changed to one of high densities in the deep soils to low densities in surface soils. Population density gradients were not observed 104 days after fumigation. The pathogen invaded the soils from the outer edges of the bed. However, some of the inoculum source appeared to have originated from below 20 cm, which may have been beyond the zone of effective fumigation.

Inoculum that originated in the outer edges of the bed could have been due to the colonization of the soils by the pathogen population in the nonfumigated access rows or by survivors in the outer edges of the fumigated bed. Although the pathogen was not detected in the outer edges of the bed 6 days after fumigation, the fact that there may have been survivors cannot be discounted, and is to be expected when one considers that the outer edges of the bed are fumigated by one chisel with one dispersal gradient only, whereas the rest of the bed is fumigated with overlapping dispersal gradients from chisels on each side.

Hopkins and Elmstrom (5) reported that fumigation of a 186-cm-wide bed reduced Fusarium wilt of watermelon more effectively than fumigation of a 76-cm-wide bed. Also, when a 186-cm-wide bed was fumigated to a depth of 40 cm (twice the standard depth), disease control was further increased, but not significantly ($P = 0.05$). The effect of deeper fumigation on the 76-cm-wide bed was not determined. The experimental computer model indicated that an increase in the depth of the fumigation zone maintained a greater pathogen-free area of soil than did the increase in width of fumigation. The model also indicated that the combined increase in depth and width of fumigated soil would give the best control of pathogen recolonization.

Rate of spread of a pathogen is affected by biotic and abiotic soil factors. Park (9) reported that *F. oxysporum* does not move through soils by growth, but by mechanical movement of conidia in water. However, movement of water in raised beds under polyethylene mulch is restricted to capillary action. In these situations, the use of broad-spectrum soil fumigants favors the

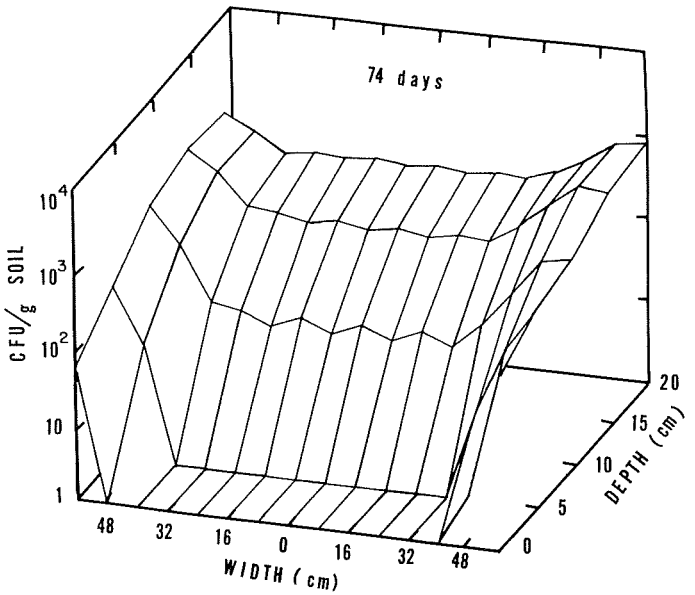

Fig. 8. Population density of *Fusarium oxysporum* f. sp. *melonis* from an experimental computer model 74 days after fumigation, when the width of fumigation was 120 cm rather than 72 cm.

