Oxygen and Carbon Dioxide Concentration Effects on the Growth and Reproduction of Aphanomyces euteiches and Certain Other Soil-Borne Plant Pathogens

D. J. Mitchell and J. E. Mitchell

Postdoctoral Fellow and Professor of Plant Pathology, respectively, University of Wisconsin, Madison 53706. Present address of senior author: Department of Plant Pathology, University of Florida, Gainesville 32601. Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison 53706. Project No. 1281.

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ABSTRACT

The growth of *Aphanomyces euteiches* was compared with that of *Fusarium solani* f. sp. *pisi*, *Phytophthora megasperma*, *Pythium irregulare*, *Rhizoctonia solani*, and *Sclerotium rolfsii* under atmospheres containing 1, 5, or 20% O_2 plus 1, 5, or 15% CO_2. *F. solani* f. sp. *pisi* and *S. rolfsii* were most tolerant to low O_2 levels and grew well at 1% O_2; *A. euteiches* and *R. solani* were intermediate and maintained 50% or more of their growth in air at 1% O_2; the growth of *P. megasperma* and *P. irregulare* at 1% O_2 was reduced to less than 20% of that in air. *S. rolfsii* grew faster in an atmosphere containing 5% O_2 than in air at all levels of CO_2. *F. solani* f. sp. *pisi* was the most tolerant and *R. solani* was the most sensitive to high CO_2 levels. The growth of *P. megasperma* at 5% O_2 and of *P. irregulare* at 1 and 5% O_2 was stimulated by the addition of 5% CO_2.

Oospore production by *A. euteiches*, *P. megasperma*, and *P. irregulare* under 5% O_2 in the absence of nutrients was 65, 55, and 26%, respectively, of that in the air control, and a few oospores were formed even when the O_2 concentration was reduced to 1%. Oospore production by *A. euteiches* was stimulated by 5% CO_2 at all levels of O_2. Oospore production by *P. irregulare* was stimulated by 5% CO_2 only at 5% O_2, but was consistently inhibited in *P. megasperma* by any increase in CO_2 above that in air. Inhibition of oospore formation by 15% CO_2 was virtually complete at 1 and 5% O_2 with all three organisms.

Zoospore formation by *A. euteiches* was maximum in air and decreased with decreasing O_2 or increasing CO_2 levels. When the pH was controlled, a few zoospores were formed under all atmospheres tested. The response of pea roots under comparable conditions is discussed with respect to the incidence of pea root rot.

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Additional key words: soil atmosphere, soil environment.

The amount of root infection and subsequent disease development, measures the integrated effects of soil environmental factors on both the host resistance and the pathogen activity. Many root diseases increase under conditions of poor soil aeration, but it is not possible under natural conditions to determine whether the increased disease is due to altered resistance of the roots, altered growth and sporulation of the pathogen, or to a response of the pathogen to the changed functions of the host.

The effect of aeration on fungal growth and

metabolism has been thoroughly reviewed (18), and it is evident that the response of specific organisms in specific environmental circumstances must be considered and that generalizations have little value. The response of each specific organism under the range of gas concentrations of interest must be known. Different growth processes in one organism may have different requirements, so all must be studied if the behavior of an organism is to be understood.

It is difficult to simulate the supply of a gas experimentally and stabilize other factors. The concentration of the gas in the soil atmosphere at a microhabitat will be a function of the rate at which the gas can diffuse into that space, and by the rate at which that gas is being used in biological activity. The rate of exchange of CO_2 will be much more rapid than that of O_2 (7). The gas exchange rate will vary greatly between microhabitats in the soil and will be drastically altered by invading root tips. Control of atmosphere composition appears to be a suitable and acceptable substitute and is used in the work discussed below.

It is not possible to determine gas concentrations in pore spaces around specific microhabitats, but it is reasonable to assume that O_2 will range from 21% down to 0%, and CO_2 from 0.03 up to 15% and possibly more under exceptional conditions (8, 13). Oxygen and carbon dioxide must be considered together for only under extremely unusual conditions would one be absent or vary independently of the other. Information concerning the responses of organisms to gas mixtures within these ranges of concentrations provides a useful guide to the activity that might be expected at specific microhabitats in the soil.

