Effect of Oxygen, Carbon Dioxide, and Ethylene on Growth, Sporulation, and Production of Microsclerotia by Verticillium dahliae

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ABSTRACT

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Verticillium dahliae cultured in decreasing O_2 (20.9 to 0.06%) and nearly constant CO_2 (about 0.03%) concentrations, produced maximum radial growth and sporulation at 1.9 and 2.7% O_2, respectively; growth was nil at 0.06% O_2. The production of microsclerotia (MS) was maximum at 20.9% O_2 and decreased progressively with each decrease in O_2 concentration. Under constant O_2 (17-18%) and increasing CO_2 concentrations (ranging from 0.03 to 20.9%), maximum radial growth and sporulation were produced at 6.2 and 0.03% CO_2, respectively. In 20.9% CO_2, radial growth was essentially the same as at 0.03%, but sporulation was reduced to about 20% of the maximum. The production of MS decreased with each increase in CO_2 concentration and was completely inhibited at about 12.2% CO_2. Under different mixtures of O_2 and CO_2, ranging from 20.9% O_2 and 0.03% CO_2 to 0.5% O_2 and 20.5% CO_2, radial growth was maximum at 16.5% O_2 and 4.5% CO_2, whereas conidia and MS production were maximum in normal air. Under 0.5% O_2 and 20.5% CO_2, radial growth was reduced to 17.5% of maximum and very few conidia were produced. The production of MS decreased progressively with each decrease in O_2 and corresponding increase in CO_2 concentration, and was completely inhibited at 10% O_2 and 11% CO_2. Infected tomato stems exposed to normal air, 10% O_2 and 11% CO_2, or 2.5% O_2 and 20% CO_2, produced the maximum, 10% of the maximum, and no MS, respectively. Infected stems, that were exposed to the inhibitory gas mixtures for 10 days, resumed production of MS after return to normal air, and produced within 10 days about 30-40% of the numbers of MS produced by stems exposed continuously to normal air. Exposure of cultures to ethylene concentrations ranging from 0 to 35 μliters/liter did not affect growth, sporulation, or MS production. Microsclerotia produced under the different atmospheres were equally viable. Also, MS produced under normal air, and then exposed to low O_2 and high CO_2 concentrations for up to 3 mo, survived as well as MS exposed continuously to normal air.

Additional key words: tomato, vascular wilt disease, microbial ecology, soilborne pathogens.

Although various investigators (4, 5, 13, 14, 15, 23) have reported on the influence of aeration on growth and survival of *Verticillium dahliae* Kleb., detailed studies on this subject are lacking. Menzies (14) and Nadakavukaren (15) reported that microsclerotia (MS) were eliminated from soil by flooding and they attributed this to the induced anaerobiosis. Wilhelm (23) and Green (5) reported that the population of *V. dahliae* in soils decreased with increasing depth, and that the heaviest infestation was located in the upper 30 cm of soil. Although aeration was not discussed at length by these authors, Green (5) suggested that the abrupt reduction of inoculum in soil at depths below 30 to 45 cm corresponded "to the lower limits of the partially oxidized topsoil of most of the soils sampled." Brinkerhoff (4) suggested that low O_2 concentrations inhibited the formation of MS because MS did not develop in infected cotton leaves incubated in sealed glass jars, but were formed abundantly in leaves incubated in jars closed with cotton plugs. Luck (13) reported that MS did not develop when the CO_2 content was lower than that in normal air and suggested that CO_2 fixation was involved in the formation of MS.

Production of MS by *V. dahliae* was inhibited in infected tomato stems incubated in saturated soil (10). In preliminary experiments, when test tubes were filled with sterilized quartz sand and saturated with liquid medium containing conidia of *V. dahliae*, MS formed only on top of the sand and around air bubbles trapped at various depths within the sand. These observations and the reports by others led us to examine the effect of various concentrations of O_2 and CO_2 on the growth, sporulation, MS development, and survival of *V. dahliae*. Ethylene, a volatile factor recently implicated in soil fungistasis (18) and in soil biology generally (19), also was examined in relation to its effects on growth, sporulation, and production of MS by *V. dahliae*. The effect of the above-mentioned gases on the production of MS was given particular emphasis. A preliminary report of this study has been published (9).