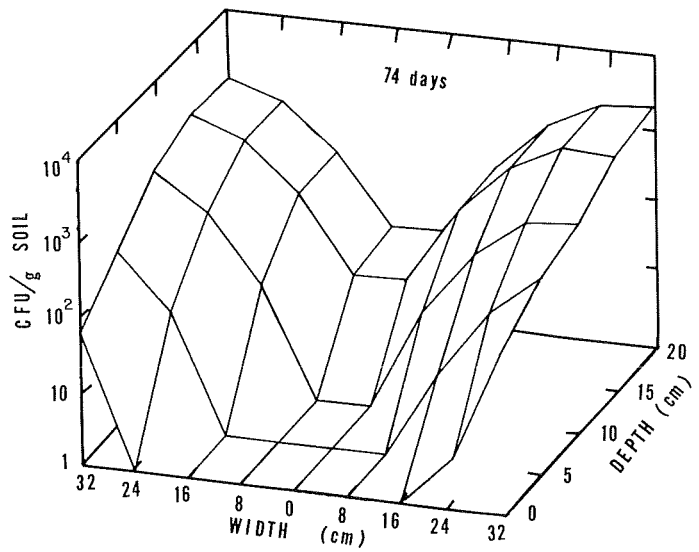

Fig. 7. Population density of *Fusarium oxysporum* f. sp. *melonis* from an experimental computer model 74 days after fumigation, when the depth of fumigation was 30 cm rather than 20 cm.

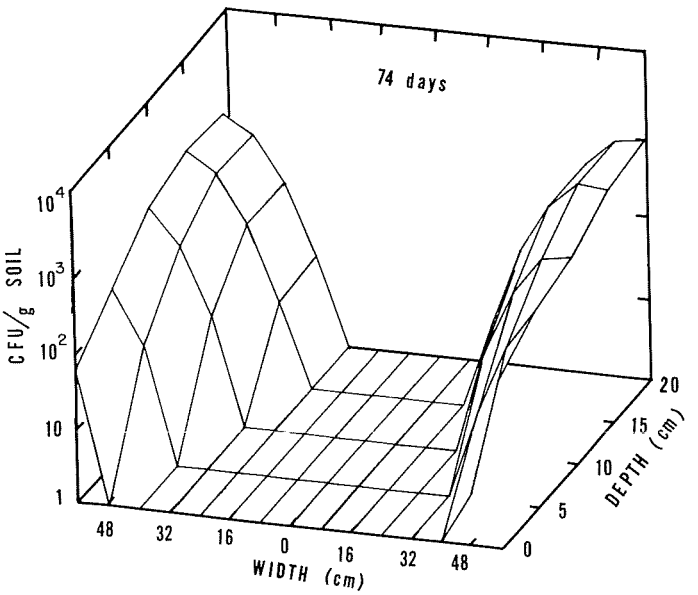

Fig. 9. Population density of *Fusarium oxysporum* f. sp. *melonis* from an experimental computer model 74 days after fumigation, when the width of fumigation was 120 cm and the depth of fumigation was 30 cm rather than 72 cm and 20 cm, respectively.

actual growth of the pathogen through the release of soil nutrients and the elimination of the extremely competitive biotic factors within the soil community. The ability of *F. oxysporum* to utilize and colonize soil substrates is dependent upon the level of competition in the soil ecosystem. Park (9) described *F. oxysporum* as a pioneer fungus because it could not colonize organic matter previously colonized by other soil organisms. Bollen (1) reported that *F. oxysporum* was one of the first organisms to recolonize treated soils. Hine (4) found that saprophytic abilities of *F. oxysporum* f. sp. *niveum* depended on the number of microorganisms in the soil and on the apparent saprophytic aggressiveness of the isolate. In this study, *F. oxysporum* f. sp. *melonis* spread rapidly through soils treated with a broad-spectrum biocide.

Pugh (10) has applied the concepts of low and high stress, and low and high life disturbance strategies, originally developed for higher plants, to the kingdom of fungi. *F. oxysporum* f. sp. *melonis* appears to belong in the low-stress/high-disturbance category because it has a high growth rate and rapid germination of propagules when exposed to fumigated soil (high disturbance) with few competitors (low stress). With addition of microorganisms to fumigated soils before reinvasion of the pathogen, the soil environment would be shifted to a high-stress/high-disturbance category, which should inhibit the saprophytic abilities of the pathogen (8). The utilization of these basic ecological principles will be used in further investigations to control Fusarium wilt of muskmelon.

In conclusion, *F. oxysporum* f. sp. *melonis* is able to recolonize soils treated with broad-spectrum biocides due to an increase in actual numbers of propagules and to an increase in area occupied by the organism. This ability depends on the absence of soil competitors and identifies the mechanism by which Fusarium wilt of muskmelon is a severe disease in fumigated soils.

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