The present paper reports the effect of O_2 and CO_2 concentrations that would be encountered in the soil atmosphere on growth and reproduction of *A. euteiches*. The effect of the atmosphere composition on growth and oospore formation of the fungus was compared with the effect on growth and development of other soil-borne pathogens to provide a better understanding of the relative behavior of these different organisms.

MATERIALS AND METHODS.—The fungi studied were hyphal tip isolates obtained from the collection of soil-borne plant pathogens of the Department of Plant Pathology, University of Wisconsin, Madison, and were isolate Q of *A. euteiches* Drechs. isolated from pea (*Pisum sativum* L.), *F. solani* (Mart.) Appel & Wr. f. sp. *pisi* (F. R. Jones) Snyd. & Hans. also isolated from pea, isolate MM-5 of *P. megasperma* Drechs. isolated from alfalfa (*Medicago sativa* L.), *P. irregulare* Buis. also isolated from alfalfa, *R. solani* Kuehn isolated from carrot (*Daucus carota* L.), and *S. rolfsii* Sacc. isolated from peanut (*Arachis hypogaea* L.).

For growth studies, 15-ml aliquots of fresh potato-dextrose broth (PDB) were dispensed into 250-ml Erlenmeyer flasks each of which was equipped with a rubber stopper having a gas inlet and outlet. After the medium was adjusted to pH 6.5 and autoclaved for 15 min at 121 C, each of four flasks was inoculated with a 7-mm disk cut from the margin of a 4-day-old potato-dextrose agar culture of one of the six fungi. Tubing through which flowed a stream of humidified gas of the desired composition at 20 ml/min was connected to each of the six series of four flasks that were joined by Tygon tubing. The effect on growth of each fungus of different concentrations of O_2 and CO_2 was compared with that of duplicate cultures maintained under air (20.9% O_2, 0.03% CO_2, and 78.1% N_2) at the same flow rate. All cultures were incubated in a controlled environment chamber maintained at 16 hr of daylight [21,520 lux (2,000 ft-c) at the level of the cultures] at 28 C followed by 8 hr of darkness at 16 C. Mycelial mats of all fungi were removed from the growth chamber after 8 days (except those of *P. megasperma* which were incubated for 14 days), and collected on tared Whatman No. 1 filter paper disks, washed with 100 ml of distilled water, oven-dried at 80 C for 24 hr, and weighed.

The effect of atmosphere composition on oospore development was determined using washed, young mycelia in which no reproduction structures were evident at the initiation of the test period. Cultures were started in 15-ml aliquots of V-8 juice broth (10% Campbell's V-8 juice, 1.5 g/liter of $CaCO_3$) dispensed into 500-ml screw-capped bottles, each of which was equipped with a rubber stopper having a gas inlet and outlet. The bottles were joined in series of three with Tygon tubing, and glasswool filters were attached at each end of the series. The bottles were autoclaved for 15 min at 121 C, after which each bottle was inoculated with two 5-mm mycelial disks from the margin of a 4-day-old colony growing on V-8 juice agar (17 g of agar/liter of V-8 juice broth medium). The cultures of *P. irregulare* were incubated for 48 hr and those of *A. euteiches* and *P. megasperma* for 72 hr in darkness in a controlled environment chamber maintained for 16 hr at 28 C followed by an 8-hr period at 16 C. In order to distinguish the influence of O_2 and CO_2 on oospore production from the effects on growth, mycelial mats of the test fungi were washed after the initial growth period. The V-8 juice broth was drained from the bottles and the mycelial mats aseptically washed four times during a 2-hr period with 50-ml aliquots of glass-distilled water. The mycelial mats of *P. megasperma* and *P. irregulare* were each finally suspended in 15-ml of distilled water (brought to pH 6.0 with dilute KOH), whereas those of *A. euteiches* were each suspended in 15-ml of corn-extract broth (45 g sweet corn seed/liter of distilled water, pH 6.0) because this organism formed few oospores in distilled water. A line through which flowed a stream of humidified gas of the desired composition at 30 ml/min was then connected to each of three series of three bottles each. Duplicate series of bottles were maintained as controls under air at the same flow rate. The mycelial mats were removed from the bottles after 10 days of incubation in darkness in the controlled environment chamber, and homogenized in a glass tissue grinder. The homogenates were

diluted to 10-ml volumes and vibrated on a mechanical mixer for 15 sec to disperse the oospores in the suspension. The oospores in each of four samples from each bottle of *A. euteiches* and *P. irregulare* were counted in six fields of a standard haemocytometer. Oospores produced by *P. megasperma* were counted directly in ten 20-μliter samples from each oospore suspension. The effects of O_2 and CO_2 concentrations on oospore production were evaluated by comparison to spore formation in the flowing air controls.