MATERIALS AND METHODS

Preparation of gas mixtures.—Mixtures of gases of the desired composition were prepared by metering together appropriate amounts of compressed air, compressed CO_2, liquid N_2, and ethylene. Metering was accomplished by the use of capillary tubes of known resistances to gas flow and a constant-gas pressure maintained by allowing excess gas to bubble through a 130-cm column of water. The concentrations of O_2, CO_2, and N_2 (percentage of gas volume) were determined with a Carle-8000 gas chromatograph (Carle, Inc., Fullerton, CA 92631) equipped with a thermal-conductivity detector and molecular-sieve and silica-gel columns. A Carle-211 gas chromatograph, equipped with a flame-ionization detector and alumina column was used to determine ethylene concentrations (μliters/liter). Cultures of the fungus or infected host tissues were exposed to the different gas mixtures in 10-liter cylindrical glass chambers fitted with metal covers with inlet and outlet openings. For improvement of gas exchange, lids of the petri plates were raised slightly by inserting a sterilized paper clip between the top and bottom parts of each plate. The gas mixtures were humidified prior to introduction into the sample chamber. The flow rates in all experiments were maintained at 10 liters/hr. All experiments were carried out in a controlled-temperature room at 21 C, in the dark.

In vitro experiments.—A single-spore-derived isolate of *V. dahliae* (45-1) obtained from a field-infected tomato plant was used in all in vitro experiments, unless specified otherwise. The fungus was maintained in tube slant cultures on potato-dextrose agar (PDA) at 22-24 C. For determination of rates of radial growth, the fungus was grown in plastic petri plates, each containing 25 ml of PDA. Inoculum was prepared in PDA plates by spreading 1 ml of a concentrated conidial suspension onto the surface of the medium; after incubation for 5 days at 24 C, 6-mm-diameter agar disks were excised with a sterile cork borer and used to inoculate the center of each plate. Colony diameters from five plates per treatment were measured after incubation for 15 days in the modified-atmosphere chambers.

The production of conidia and MS in petri plate cultures of the fungus on quartz sand wetted with potato-dextrose broth (sand-PDB) was determined as described elsewhere (10). After incubation for 15 days, the content of each of five plates per treatment was blended (Waring Blendor at high speed for 0.5 min) in 200 ml of sterile-distilled water. Numbers of conidia and MS in portions of the suspension were determined with a haemocytometer or a nematode-counting chamber, respectively (10).

The influence of decreased O_2 and increased CO_2 concentrations on survival of MS was determined with MS produced on the sand-PDB substrate under normal air. Sand cultures with numerous MS were subjected to various modified atmospheres for up to 3 mo. At prescribed times, five plates were removed from each atmosphere treatment and the viability of MS was determined by culturing on modified pectate agar media, as described elsewhere (10).

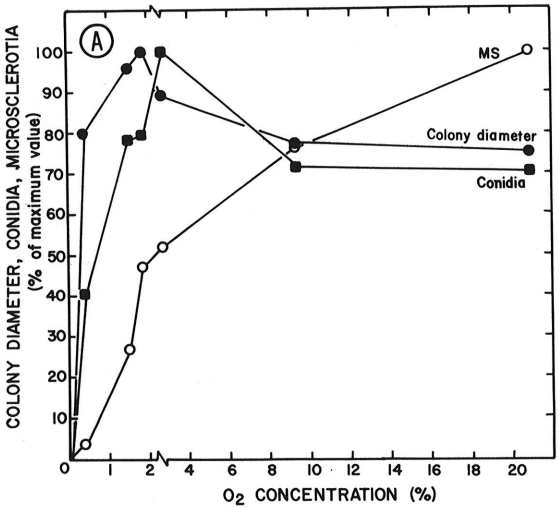
The initial pH of both the PDA and the sand-PDB media was about 6.5 and no significant changes had occurred at the end of the 15-day incubation period. However, in the sand cultures exposed to the gas mixtures for 3 mo (MS survival test), the final pH had increased considerably and varied from 7.8 in cultures exposed to the highest (20%) CO_2 level to 8.6 in cultures exposed to normal air.

