Effect of Flooding on the Soil Gas Composition and the Production of Microsclerotia by Verticillium dahliae in the Field

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ABSTRACT

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The production of microsclerotia (MS) in tomato tissues infected with *Verticillium dahliae* was examined in soil subjected to different water treatments (no irrigation, one irrigation, and 10, 20, and 40 days of continuous flooding) under field conditions. About equal numbers of MS were produced in the dry and one-irrigation treatments. No, or very few, MS were produced during the flooding and this inhibition was due in large part to decreased O_2 and increased CO_2 concentrations in the flooded soil. Upon drainage, the concentrations of O_2 and CO_2 returned rapidly to normal atmospheric levels and MS production resumed. The numbers of MS eventually produced in the 10-, 20-, and 40-day flooding treatments were 90, 44, and 46%, respectively, of the average numbers in the nonflooded treatments. The reduction was significant ($P=0.05$) only in the 20- and 40-day flooding treatments. Ethylene, at levels ranging from traces to about 6.5 μliters/liter was detected in all treatments. Highest concentrations occurred in flooded or nearly saturated soils with added plant debris, but there was no indication that ethylene affected the production of MS.

Additional key words: tomato, soil microbiology, biological control.

Microsclerotia (MS) of *Verticillium dahliae* Kleb. are formed in moribund tissues of diseased plants when these are incorporated into the soil at the end of the growing season (5, and N. Ioannou, *unpublished*). Attempts to reduce the number of MS in soil have focused primarily on factors that affect longevity (3, 6, 15, 17). Factors associated with the initial production of MS have received little attention, although Menzies (16) pointed out that they also are important and may be easier to manipulate. In earlier laboratory work (12) it was observed that infected tomato stems incubated in saturated soil produced insignificant numbers of MS compared to stems incubated in soil at slightly reduced water potential (ψ) values. Further studies (11) showed that the production of MS in vitro and in infected tomato stems also was inhibited by low O_2 and high CO_2 concentrations. In the present study, the production of MS was examined in the field under different irrigation and flooding treatments. The effect of soil water on the gas composition of the soil atmosphere was given particular emphasis because of the dramatic effects of O_2 and CO_2 concentrations on MS production (11). Although ethylene in laboratory experiments did not affect growth, sporulation, or production of MS by *V. dahliae* (11), the production of this gas in soil also was examined in the present study. This was done because there is some evidence that ethylene is a major factor in soil biology (20, 21, 22, 23) and to determine the effect of soil conditions on its production (14).

MATERIALS AND METHODS

Placement of infested debris in soil.—Near Davis, California, the effect of soil flooding on the production of MS was studied in a field plot, which had been fallowed for the previous 5 yr, and in which the native population of *V. dahliae* MS was very low (0.2 -0.3 MS per g of air-dry soil). The soil was a well-drained Yolo loam with pH value 7.2 - 7.5 [in 1:2.5 soil-water suspension (4)] and organic matter content of about 0.65% [determined by the Walkley-Black chromic acid oxidation method (4)]. During the spring of 1975, the field was rototilled twice to control weeds and during the summer it was dry-fallowed. Twenty basins, each surrounded by a levee approximately 0.6-m high and 1-m wide, were built in the fall of 1975. Individual basins were 3×3 m (internal dimensions) and were separated by 2-m-wide walkways. To obtain infected tissues for inoculum, tomato plants (*Lycopersicon esculentum* 'VF-145-B-7879') were grown in a nearby heavily-infested plot (inoculum density about 50 MS per g of air-dry soil). After fruit harvest, the vines were collected and shredded with a corn harvester, and 0.15 m^3 of the shredded plant material was spread on the surface of each basin and covered to a depth of 10-12 cm with soil removed earlier from the surface of each basin. After placement of the plant material in the soil, five different water treatments; i.e., no water, one 12-hr irrigation, and 10-, 20-, and 40 days of continuous flooding, were applied. Loss of water from evaporation and downward percolation during flooding was replenished by continuous inflow of fresh water which was adjusted several times each day to maintain 10-20 cm of standing