Zoospore production was determined using isolate PR-4 of *A. euteiches* maintained on potato-dextrose agar (PDA) and transferred every 4 days. Test mycelia were grown in peptone-glucose broth (PGB, 2% peptone and 0.5% glucose) autoclaved for 15 min at 121 C. The basal salt replacement solution (BSS) used for zoospore production (15) consisted of 1.75×10^{-3} M $CaCl_2 \cdot 2H_2O$, 10^{-3}M KCl, and 10^{-3}M $MgSO_4 \cdot 7H_2O$. The pH was adjusted to 6.2.

Since high CO_2 levels may result in decreased pH in the replacement solutions that in turn could inhibit spore formation, it was important to maintain a favorable pH throughout the test period. Direct CO_2 effects were differentiated from indirect CO_2 effects on hydrogen ion concentration by incorporating buffer into the replacement solution to maintain a pH of approximately 6.2 in one experiment and comparing results with those in a second set of experiments identical except that the buffer was omitted. For studies in which a stable pH was maintained, 0.01 M 2-(N-morpholino)-ethanesulfonic acid (MES) was added as a buffer to the BSS and the pH was adjusted to 6.2 with 6 N KOH.

Cultures for zoospore production were initiated by inoculating each of nine 250-ml Erlenmeyer flasks containing 30 ml of sterile PGB with four 5-mm plugs from the margin of a 72-hr-old PDA culture of *A. euteiches*. After 24 hr of growth in the dark at room temperature (25-27 C), the contents of each flask was transferred to a 250-ml side arm filtering flask fitted with a rubber stopper having a gas inlet and outlet. Gas of the desired composition was bubbled at 100 ml/min through 500-ml of BSS in a 1-liter Erlenmeyer flasks, and then passed via Tygon tubing over the cultures in the filtering flask. After exposing the cultures and the BSS to the desired atmosphere for 30 min, the PGB was drained through the side arm and connective tubing into a 1-liter collection flask. The flask containing the constantly aerated BSS was then tipped to allow 50 ml of BSS to pass through the gas line and into the culture flask. The mycelia were washed five times during a 5-hr period with 50-ml aliquots of BSS. In each experiment, three flasks were exposed to the desired atmosphere, three flasks were exposed to filtered air at the same flow rate, and three flasks were stoppered with cotton plugs. All cultures were maintained in a growth chamber under 12 hr of daylight [21,520 lux (2,000 ft-c) at the level of the cultures] at 28 C followed by 8 hr of darkness at 16 C. After 20 hr of incubation in the BSS, 10-ml aliquots of BSS were removed from the cultures and vibrated on a mechanical mixer for 15 sec to break spore clumps and to induce motile zoospores to encyst. The number of zoospores was determined by counting six fields of each of four samples from each flask in a standard haemocytometer. The effects on cultures exposed to various O_2 and CO_2 concentrations were evaluated by comparison with those in cultures exposed to air blowing at the same rate.

Atmospheres used in all experiments contained 1, 5, or 20% O_2 plus 0, 5, or 15% CO_2. Gases were purchased as mixtures of O_2, CO_2, and N_2 from the Matheson Company. Constant gas flows of 20 ml/min were obtained by using a capillary flowmeter apparatus similar to that developed by Claypool & Keefer (2). The concentrations of O_2, CO_2, and N_2 were determined by periodically analyzing the effluent gases from the culture flasks by gas chromatography. The flow rates (20 ml/min) of the gas streams through each flask were sufficient to maintain constant levels of O_2 and CO_2. The data presented in this paper are means of two replicated experiments. In every case where concentration of O_2 and/or CO_2 is given, the N_2 concentration, though not stated, constituted the balance of the atmosphere.