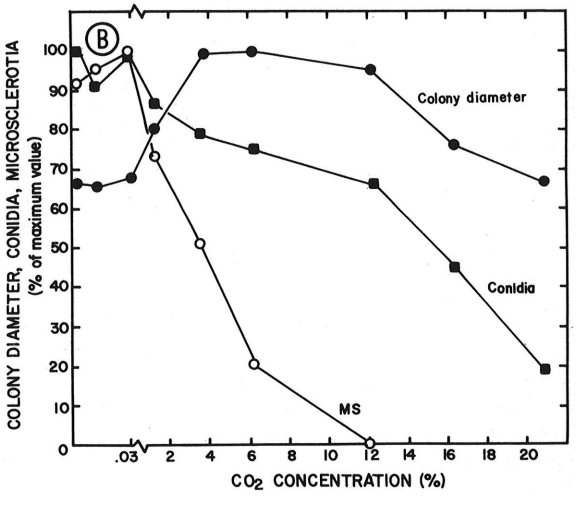
Experiments with infected host tissues.—Five highly virulent isolates of *V. dahliae* were used to inoculate tomato seedlings (8 days old) in the greenhouse by dipping their roots for 5 min into a conidial suspension containing approximately 10^5 conidia/ml. After 5 wk, plants of uniform size and with typical "wilt" symptoms were collected and the lower stem portions (between ground-level and the cotyledonary node) were used in the modified-atmosphere experiments. Each stem section was divided into three 2-cm-long segments and each segment was exposed to one of three different gas mixtures. Each atmosphere included 18 stem segments per isolate. The production of MS was determined after 10 days by assaying six segments from each treatment. The remaining 12 segments from each treatment were transferred to chambers containing normal air and assayed after an additional incubation for 10 and 20 days (six segments for each assay). The method of assay was similar to that described for determination of MS production in culture. Each stem segment was blended in 20 ml of water for 1 min (Waring Blendor with a microcontainer, at high speed) and MS from at least three 1-ml portions were counted using a nematode-counting chamber and a dissecting microscope.

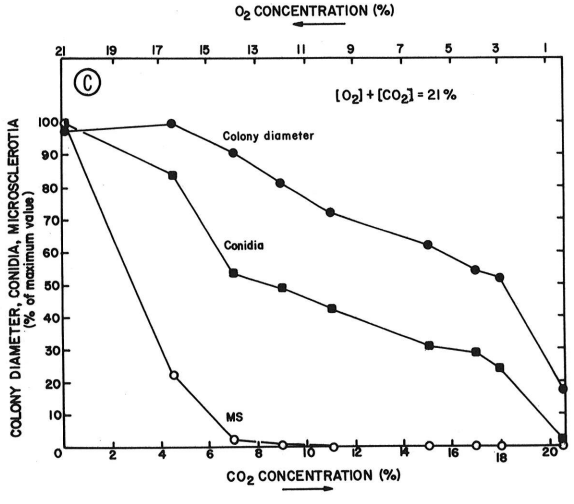
The infected stem segments were exposed to the test atmospheres either after surface disinfestation (0.5% NaOCl for 5 min) or in the presence of soil microflora. Surface-disinfested stem segments were rinsed with sterile-distilled water and plated on the surface of sterilized quartz sand moistened with autoclaved soil extract in glass petri plates. Incubation in the presence of soil microflora was done similarly, except that the stem segments were soaked in nonsterilized soil extract for 5 min before placement on the surface of sand moistened with nonsterilized soil extract. Soil extract was prepared by mixing two volumes of water with one volume of field soil (Yolo loam) and filtering the mixture through a filter paper disk on a Büchner funnel under vacuum.