water in the flooded plots. Each treatment was replicated three times in a completely randomized design. Five basins in which no plant material was added, also received the water treatments to allow a comparison of gas composition in soil with and without the infested plant debris. The following parameters were monitored at a depth of 10-12 cm (debris layer) for 75 days or longer: soil ψ, gas composition of the soil atmosphere, temperature, pH, and rate of organic matter decomposition. The production of MS was determined at intervals for 7 mo (September 1975 to April 1976).

Measurement of water potential.—Soil ψ was determined in situ with thermocouple psychrometers (Type PT51, Wescor, Inc., Logan, UT 84321). The ceramic tip of each thermocouple psychrometer was positioned in the layer of infested debris, at the center of each plot, and left in place for the duration of the experiment. Psychrometer lead wires extended underground to the edge of the plot from where readings were taken using a Peltier cooling current (2); readings were corrected for temperature (2).

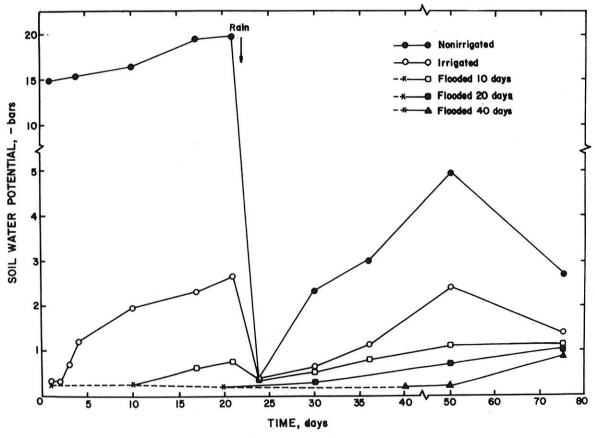

Fig. 1. Soil water potentials in field plots under different irrigation and continuous-flooding treatments. Water potentials were determined with thermocouple psychrometers buried at 10-12 cm deep. Each point represents the average of three values determined from three replicated plots. Note that a heavy rain (4.35 cm) on the 22nd day saturated all treatments.

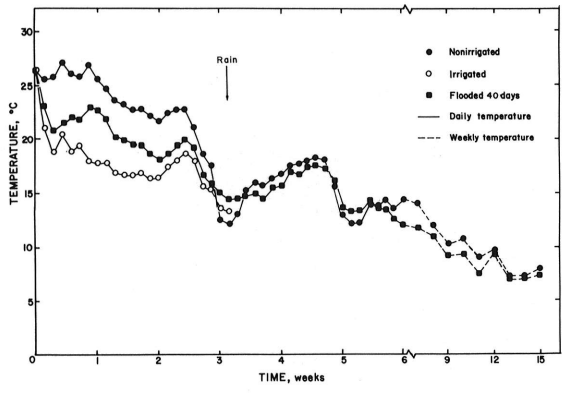

Fig. 2. Soil temperatures at a depth of 10-12 cm in field plots subjected to different water treatments. Soil temperatures were continuously recorded with soil thermographs and average daily (for the first 6 wk, starting 18 December) and weekly (for the last 9 wk, ending 31 December) temperatures calculated. Soil temperatures in the 10- and 20-day flooding treatments were very close to those in the 40-day treatment. Soil temperatures in the irrigated treatment after the 3rd wk were essentially the same as those in the nonirrigated treatment.

Gas sampling and analysis.—Gas samples were taken from the soil with a sampling device of the "equilibrium type" (24). Briefly, the sampler consisted of a gas reservoir which was buried in the soil at a depth of 10-12 cm and a collection tube that extended above the soil surface. The reservoir consisted of an inverted plastic (polystyrene) funnel (63-mm maximum diameter and 80-ml total capacity) and the collection tube was a capillary pipette (25-cm long and 0.5-ml total capacity). The two parts were joined with a short piece of Tygon tubing. A piece of surgical tubing (3-4 cm long), sealed at one end with a glass plug, was attached to the top end of the collection tube as a sampling port.