RESULTS.—*Growth.*—The comparative response of all six organisms to changes in composition of the soil atmosphere could be assessed by measuring the dry matter produced in culture under the range of test atmospheres used. Dry weights of all fungi tested

TABLE 1. Effects of oxygen and carbon dioxide concentrations at the culture surface on growth in potato-dextrose broth by *Aphanomyces euteiches, Fusarium solani* f. sp. *pisi, Phytophthora megasperma, Pythium irregulare, Rhizoctonia solani*, and *Sclerotium rolfsii*

Species	Growth at various gas concentrations (% O_2 :% CO_2)							
	1:0	1:5	1:15	5:0	5:5	5:15	20:5	20:15
A. euteiches	50 [a] ± 1.8	40 ± 2.0	18 ± 1.3	82 ± 0.5	64 ± 2.5	32 ± 2.1	95 ± 0.6	68 ± 3.1
F. solani f. sp. *pisi*	86 ± 0.9	81 ± 1.2	86 ± 1.7	97 ± 0.5	82 ± 6.3	78 ± 0.8	84 ± 1.9	76 ± 2.2
P. megasperma	19 ± 2.3	15 ± 1.1	19 ± 0.9	44 ± 2.2	52 ± 3.9	29 ± 2.6	92 ± 1.1	86 ± 2.3
P. irregulare	17 ± 4.9	26 ± 2.8	10 ± 1.3	63 ± 4.9	78 ± 6.2	34 ± 3.1	96 ± 1.8	62 ± 2.1
R. solani	61 ± 7.2	32 ± 9.1	11 ± 3.2	75 ± 6.7	49 ± 8.1	15 ± 2.4	78 ± 4.1	14 ± 1.6
S. rolfsii	86 ± 1.7	79 ± 0.8	27 ± 1.2	105 ± 1.6	92 ± 3.3	72 ± 2.1	79 ± 1.9	54 ± 4.2

[a] Growth in air (20.7% O_2, 0.03% CO_2) = 100; based on two experiments with four replications per experiment. Variation expressed as standard error.

TABLE 2. Effects of oxygen and carbon dioxide concentrations above replacement medium on oospore production by *Aphanomyces euteiches, Phytophthora megasperma*, and *Pythium irregulare*

Species	Oospore production[a] at various gas concentrations (% O_2 :% CO_2)							
	1:0	1:5	1:15	5:0	5:5	5:15	20:5	20:15
A. euteiches	11[a] ± 1.2	65 ± 6.1	tr	65 ± 2.5	80 ± 4.7	tr[b]	121 ± 9.2	37 ± 3.1
P. megasperma	9 ± 0.8	6 ± 2.2	tr	55 ± 2.3	14 ± 1.9	tr	95 ± 2.9	53 ± 1.7
P. irregulare	5 ± 1.1	8 ± 1.9	tr	26 ± 3.1	43 ± 4.3	tr	28 ± 1.7	2 ± 0.6

[a] Percent of oospore production in air (20.7% O_2 :0.03% CO_2). Based on two experiments with four replications/experiment. Variation expressed as standard error.
[b] tr = less than 1% of the air control.

(except *S. rolfsii*) were maximum in air, but decreased as the O_2 level in the atmosphere decreased from that in air (Table 1). Growth of *S. rolfsii* was slightly greater at 5% O_2 than in air at all levels of CO_2. The growth of *P. megasperma* and *P. irregulare* in an atmosphere of 1% O_2 was only 19% and 17% of their growth in air, but growth of the rest of the fungi was inhibited less than 50% at that level of O_2. The growth of *F. solani* f. sp. *pisi* and *S. rolfsii* in 1% O_2 was 85% that in air.