RESULTS

Effect of oxygen concentration in vitro.—Oxygen concentrations were decreased from 20.9% (normal air) to nearly zero (0.06%) while CO_2 was held nearly constant at about 0.03% (normal atmospheric level). The fungus grew, sporulated, and produced MS at all O_2 concentrations tested except 0.06% (Fig. 1-A). Growth and sporulation responded similarly to decreased O_2 levels, and were maximum at 1.9 and 2.7% O_2, respectively; however, further decreases in O_2 concentration caused a decline in both growth and sporulation, and both were completely inhibited at 0.06%. In contrast to radial growth and sporulation, production of MS was maximal at the highest O_2 concentration (20.9%) and decreased progressively with each decrease in O_2 concentration; at 1.9% O_2 the







production of MS decreased to less than 50% of the maximum, but complete inhibition occurred only in 0.06% O_2, wherein the fungus was unable to grow (Fig. 1-A). Decreased production of MS with decreasing O_2 concentration also was observed in the agar cultures used for determination of radial growth. However, low O_2 concentrations appeared to affect the production of MS more in agar than in sand cultures. For example, at 0.4% O_2, MS production in agar cultures was completely inhibited, and at 1.5% O_2 the few MS produced were detectable only with microscopic examination. At 1.9 and 2.7% O_2, MS in many of agar cultures were produced in distinct black sectors which often had smaller radii than the white sectors (Fig. 2). Radial growth in these plates was determined by averaging measurements of several colony diameters.

Effect of carbon dioxide in vitro.—Concentrations of CO_2, both higher and lower than the normal atmospheric

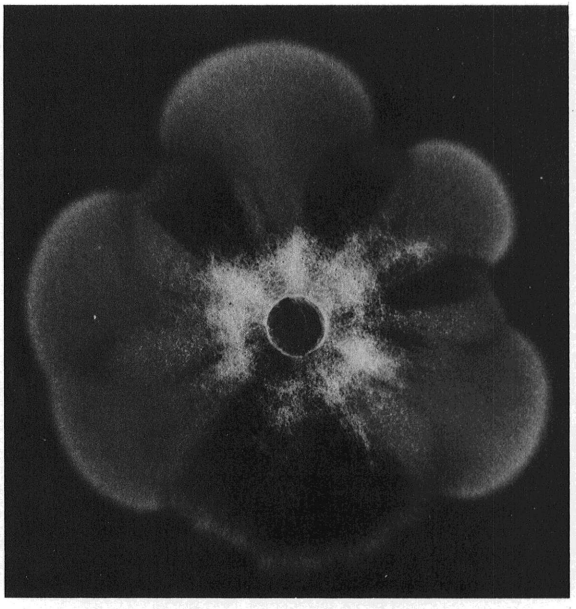


Fig. 2. A colony of *Verticillium dahliae* grown on potato-dextrose agar for 10 days at 2.7% O_2. Notice the black and white sectors and the greater colony radius in the white sectors.

Fig. 1-(A to C). Effect of various concentrations of oxygen and carbon dioxide on radial growth, sporulation, and production of microsclerotia by *Verticillium dahliae*. For radial growth determinations, the fungus was grown on potato-dextrose agar, and for conidia and microsclerotia production, on quartz sand amended with potato-dextrose broth. Measurements were taken after 15-days incubation at 21 C in the dark. **A)** Carbon dioxide concentration was held constant at 0.03% and oxygen concentration decreased from 20.9 to 0.06%. **B)** Oxygen concentration was held constant at 17-18% and carbon dioxide concentration increased from 0.0026 to 20.9%. **C)** Both oxygen and carbon dioxide concentrations were varied inversely so that the sum of the two concentrations in each treatment equalled 21%.

level (0.03%), were tested with a near-constant concentration of O_2 (17-18%). Levels of CO_2 lower than 0.03% (obtained by bubbling air through a series of five flasks containing 1 N KOH) did not affect significantly the growth, sporulation or production of MS (Fig. 1-B). The radial growth increased with increasing CO_2 concentrations from 0.03% to about 3.5% and was maximal at 3.5 to 12.2% CO_2. With further increases in CO_2 concentration, the radial growth decreased slightly, but even at 20.9% CO_2, growth was about the same as at 0.03% (Fig. 1-B). Successive increases in CO_2 concentrations (above 0.03%) caused a near-linear decrease in the production of both conidia and MS (Fig. 1-B). However, the production of MS was affected to a much greater extent than sporulation by increased CO_2; thus, at 6.2% CO_2 production of MS was reduced to approximately 20% of that at 0.03%, and at 12.2% CO_2 no MS were produced whereas a few conidia were produced even at the highest CO_2 concentration tested (20.9%).