Five samplers were installed in each basin (one in the center and one in each corner, 1-m from the border). Samples were extracted from the gas reservoir (the contents of which were assumed to be in equilibrium with the soil gas and/or liquid phase) through the surgical tubing on the top of the collection tube using a syringe fitted with a hypodermic needle. About 5 ml of gas was first extracted and discarded to flush the syringe and the collection tube and then two samples were taken and kept for analysis. The first sample (10-ml) was extracted with a 20-ml glass syringe and used for O_2, CO_2, and N_2 analyses, and the second (1-ml) was taken with a 1-ml plastic syringe and used for ethylene analysis. Analyses were made by gas chromatography, as described elsewhere (11), usually within 2-3 hr after collection. During this time interval the syringes containing the samples were sealed gas-tight by inserting the tip of the hypodermic needle in a silicone septum and stored at low temperature (in an ice chest).

Measurement of soil temperature and pH.—Soil temperatures at a depth of 10-12 cm were recorded continuously with soil thermographs (Model 2200, Marshalltown Manufacturing Inc., IA 50158). An instrument was installed in one plot of each treatment. Soil samples were collected periodically and pH was determined electrometrically in 1:2.5 soil-water suspensions (4).

Organic matter decomposition.—Soil cores (9 cm in diameter and 18-20 cm deep) from five different sites in each plot were taken at each sampling time and bulked into a composite sample. Samples were taken immediately after the debris was placed in soil, and after 30 and 75 days. Each composite sample was suspended in 20 liters of 5% Calgon solution (Consumer Products Co., Inc., Pittsburgh, PA 15230) and stirred vigorously several times over a 2-hr period to disperse soil aggregates. The suspension then was washed through a series of three sieves (5-, 2-, and 1-mm openings, respectively) for 10 min to eliminate all mineral soil particles and organic material with diameter of less than 1 mm. Pieces of organic material larger than 1 mm, which were retained on the sieves, were separated from sand particles and rocks by flotation, and their dry weight was

arbitrarily considered as "undecomposed debris."

Production of microsclerotia.—Soil samples, collected as described above, were air-dried for 15 days at 22-24 C, pulverized in a revolving-jar mill (3) for about 25 min, and triplicate 15-g samples from each composite sample were assayed with the modified (12) wet-sieving technique (10). All plots were sampled once prior to placing the infested debris in soil (to determine the native population of *V. dahliae*) and then the nonflooded plots were sampled again after 15, 75, and 220 days. No samples were taken during flooding; thus, the production of MS in the flooded plots was determined at the end of each flooding period (5 days after flooding was terminated) and again 75 and 220 days after burial of the infested debris in soil.