Dry weights of *A. euteiches, R. solani*, and *S. rolfsii* decreased with increasing CO_2 concentrations. The growth of *P. irregulare* in atmosphere containing 1 or 5% O_2 was stimulated by the addition of 5% CO_2 as was that of *P. megasperma* at 5% O_2 with 5% CO_2. *F. solani* f. sp. *pisi* grew as well at 1% O_2 plus 5 or 15% CO_2 as it did at 1% O_2 without added CO_2. The dry weight of *F. solani* f. sp. *pisi* was at least 76% of the air controls under all atmospheres tested. All of the remaining fungi were inhibited 70% or more at 1% O_2 plus 15% CO_2. The reaction of the medium did not appear to be a factor in the tests since the final pH of the filtrates from cultures exposed to various concentrations of O_2 and CO_2 differed from the pH of filtrates of control cultures exposed to air by less than 0.4 pH units for *A. euteiches*, 0.9 for *F. solani* f. sp. *pisi*, 0.3 for *P. megasperma*, 0.8 for *P. irregulare*, 0.9 for *R. solani*, and 0.4 for *S. rolfsii*. In no case was the final pH at a level inhibitory to the test organism.

TABLE 3. Effects of oxygen and carbon dioxide concentrations above basal salt replacement solution on zoospore production by *Aphanomyces euteiches* isolate PR-4

Gaseous phase			Zoospore production[a] and final pH of solution[b]			
			Basal salt		Basal salt + buffer[b,c]	
O_2 (%)	CO_2 (%)	N_2 (%)	(%)	(pH)	(%)	(pH)
0	0	100	0.0	6.4	0.0	6.1
1	0	99	1.9 ± 6.3	6.5	1.9 ± 0.4	6.2
1	5	94	0.7 ± 0.2	5.3	0.4 ± 0.3	6.1
1	15	84	0.0	4.7	0.8 ± 0.3	6.0
5	0	95	23.0 ± 1.8	6.4	26.0 ± 3.1	6.1
5	5	90	12.0 ± 0.3	5.1	27.0 ± 0.6	6.1
5	15	80	0.0	4.7	11.0 ± 1.2	6.0
20	0	80	100.0 ± 7.2	6.5	100.0 ± 4.2	6.2
20	5	75	63.0 ± 9.3	5.3	85.0 ± 6.7	6.1
20	15	65	3.0 ± 1.1	4.8	47.0 ± 2.6	6.0

[a] Zoospore production in air (20.7% O_2 :0.03% CO_2) = 100%. Based on two experiments with three replications/experiment. Variation expressed as standard error.
[b] Initial pH of basal salt solution = 6.2.
[c] Buffer = 0.01 M 2-(N-morpholino)-ethanesulfonic acid.

Oospore production.—The numbers of oospores formed per culture in the air controls by *A. euteiches*, averaged 3.2 × 10^5 (range 1.9 - 4.5), for *P. megasperma*, 1.85 × 10^5 (range 1.0 - 2.7), and for *P. irregulare*, 5.6 × 10^5 (range 4.2 - 6.9). Oospore production by all three organisms decreased as the O_2 concentrations in the atmosphere flowing over the cultures were reduced (Table 2). Oospore formation by *A. euteiches* and *P. megasperma* at 5% O_2 was 65 and 55%, respectively, of that in air, but oospore production by *P. irregulare* in an atmosphere containing 5% O_2 was reduced to 26% of that in air. When the O_2 content of the atmosphere was reduced to 1%, oospore production by all of the fungi was reduced to approximately 10% or less of the air controls.

The presence of 5% CO_2 stimulated the production of oospores by *A. euteiches* at all levels of O_2 and by *P. irregulare* at 5% O_2. At this CO_2 level oospore production by *P. megasperma* was reduced at all O_2 levels as it was by *P. irregulare* at 1 and 20% O_2. Oospore production by all of the fungi at 15% CO_2 plus 1 or 5% O_2 was less than 1% of that in the controls in air. With 20% O_2 and 15% CO_2, oospore production by *P. irregulare* was almost completely inhibited, whereas, that of *A. euteiches* or *P. megasperma* was still 30 and 50% of the air controls, respectively.

Zospore production.—Zoospore production by *A. euteiches*, isolate PR-4, was maximal in control flasks exposed to flowing air (6.2 - 10.8 × 10^4 zoospores/ml, Table 3). The effect of flowing air was negligible, however, for approximately the same number of spores was produced in cotton-stoppered flasks as in flasks aerated at the rate of 100 ml/min. Approximately one-fourth as many zoospores were produced in an atmosphere containing 5% O_2 as in air. When the O_2 concentration was reduced to 1%, zoospore production was only 2% of the air control and no zoospores were released under an atmosphere

of N_2. Approximately the same number of zoospores were produced in buffered and unbuffered BSS at each concentration of O_2 without added CO_2.