Effect of mixtures of oxygen and carbon dioxide in vitro.—In this experiment, the concentrations of both O_2 and CO_2 were varied simultaneously so that the sum of the two gases in each treatment equalled approximately 21%; i.e., as O_2 concentrations were decreased from 21 to 0.5%, CO_2 concentrations were increased from 0.03 to 20.5%. Colony diameter in the 16.5% O_2-4.5% CO_2 mixture was essentially the same as in normal air, but decreased slightly with each additional decrease in O_2 and corresponding increase in CO_2 concentrations (Fig. 1-C). Production of conidia was affected by the different mixtures in about the same way. In contrast, production of MS decreased sharply with each decrease in O_2/CO_2 ratio, and at 14% O_2 and 7% CO_2, MS production was reduced to 3% of that in normal air (Fig. 1-C). Very few MS were produced in 12% O_2 and 9% CO_2 (only one out of five replicate plates had a few MS) and no MS were detected in the 10% O_2-11% CO_2 treatment (Fig. 1-C).

Effect of ethylene in vitro.—The effect of five different levels of ethylene, ranging from 0 to 35 μliters/liter, was tested under the normal atmospheric concentrations of O_2 (20.9%) and CO_2 (0.03%) and under the modified concentrations of 17% O_2 and 5% CO_2. This was done to determine the possible existence of synergistic or counteracting effects between ethylene and O_2-CO_2 concentrations which were limiting to MS production. Two isolates (511-4 and 45-1) were used with essentially the same negative results: ethylene levels of up to 35 μliters/liter did not induce any suppressive or stimulatory effects on growth, sporulation, or MS production, under either O_2-CO_2 mixture used. The effect of the two O_2-CO_2 mixtures was similar to that already described (Fig. 1-C).

Effect of oxygen and carbon dioxide concentrations on production of microsclerotia in infected host tissues.—The following three O_2-CO_2 combinations were tested: (i) 20.9% O_2-0.03% CO_2 (normal air); (ii) 10% O_2-11% CO_2; and (iii) 2.5% O_2-20% CO_2. After incubation for 10 days in the three gas mixtures, mycelial growth of all isolates on the surface-disinfested tissues was about equal in all treatments. Profuse sporulation was observed with a dissecting microscope in all three treatments, but no spore counts were made. In the presence of the soil microflora, the fungus grew and sporulated in all treatments, but growth and sporulation (assessed visually) were greatest in the 10% O_2-11% CO_2 treatment. No MS were formed in 2.5% O_2-20% CO_2 in either surface-disinfested tissues or tissues incubated in the presence of soil microflora. The average MS production in 10% O_2 - 11% CO_2, as compared with the respective normal air controls, was reduced by approximately 90% in the surface-disinfested tissues and by about 86% in tissues incubated with soil microflora (Table 1). However, the percentage reduction was variable among isolates and ranged from 86 to 100% in surface-disinfested tissues and from 75 to 97% in tissues incubated with soil microorganisms. In general, the percentage of reduction was greater for those isolates which produced fewer MS under normal atmospheres.

Upon return of the infected tissues from the gas mixtures to normal air, MS were formed within 10 days in amounts equivalent to 30-40% of those produced in the respective continuous-air controls (Table 1). The numbers of MS produced after transfer to normal air were not affected by the O_2-CO_2 levels of the previous 10-day incubation (Table 1). However, there was great variation in the response of different isolates; some isolates produced as many as 60%, and others as few as

TABLE 1. Production of microsclerotia in *Verticillium dahliae*-infected tomato stems exposed to various concentrations of oxygen (O_2) and carbon dioxide (CO_2) for 10 days, and then transferred to normal air for 10 additional days

Gas composition				Microsclerotia produced per 2-cm stem segment[a] ($\times 10^3$)			
1st-10th day		11th-20th day		Surface-disinfested[b]		Non-surface-disinfested[c]	
O_2	CO_2	O_2	CO_2	10th day	20th day	10th day	20th day
(%)	(%)	(%)	(%)	(no.)	(no.)	(no.)	(no.)
20.9	0.03	20.9	0.03	9.1[d] (0.7-14.0)	8.4 (0.5-14.8)	2.2 (1.7-3.0)	2.3 (1.8-2.9)
10.0	11.0	20.9	0.03	1.0 (0-1.9)	3.3 (0.03-7.0)	0.3 (0.1-0.7)	0.8 (0.2-1.1)
2.5	20.0	20.9	0.03	0	2.8 (0.09-5.3)	0	0.9 (0.7-1.2

[a]Stem segments were obtained from greenhouse-inoculated tomato plants.