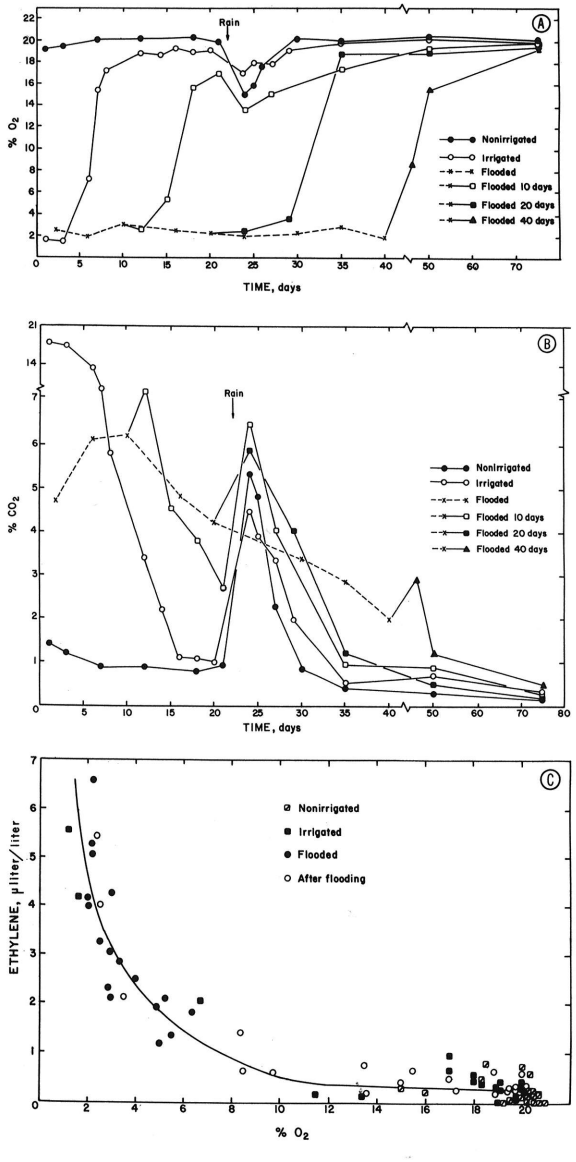

Fig. 3.-(A to C). Levels of oxygen, carbon dioxide, and ethylene (volume/volume) in field plots subjected to different irrigation and flooding treatments. Each point represents the average value from analyses of 15 different gas samples taken from five sites in each of three replicated plots. Gas samples were taken from equilibration chambers placed in soil at a depth of 10-12 cm. **A)** Oxygen levels. **B)** Carbon dioxide levels. **C)** Coordinate plot of levels of ethylene (µliters/liter) and oxygen (percent volume). The curve of best fit is described by the equation $Y = 5.571\ X^{-.854}$.

RESULTS

Soil water potential, temperature, and pH.—During the first 20 days, soil ψ in the nonirrigated treatment decreased slowly from −15 to −19 bars (Fig. 1). During the same period, soil ψ in plots that received only one irrigation at the beginning of the experiment had decreased to −2.7 bars. A heavy rain (4.35 cm) on the 22nd day saturated both treatments (Fig. 1), but after the rain, soil ψ decreased faster in the no-irrigation than in the one-irrigation treatment. After termination of flooding, soil ψ in the flooded plots changed very slowly and at 75 days had decreased only to about −1 bar (Fig. 1).

Soil temperatures in all treatments decreased gradually from September to December (Fig. 2). During the first 2-3 wk the temperature of the irrigated soil was consistently lower by 5-6 C than that of the dry soil; temperatures in the flooded treatments were consistently intermediate (Fig. 2). Flooding had a buffering effect against abrupt changes in soil temperature; for example, at the end of the 3rd wk the temperature of the dry soil dropped from 22.8 to 12.5 C within 4 days, whereas that of the flooded soil dropped only from 20 to 15 C (Fig. 2). The temperature decrease at the end of the 3rd wk occurred concurrently with a heavy rain which saturated all of the nonflooded treatments; from then on, temperatures in the one-irrigation treatment were very close to those in the no-irrigation treatment and for this reason, are not shown in Fig. 2. The temperatures of the flooded soil continued to be lower (by about 1 C) than those of nonflooded soil, even after flooding was terminated (end of the 6th wk).

Soil pH, determined over a 75-day period, held consistently at 7.1 - 7.5 in all treatments.