Zoospore production decreased with increasing CO_2 concentrations with 1, 5, or 20% O_2; a few zoospores (at least 300/ml) were formed at all CO_2 concentrations except 15% in combination with 1% or 5% O_2 in unbuffered BSS in which case pH was reduced below 5.0 and no zoospores were formed.

The decrease in pH in the unbuffered BSS under atmospheres containing 5 or 15% CO_2 shown in Table 3 could be limited to less than 0.2 units from the initial pH of 6.2 during the 20 hr of incubation under gas by the addition of 0.01 M MES to the BSS. The greater spore production in buffered than in unbuffered BSS under atmospheres containing 5 or 15% CO_2 (except at 1% O_2 plus 5% CO_2) appeared to be related to the pH control.

DISCUSSION.—The response of *A. euteiches* to mixtures of N_2 with O_2 and/or CO_2 in proportions typical of what might be expected to occur in pore spaces in the soil has been compared with the response of five other well known root pathogens. Three growth patterns were evident among the six organisms with respect to growth at reduced O_2 concentrations. *F. solani* and *S. rolfsii* were most tolerant to reduction in O_2 concentration with the latter growing faster at 5% than at 20% O_2 and the growth of both inhibited less than 20% at 1% O_2. *A. euteiches* and *R. solani* were intermediate with growth being 50 and 60% of the control, respectively, when the O_2 concentration was reduced to 1%. *P. irregulare* and *P. megasperma* were the most sensitive to low O_2 concentrations and grew at less than 10% of the control rate when exposed to 1% O_2.

F. solani, alone, was almost insensitive to the increase of CO_2 to 15%. The isolate of *R. solani* used was the most sensitive in being completely inhibited by 15% CO_2 at all O_2 levels. *A. euteiches, R. solani*, and *S. rolfsii* were similar in showing reduced growth with each increase in CO_2 at all O_2 levels. This was not uniformly true with *P. irregulare* or *P. megasperma* for there was evidence of stimulated growth by 5% CO_2 at 1 and 5% O_2 with the former and at 5% O_2 with the latter. *P. megasperma* was similar to *F. solani* in being relatively insensitive to up to 15% CO_2 in the presence of 20% O_2. *P. irregulare* was similar to *A. euteiches* and *S. rolfsii* in being markedly inhibited by 15% CO_2, particularly at lower levels of O_2.

The results reported above are similar in many respects to those reported for the same organisms elsewhere. Some differences are notable. Sherwood & Hagedorn (17) found a stimulation of growth of *A. euteiches* in cultures sealed at the time of inoculation under an atmosphere of 5% O_2 as compared with the rate for those sealed under air. It is difficult to compare the two studies since the CO_2 and O_2 concentrations were maintained at a constant level in the present study, but would have changed as the fungus grew in the sealed flasks. The difference between isolates of *A. euteiches* in this regard has not been studied. Brown & Kennedy (1) found that *Pythium ultimum* grew as well in Czapek-Dox yeast extract broth at 4% O_2 as it did in air, and that at least 65% of the growth in air was attained in an atmosphere containing as little as 1.3% O_2. The greater inhibition of *P. irregulare* at 1% O_2 than Brown & Kennedy reported for *P. ultimum* (1) could well have been caused by the difference in transport of O_2 in liquid media in still and shaker cultures.

The stimulation in growth of *S. rolfsii* by 5% O_2 which was recorded here at all levels of CO_2 did not occur under the conditions used by Griffin & Nair (9). Although the conditions differed in that the latter used potato dextrose agar rather than broth and depended on a diffusion column to maintain a constant gas concentraion, the difference in isolates seems to be a more likely reason for the difference in results. The two cultures differed also in that sclerotial production occurred at 5% O_2 in the present work but only above 15% O_2 in that of Griffin & Nair (9). Higher CO_2 levels were found in both studies to be inhibitory to this fungus.

Most *Phytophthora* spp. grow well in O_2 concentrations as low as 1%; some grow better in 5.0% O_2 than in air (3, 13). In view of this tolerance of other *Phytophthora* spp. to low O_2 levels, it is of interest that the growth of the isolate of *P. megasperma* used was inhibited more than 80% at 1% O_2 over that in the air control. The results of this study do substantiate the observations of Mitchell & Zentmyer (13) that maximum growth in liquid media occurs when the cultures are exposed to air.