[b]Stem segments were surface-disinfested with 0.5% NaOCl for 5 min and plated in glass petri dishes containing sterilized quartz sand moistened with autoclaved soil extract.

[c]Stem segments were soaked for 5 min in nonautoclaved soil extract and plated in glass petri dishes containing nonsterile quartz sand moistened with nonautoclaved soil extract.

[d]Average values from five different fungal isolates, six replicates per isolate. Numbers in parentheses indicate the range of values obtained from the different isolates.

7% of the number of MS produced by the respective controls exposed continuously to air. Numbers of MS produced within 20 days after return to normal air were about the same as had been produced within 10 days after return to normal air.

Effect of oxygen, carbon dioxide, and ethylene on viability of microsclerotia.—Microsclerotia produced in culture and in host tissues under various levels of O_2-CO_2 and ethylene had about the same germination percentages as did MS that had been produced under normal air. The germinability of MS in the different experiments varied within the range of 70 to 100%, but in all cases there were no significant differences in the percentage germination of MS produced under the different atmospheres in the same experiment.

The effect of decreased O_2 and increased CO_2 concentrations on the long-term survival of MS also was examined. Microsclerotia were produced under normal-atmosphere conditions on sand-PDB substrate in petri plates. After growth for 15 days at 21 C the cultures were subjected to the same three atmospheres mentioned above for host tissues: (i) 20.9% O_2-0.03% CO_2 (normal air); (ii) 10% O_2-11% CO_2; and (iii) 2.5% O_2-20% CO_2. There were no significant changes in the apparent viability of MS in any of the treatments during the 1st mo of exposure. However, during the 2nd and 3rd mo, the percentage of MS that germinated decreased slightly in all three treatments. At the end of the 3rd mo, the percentages of MS germination in treatments i, ii, and iii were 21, 27, and 17%, respectively; these percentages are not significantly different ($P = 0.05$).

DISCUSSION

Many fungi possess the ability to grow at very low levels of O_2 (21). In this study, the radial growth of *V. dahliae* was maximal at O_2 concentrations considerably less than that in normal air (Fig. 1-A). However, this response may not be due to a direct effect of the reduced O_2 concentration on linear growth, but to an indirect effect resulting from reduction in production of MS. Variants of *V. dahliae* that have lost the ability to produce MS grow faster than the MS-producing strains from which they originated (17). The larger radius of white- as opposed to black colony sectors (Fig. 2) also indicates that decreased MS production can result in increased radial growth.

Pilkington and Heale (17) reported that uptake of O_2 was increased during formation of the dark, resting mycelium of *V. albo-atrum* and that hyaline variants used less O_2 per unit dry weight of mycelium produced than did the dark ones. They (17) attributed this phenomenon to the increased energy requirements for the synthesis of resting mycelium or to a greater oxidation potential necessary for synthesis of dark pigment. An increased demand for O_2 during formation of MS of *V. dahliae* may explain why decreased O_2 affects MS production more than radial growth and sporulation (Fig. 1-A). The production of MS also was sharply curtailed by increased CO_2 concentrations, in contrast to radial growth, which increased with increased CO_2 concentrations up to about 12% (Fig. 1-B). Similarly, production of sclerotia by *Sclerotium rolfsii* was much more sensitive to decreased O_2 and increased CO_2 concentrations than was its mycelial growth (7). Also, formation of chlamydospores in *Fusarium oxysporum* and *F. solani* was inhibited by high levels of CO_2, in contrast to mycelial growth, which was favored by increased CO_2 (2, 3, 16, 20). Similar morphogenic effects of O_2 and CO_2 have been noted with other fungi (8, 21). The need for more O_2 for production of MS in agar than in sand cultures may be due to a slower rate of O_2 diffusion in the agar cultures; *Verticillium dahliae* always forms MS under the surface of agar media, but in the sand cultures MS were formed predominantly on the top of the substrate.