Levels of oxygen, carbon dioxide, and ethylene.—Oxygen, and CO_2 concentrations (by volume) in the dry treatment were always about 20 and 1%, respectively, except for a transient change following 4.35 cm of rain on the 22nd day (Fig. 3-A, B). Lowest O_2 (1.5%) and highest CO_2 (18%) concentrations occurred in the one-irrigation treatment, 1-3 days after irrigation (Fig. 3-A, B). However, these near-anaerobic conditions did not last, and during the next 3-4 days, O_2 and CO_2 concentrations changed rapidly to levels nearly the same as those of the dry treatment (Fig. 3-A, B). During flooding, the O_2 concentration was consistently low; i.e., about 2% (Fig. 3-A). After flooding was terminated (10th, 20th, and 40th day, respectively), O_2 concentrations remained low for several days, but then increased rapidly to levels slightly lower than those of the nonflooded treatments (Fig. 3-A).

The concentration of CO_2 was about 5 to 6% during the first 15 days of flooding, but then decreased slowly, and after 40 days of continuous flooding, was about 2% (Fig. 3-B). Upon drainage (after 10, 20, and 40 days, respectively), the CO_2 concentrations increased significantly for several days, but then decreased rapidly

to levels not appreciably higher than those of the nonflooded treatments (Fig. 3-B).

Similar patterns of changes in O_2 and CO_2 concentrations occurred in the respective no-debris controls. However, the levels of O_2 were always higher and the levels of CO_2 always lower than the values in the corresponding plots with plant debris. For example, in the flooded no-debris controls the concentrations of O_2 ranged between 3 and 6%, and of CO_2 between 0.7 and 1.3%. The lowest O_2 and highest CO_2 concentrations in the one-irrigation treatment also occurred 1-3 days after irrigation, but the respective levels were 11.5 and 7.3%.

Ethylene, at levels ranging from trace-amounts to a maximum of 20 μliters/liter, was detected in almost all gas samples from the different plots. When averaged, however, maximum ethylene levels did not exceed 6.5 μliters/liter. Relatively high ethylene levels (3.0 - 6.5 μliters/liter) occurred shortly after irrigation, during the periods of flooding, and shortly after flooding in the plots with added plant debris. In the absence of plant debris, appreciable ethylene levels (1-3 μliters/liter) were detected only during the periods of flooding. However, trace-amounts of ethylene were detected even in soils with $\psi = -15$ to -19 bars.

There was a close correlation between ethylene and O_2 concentrations; the relation between the two was negative-curvilinear (Fig. 3-C), described by the equation $Y = 5.571\ X^{-0.854}$, where Y = ethylene concentration (μliters/liter) and X = percentage of O_2 concentration (by volume). After logarithmic transformation, the data fit a negative-linear relationship described by the equation $\log Y = 0.746 - 0.854 \log X$ ($r = 0.585$, significant at $P = 0.05$).

Decomposition of plant debris.—The rate of organic matter decomposition was fastest in the one-irrigation treatment; after 75 days about 85% of the debris that was originally added had decomposed. The lowest rate of decomposition was in the 40-day flooding treatment wherein only 57% of the tissue had decomposed after 75 days. During the same time-period, decomposition in the dry, 10-, and 20-day flooding treatments was 64, 72, and 66%, respectively.

Production of microsclerotia.—The numbers of MS recovered from the dry and irrigated plots were statistically equal ($P = 0.05$) at all sampling times (Table 1). In the dry treatment, all MS apparently were produced within 15 days after the infested debris was buried in soil, as indicated by the lack of significant differences between numbers of MS in the assays made after 15, 75, and 220 days. In the one-irrigation treatment, a few MS apparently were produced between the 15- and 75-day samplings, but, again, most were produced during the first 15 days (Table 1). Very few MS were produced in any of the three flooding treatments while the plots were flooded, but after flooding, their numbers, as determined after 75 days, had increased significantly (Table 1). The numbers of MS that were produced after 220 days in the 10-, 20-, and 40-day flooding treatments were 90, 44, and 46%, respectively, of the average number produced in the nonflooded treatments. However, the reductions were significant ($P = 0.05$) only in the 20- and 40-day flooding treatments.