The stimulation of *P. megasperma* at 5% O_2 with 5% CO_2 is similar to that noted for several other *Phytophthora* spp. (11, 13). In those studies the growth increase on solid media due to 5% CO_2 was evident at 1, 5, and 20% O_2, but most striking at 1% O_2. The response of the fungi to changed levels was most evident on liquid media in both studies.

Of the six fungi included in this study, the isolate of *R. solani* was the most sensitive to high levels of CO_2, its growth being inhibited 85% or more at 15% CO_2. Papavizas & Davey (16) reported a considerable inhibition of the saprophytic activity of *R. solani* in soil by 10% or 20% CO_2, and a drastic inhibition by 30% CO_2. Durbin (4) studied 33 heterogeneous clones of *R. solani* and found that subterranean clones were inhibited approximately 31% by mixtures of 20% CO_2 with 20% O_2, but were more tolerant to high CO_2 levels than were soil surface or aerial clones, which were inhibited approximately 52% and 80%, respectively. The culture of *R. solani* used in this study, originally isolated from carrot roots, was more sensitive to high CO_2 levels under the conditions employed in this study than were any of the clones tested by Durbin.

Oospore production by the organisms in this test was more sensitive to oxygen concentration than has been reported previously. Mitchell & Zentmyer (14) found that six species of *Phytophthora* produced more oospores on a glucose-nitrate agar medium at 1% or 5% O_2 than in air, but *P. megasperma* in this study produced about half as many oospores at 5% O_2 as in air and formed less than 10% as many

oospores at 1% O_2 as in air. Mitchell & Zentmyer (14) did not include *P. megasperma* in their study, but *P. megasperma* var. *sojae*, which is closely related to *P. megasperma* (20), responded similarly to other species of *Phytophthora* at low O_2 levels. The constant temperature of 20 C used by Mitchell & Zentmyer (14) and diurnal alternating temperatures of 28 C-16 C for the light and dark periods of this study were a possible but not a probable cause of the difference in oospore production under low O_2 levels found in the two studies. It appears reasonable to assume that the different effects of low O_2 levels on oospore production found in this and previous studies are probably due to the availability of nutrients for continued growth of the mycelium on which oospores form. Since colonies of the fungi observed in this study had sparse growth (48 or 72 hr), were not in active growth stages, and were treated in shallow layers of wash solution and thus well exposed to the atmosphere, it is doubtful that the gas diffusion rate would have been a factor in oospore production.

The fact that *A. euteiches* formed greater numbers of oospores at 1, 5, or 20% O_2 upon the addition of 5% CO_2 to the atmosphere, suggests that this effect of CO_2 is a phenomenon of some significance. *P. megasperma* produced fewer oospores as the CO_2 concentration increased and thus behaved like the *Phytophthora* spp. studied by Mitchell & Zentmyer (14).

While only a few oospores were formed by any of the fungi in atmospheres containing either 1 or 5% O_2, plus 15% CO_2, oospore production by *A. euteiches, P. megasperma*, and *P. irregulare* was 37, 53, and 2%, respectively, of the air controls at 20% O_2 plus 15% CO_2. It is of interest that Mitchell & Zentmyer (14) found that oospore production by *Phytophthora* spp. under the conditions employed in their study was generally inhibited more by 15% CO_2 when the O_2 level was 5 or 20% than when the O_2 concentration was 1%. Thus, it appears that the effects of high CO_2 levels on oospore production may also be influenced by the physical nature of the medium, by gas diffusion, and by the availability of nutrients for continuing growth of the fungal thallus.

Since at least a few oospores were formed by all three of these homothallic fungi in atmospheres containing concentrations of O_2 as low as 1% and CO_2 concentrations as high as 15%, it is doubtful that the levels of O_2 and CO_2 that are commonly found in plant tissue or debris would be severely limiting factors in oospore formation by *A. euteiches, P. megasperma*, or *P. irregulare*.