Despite Luck's (13) report that MS did not form at CO_2 concentrations less than that of normal air (0.03%), our results showed that neither growth, sporulation, nor production of MS were decreased by low concentrations of CO_2 (Fig. 1-B). As pointed out by Hutchinson (8), incubation of living tissue under CO_2-free atmospheres is virtually impossible because of the production of respiratory CO_2. The concentrations of CO_2 less than 0.03% plotted in Fig. 1-B were determined from gas samples taken before the gas mixtures entered the sample chambers; the composition of gas leaving the sample chambers was initially identical to that of samples taken from the inlets, but later the CO_2 concentrations at the outlets were greater than concentrations at the inlets, probably owing to production of respiratory CO_2. For example, 1 day before the experiment was terminated, the lowest CO_2 concentration at the outlet was 0.02%, which is only slightly less than the concentration in normal air. Although the effect of a complete lack of CO_2 is still unknown, it is certain that the fungus can grow, sporulate, and produce MS at levels of CO_2 considerably lower than that in normal air.

The composition of the soil atmosphere is greatly affected by the amount of soil water; in wet soils, as concentrations of O_2 decrease, the CO_2 concentrations increase so that the sum of the two is about 21% (6). The effect of these simultaneous, inverse O_2-CO_2 changes on MS production and sporulation was similar to that produced by increases in CO_2 alone, except that MS production was affected more by concomitant reductions of O_2 and increases of CO_2 (Fig. 1-A-C). Radial growth also was affected more by concomitant changes than by separate changes in either O_2 or CO_2 (Fig. 1-A-C). Similar responses have been reported for other fungi (11, 12, 22).

Sand cultures left on a laboratory bench, after removal from the inhibitory gas mixtures, produced some MS but fewer than were produced under continuous exposure to normal air. Tomato stems, incubated for 10 days under inhibitory gas mixtures and then transferred to normal air, produced about 60-70% fewer MS than were produced under continuous normal air (Table 1). The results were similar whether the stems were surface-disinfested or incubated with the soil microflora; this indicates that a similar depression in MS production can be expected in the field. The duration of incubation under inhibiting atmospheres also may affect the number of MS produced upon transfer to normal air, but this factor was not examined in this study.

Ethylene is commonly produced in soil (18, 19), and has been shown to be fungistatic at concentrations of about 1 μliter/liter (18). Although the effect of ethylene on the germination of MS of *V. dahliae* was not examined in the

present study, levels of ethylene up to 35 μliters/liter caused no apparent effect on growth, sporulation, or production of MS in vitro. Nevertheless, these in vitro results do not rule out the possibility that under field conditions ethylene may affect the growth and production of MS by *V. dahliae* indirectly; i.e., by its influence on other soil organisms (18, 19) or by inducing the production of other volatile fungistatic factors in the soil (1).

In contrast to MS formation, their viability was not affected by 3-mo exposure to low O_2 and high CO_2 concentrations. Menzies (14) reported that MS did not survive 6 wk of flooding in soil and suggested that a fungicidal compound, produced by anaerobic fermentation, was responsible for this phenomenon. Such an indirect effect of reduced conditions in the field is possible, but it would be affected by several biological and physicochemical characteristics of the particular soil under study. For example, Ioannou (*unpublished*) failed to demonstrate any reduction in the numbers of viable MS of *V. dahliae* after continuous flooding of Yolo loam soil for 40 days.

The inhibition of production of MS by increased CO_2 and decreased O_2 concentrations may provide a means for reducing the inoculum density of *V. dahliae* in the field. In infected tomato plants, MS are formed primarily in the peripheral cortical tissues and in the pith of stems incorporated in the soil at the end of the growing season (N. Ioannou, *unpublished*). Any cultural practice, such as flooding, that increases CO_2 and decreases O_2 concentrations in the soil during the critical stage of MS development should inhibit the formation of MS. However, additional field experimentation is needed to determine feasibility and efficacy of this approach to disease control.