DISCUSSION

Although soil aeration was curtailed drastically by flooding (Fig. 3-A, B), total anaerobiosis of the whole soil mass was never attained in any of the flooded plots. Oxygen, at low levels, was consistently present and the concentration of CO_2 never exceeded 6%; the remainder of the gas mixture was mostly N_2, which indicates no evolution of large amounts of gaseous products of anaerobic fermentation (1, 18). Lack of complete anaerobiosis at a depth of 10-12 cm in flooded soil is inconsistent with theoretical predictions and experimental results by others (8, 18). However, the above studies involved systems with stagnant water, whereas in our flooding experiments there was a continuous supply of fresh (oxygenated) water to replenish losses from evaporation and downward percolation. Although the total amount of oxygenated water applied to the plots was not determined, apparently enough O_2 was supplied to support aerobic respiration in most of the soil mass. Aerobic respiration in soil is not curtailed until very low levels of O_2 are reached (8). Even in the presence of stagnant water, a thin layer of soil at the soil-water interface is primarily aerobic because of O_2 dissolved in water (18).

Despite the lack of complete anaerobiosis in the whole soil mass, O_2-free zones undoubtedly existed in the flooded soil because methane and several other low-molecular weight hydrocarbons, products of anaerobic fermentation (1, 18, 23), were consistently detected at concentrations comparable to those reported for ethylene (Fig. 3-C). Trace-amounts of these gases also were detected occasionally from nonflooded plots. Thus, anaerobic microsites (8, 9, 21) probably existed even in

TABLE 1. Effect of different irrigation and flooding treatments on the production of *Verticillium dahliae* microsclerotia in infected tomato tissues buried in soil at a depth of 10 to 12 cm

Water treatment	Microsclerotia per gram of air-dry soil determined after the indicated time (days) of incubation						L.S.D. ($P = 0.05$)
	0[a]	15	25	45	75	220	
No irrigation	0.2[b]	4.3	...	...	4.5	3.8	0.9
One irrigation	0.3	3.5	...	...	4.6	4.4	1.0
Flooding 10 days	0.2	0.8	...	...	3.3	3.7	0.8
Flooding 20 days	0.3	...	0.5	...	1.8	1.8	0.4
Flooding 40 days	0.3	...	...	0.5	1.4	1.9	0.3
L.S.D. ($P = 0.05$)	0.2	1.1	...	...	0.9	1.4	

[a]Microsclerotia determined from soil before addition of infested debris (pre-existing in soil).
[b]Values represent means of three replicates with three subsamples per replicate.

soil in which the average O_2 concentration approximated normal atmospheric levels.

In nonflooded soil the concentrations of O_2 and CO_2 were inversely correlated so that the sum of the two was always about 21% (Fig. 3-A, B). Wood and Greenwood (25) have proved theoretically the existence of this relationship between O_2 and CO_2 concentrations in the gas phase of aerobic soils. However, in the liquid phase of aerobic soils the decrease in O_2 partial pressure (equilibrium concentration in the gas phase, expressed as fraction of 1 atmosphere) is about 20 times the corresponding increase in CO_2 partial pressure (7); this is because of the much greater solubility of CO_2 in water as compared to O_2 (7). In our study, also, great decreases in O_2 concentration were associated with relatively small increases in CO_2 concentration in flooded soil (equilibrium concentrations in the gas phase) (Fig. 3-A, B). However, the increases in CO_2 concentration observed here were considerably higher than those reported by Greenwood (7) for the liquid phase of aerobic soils. This is probably because, in the flooded soil, a certain amount of CO_2 was produced by anaerobic fermentation in O_2-free zones.

Carbon dioxide, being chemically active, forms carbonic acid, bicarbonates, and insoluble carbonates in the soil (9, 18). Loss of CO_2 as insoluble carbonates may have been one of the factors causing decline in the CO_2 concentration after about 2 wk of flooding (Fig. 3-B). Also, decreased rate of CO_2 production because of decreased amounts of readily decomposable organic material, dilution with fresh water, leaching, and escape to the atmosphere may have contributed to the CO_2 decrease with time. A similar pattern of CO_2 kinetics in flooded soil has been observed also by other workers (18). Carbon dioxide, also, is primarily responsible for the dramatic changes in soil pH observed in flooded soils (18). However, when the initial soil pH is near-neutral, as in the present study, changes are insignificant (18).