Production of zoospores by *A. euteiches* is less tolerant to reduced aeration than is sexual reproduction in *Phytophthora*. Mitchell & Zentmyer (14) found that sporangium formation by *Phytophthora* spp. exposed to different concentrations of O_2 and CO_2 was generally reduced only slightly at 5% O_2 in a N_2 atmosphere. Sporangium production by *P. cactorum* and *P. parasitica* was completely inhibited at 1% O_2, but sporangium formation by *P. capsici, P. citrophthora* and *P. palmivora* was only slightly less in 1% O_2 than in air (14). Sporangia formed on mycelial mats under any of the atmospheres tested released zoospores after the cultures were chilled under air (14). Uppal (19) found that sporangia formed by *P. colocasia, P. infestans, P. palmivora*, and *P. parasitica* in the absence of O_2 were able to germinate indirectly by zoospore release, but not directly by germ tube. Since no zoospores were produced by *A. euteiches* in this study in BSS under an atmosphere of N_2, it appears that some *Phytophthora* spp. clearly are better adapted for sporangium and zoospore formation in poorly aerated environments than is the isolate of *A. euteiches* that we used.

Llanos & Lockwood (12) observed a small increase in zoospore formation when air was supplied at the surface of the suspending liquid as compared with that which occurred in flasks not so aerated. No such increase occurred in the present work. The fact that the wash solution was replaced five times in the first 5 hr of incubation in our experiments, probably provided more aeration than did the single replacement used by Llanos & Lockwood (12). The smaller thalli and smaller volume of suspending fluid also would have reduced the demand and increased the ease of gas exchange in the nonaerated cultures used in this study.

The experiments describing the effect of various combinations of O_2 and CO_2 within limits on the behavior of pea root pathogens and other soil-borne root pathogens make it possible to visualize the behavior of each organism in a specific microhabitat with any combination of atmospheric parameters. It may well be that the true impact of reduced aeration can only be measured in terms of the relative effect of specific treatments, the host, and the pathogen. Disease severity would be expected to increase substantially if the growth or metabolism of the root were inhibited proportionately more than that of the pathogen. Geisler (5, 6) in his studies on soil atmosphere and growth of roots showed that, in general, pea root growth decreased as O_2 concentration fell below 14% with the major reduction occurring below 7% O_2. It would appear, from the limited data available, that root growth is slowed more by reduced O_2 and increased CO_2 than are growth and oospore formation by *A. euteiches*. In general, pea root growth decreased as O_2 concentration fell below 14%, but there was a definite stimulation of growth by 1-2% CO_2 at 7% O_2, and a stronger inhibition by 8% CO_2 at 7% O_2 than in the absence of O_2.

On the other hand, zoospores of *A. euteiches* with their requirement for high O_2 would appear to be inefficient agents of infection in soils where a high water potential would result in O_2 levels below 5%. The increase in CO_2 that could be expected under such conditions would further repress zoospore formation. The active oospore formation with 5% CO_2 and 1 or 5% O_2 would be compatible with the occurrence of oospores in roots in which infection is well developed and tissue decomposition has begun.

Finally, growth of mycelium of *A. euteiches* was

inhibited less than root growth at all O_2-CO_2 combinations tested. Consequently infection by mycelial invasion (direct germination of oospores or germinating cysts of zoospores) and colonization of root tissue could occur readily in soils with reduced O_2 and increased CO_2 at a high water potential. There is a limit to the degree that high water potential will favor the development of pea root rot, however, for Sherwood & Hagedorn (17) have shown that the fungus will not grow under anaerobic conditions and experience has shown that roots in saturated soil or those submerged in still water in culture tubes do not become invaded.

Assuming that the isolate of *A. euteiches* used is typical of the natural population that exists in the soil, one can conclude that this pathogen is not well adapted to operate with unusual efficiency under conditions that appear most favorable for pea root rot. In fact, the requirement for matric potentials near −0.01 bars (10) for zoospore formation would suggest that this form of reproduction may not be operative in soils since such conditions would be accompanied by greatly reduced O_2 and increased CO_2 tensions. This suggests that any advantage it may have is a relative one resulting from debilitating effect of the environment on the root. It is only when more information concerning the effect of water potential and soil atmosphere composition on root function is available that the ecological aspects of this host-pathogen relationship can be properly interpreted.

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