LITERATURE CITED

1. BALIS, C. 1976. Ethylene-induced volatile inhibitors causing soil fungistasis. Nature 259:112-114.
2. BOURRET, J. A., A. H. GOLD, and W. C. SNYDER. 1965. Inhibitory effect of CO_2 on chlamydospore formation in Fusarium solani f. phaseoli. Phytopathology 55:1052 (Abstr.).
3. BOURRET, J. A., A. H. GOLD, and W. C. SNYDER. 1968. Effect of carbon dioxide on germination of chlamydospores of Fusarium solani f. sp. phaseoli. Phytopathology 58:710-711.
4. BRINKERHOFF, L. A. 1969. The influence of temperature, aeration, and soil microflora on microsclerotial development of Verticillium albo-atrum in abscised cotton leaves. Phytopathology 59:805-808.
5. GREEN, R. J., JR. 1958. "Deep plowing" for controlling Verticillium wilt of mint in muck soils. Phytopathology 48:575-577.
6. GRIFFIN, D. M. 1972. Ecology of soil fungi. Syracuse University Press, Syracuse, New York. 193 p.
7. GRIFFIN, D. M., and N. G. NAIR. 1968. Growth of Sclerotium rolfsii at different concentrations of oxygen and carbon dioxide. J. Exp. Bot. 19:812-816.
8. HUTCHINSON, S. A. 1973. Biological activities of volatile fungal metabolites. Annu. Rev. Phytopathol. 11:223-246.
9. IOANNOU, N., R. W. SCHNEIDER, and R. G. GROGAN. 1976. Influence of aeration on the production of microsclerotia by Verticillium dahliae. Proc. Am. Phytopathol. Soc. 3:276. (Abstr.).
10. IOANNOU, N., R. W. SCHNEIDER, R. G. GROGAN, and J. M. DUNIWAY. 1977. Effect of water potential and temperature on growth, sporulation, and production of microsclerotia by Verticillium dahliae. Phytopathology 67:637-644.
11. LOCKHART, C. L. 1967. Effect of temperature and various CO_2 and O_2 concentrations on growth of Typhula sp., a parasitic fungus of strawberry plants. Can. J. Plant Sci. 47:450-452.
12. LOCKHART, C. L. 1967. Influence of controlled atmospheres on the growth of Gloeosporium album in vitro. Can. J. Plant Sci. 47:649-651.
13. LUCK, J. V. 1954. Studies on the Verticillium wilt of Mentha piperita L., with special emphasis on the causal organism, Verticillium albo-atrum R. & B. Diss. Abstr. 14:916-917.
14. MENZIES, J. D. 1962. Effect of anaerobic fermentation in soil on survival of sclerotia of Verticillium dahliae. Phytopathology 52:743 (Abstr.).
15. NADAKAVUKAREN, M. J. 1960. The effect of soil moisture and temperature on survival of Verticillium microsclerotia. Ph.D. Thesis, Oregon State University, Corvallis. 64 p.
16. NEWCOMBE, M. 1960. Some effects of water and anaerobic conditions on Fusarium oxysporum f. cubense in soil. Trans. Br. Mycol. Soc. 43:51-59.
17. PILKINGTON, S., and J. B. HEALE. 1969. Respiration and morphogenesis in Verticillium. Trans. Br. Mycol. Soc. 53:467-470.
18. SMITH, A. M. 1973. Ethylene as a cause of soil fungistasis. Nature 246:311-313.
19. SMITH, A. M., and R. J. COOK. 1974. Implications of ethylene production by bacteria for biological balance of soil. Nature 252:703-705.
20. STOVER, R. H., and S. R. FREIBERG. 1958. Effect of carbon dioxide on multiplication of Fusarium in soil. Nature 181:788-789.
21. TABAK, H. H., and W. B. COOKE. 1968. The effects of gaseous environments on the growth and metabolism of fungi. Bot. Rev. 34:126-252.
22. TOLER, R. W., P. D. DUKES, and S. F. JENKINS, JR. 1966. Growth response of Fusarium oxysporum f. tracheiphilum in vitro to varying oxygen and carbon dioxide tensions. Phytopathology 56:183-186.
23. WILHELM, S. 1950. Vertical distribution of Verticillium albo-atrum in soils. Phytopathology 40:368-376.