There has been some controversy in the literature as to whether anaerobic conditions are necessary for ethylene production in soils (14). Results from this study support the finding (20, 21, 22) that ethylene at low levels is produced even in well-drained agricultural soils, possibly in anaerobic microsites (21, 22). The negative correlation between O_2 and ethylene concentrations (Fig. 3-C) indicates that ethylene levels are usually maximal under near-anaerobic conditions. Similar relationships between O_2 and ethylene concentrations in soil have been observed in laboratory studies (21, 23).

Since some synthetic materials have been reported to give off or absorb ethylene (13, 19, 23), it was necessary to examine possible alterations in ethylene levels because of the plastic funnels used in this study as gas reservoirs. Ethylene levels at each of five different sites in both flooded and nonflooded soil were determined, over a 20-day period, using two side-by-side samplers, one with plastic and the other with a glass funnel. In no case were any significant differences detected between the levels of ethylene from the two samplers.

The production of MS was greatly inhibited under flooded conditions (Table 1). A similar inhibition was observed in the laboratory (12). In other laboratory tests (11), the production of MS was inhibited by atmospheres of low O_2 and high CO_2 concentrations. Since the levels of O_2 and CO_2 in flooded soil were determined in the gas phase (Fig. 3-A, B), a comparison between the response of the fungus under field (Table 1) and laboratory conditions (11) can be made. Although the combinations of O_2 and CO_2 encountered in flooded soil were not duplicated exactly in the laboratory tests, the concentration of O_2 in flooded soil was sufficiently low (Fig. 3-A) and that of CO_2 sufficiently high (Fig. 3-B) to account for the inhibition of MS production observed in the flooded soil (Table 1). The inhibitory effect of flooding on the production of MS lasted only so long as flooding was continued; upon drainage, the concentrations of O_2 and CO_2 returned rapidly to normal atmospheric levels and MS production resumed (Table 1). Similarly, in the laboratory tests (11), production of MS resumed when cultures of the fungus or infected tomato stems were removed from inhibitory gas mixtures to normal air. However, in both the laboratory (11) and the field (Table 1) the numbers of MS that were produced after aeration was improved were considerably reduced, compared with those produced under continuously favorable O_2 and CO_2 conditions. Thus, it appears that flooding affects the production of *V. dahliae* MS primarily by affecting the levels of O_2 and CO_2 in the soil. Nevertheless, the involvement of other unidentified factors, especially biological, cannot be excluded.

The placement of infested debris in a defined layer at a specified depth, instead of uniform incorporation into the soil, does not duplicate the usual field situation. Nevertheless, for the purpose of this study this approach obviated some of the difficulties and uncertainties imposed by the heterogeneity of the soil physical environment. Also it allowed precise monitoring of the environment at the site of MS production.

As pointed out by Menzies (16), factors that reduce populations of soilborne plant pathogens "will be useful for disease control only if they have capabilities for almost complete eradication." Because of this, short-term flooding treatments at the end of the growing season (after the infested residues are incorporated into the soil) do not appear very promising for disease control. Although the production of MS was significantly reduced by the 20- and 40-day flooding treatments, numbers sufficient to produce considerable amounts of infection of tomato plants were formed after flooding was terminated (N. Ioannou, *unpublished*). Furthermore, in other studies (N. Ioannou, *unpublished*) on the effect of flooding on survival of previously formed MS, continuous flooding for 40 days did not reduce the numbers of viable MS. Results of similar tests in other soils, climates, etc. might be different, but our results do not indicate that short-term flooding is efficacious for control of Verticillium wilt of tomato either by its effect on MS production or on MS survival.

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