

Stimulation of *Geotrichum candidum* by Low Oxygen and High Carbon Dioxide Atmospheres

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ABSTRACT

Average in vitro growth of four isolates of *Geotrichum candidum* in a buffered asparagine-yeast extract broth at 21 C was as great or greater in 1 or 3% O₂ atmospheres, with or without 3% CO₂, as in air. In an atmosphere of 3% O₂ with 3% CO₂ average growth in 24 hours was about twice that of air. Growth decreased linearly with decreasing O₂ concentrations below 3%. At concentrations of 3% or more, CO₂ repressed growth of *G. candidum* in the presence of 21% O₂. In a low O₂

atmosphere, however, 30% CO₂ was necessary to repress growth. Percent decay of tomatoes inoculated with *G. candidum* in the green, pink, and red stages of ripeness was significantly greater in the atmospheres of 3% O₂, with or without 5% CO₂, than in air. Decay development was inhibited in 0.25% O₂ with 5% CO₂; however, this concentration of O₂-injured tomatoes.

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Geotrichum candidum Lk. ex Pers. is a yeast-like fungus with a host range that includes plants, animals (7), and man (17). As a plant pathogen it causes watery soft rot of melons (3), carrots (19), tomatoes (12), peaches (2), and other commodities after harvest. Fungi in general are obligate aerobes (4). Wells and Uota (16) reported that mycelial growth of four fungi that cause postharvest rots decreased linearly with decreasing oxygen (O₂) concentrations below 4%. Growth of the plant pathogenic bacteria *Erwinia carotovora* (Jones) Holland and *Pseudomonas fluorescens* Migula, which are similarly aerobic, decreased linearly with the logarithm of decreasing O₂ concentrations (15). Some aerobic bacteria, however, such as *Bacillus stearothermophilus* Donk and *Azotobacter vinelandii* Beijerinck, attained maximum growth with less than fully-aerated conditions (5, 6).

Carbon dioxide (CO₂) at certain concentrations is known to stimulate the growth of some plant pathogenic fungi (10, 13, 16). Stimulation of growth by CO₂ may also occur when O₂ concentrations are less than normal. Lockhart (9) reported the growth of *Gloeosporium album* Osterw., was greater in 10% CO₂ with 7.5 to 10% O₂ than in air. CO₂ is known to be essential for the growth of many aerobic microorganisms (18), but at high concentrations can inhibit growth (1). There are no detailed studies, however, of the growth responses of *G. candidum* to CO₂.

Modified atmospheres can prolong the market life of many fruits and vegetables (8), partly by retarding the growth of decay-causing organisms. This report describes the stimulatory effects of low-O₂ and high-CO₂ atmospheres on the cellular growth of the yeast-like fungus *G. candidum* in an artificial medium, and on the development of decay in tomato fruits inoculated with this organism.

MATERIALS AND METHODS.—Four isolates of *Geotrichum candidum*, obtained from infected tomato fruit, were maintained on potato-dextrose agar slants at 5 C. Actively growing starter cultures were prepared in flasks containing 10 ml nutrient broth (5 g peptone and 3 g beef extract per liter), and used as a source of inoculum for growth tubes. The media were inoculated by transferring with an inoculating needle cells from the agar

slants to the flasks, and then they were incubated for 16 hours at 21 C. Optical density (OD) reading of mature starter cultures in a Bausch & Lomb spectrophotometer were about 1.5 OD units at 550 nm, equivalent to a cell count of 2.3×10^7 cells per ml.

Growth tubes containing 9 ml of medium (2.25 g asparagine, 0.123 g MgSO₄ · 7H₂O, and 3.0 g yeast extract per liter of 0.02 M potassium phosphate buffer, pH 7.0) were autoclaved, inoculated with a 2-mm diameter loopful of cells from starter cultures, and then fitted with rubber stoppers equipped with a micropipette serving as a gas inlet and aerator extending to the bottom of each tube. Tubes were connected to lines through which flowed gas streams of the desired atmospheres humidified by flow through 150 × 20-mm side-arm tubes lined with water-saturated Whatman No. 2 filter paper. Gas streams bubbled through the medium, and were adjusted to a flow rate of 8 ml per minute. Atmospheres were 0, 0.25, 0.5, 1, 3, and 21% O₂, mixed with or without 3% CO₂; 0, 3, 10, and 30% CO₂ mixed with either 3 or 21% O₂; and normal atmosphere (ambient air check). Gases were premixed with high-purity N₂ as the filler gas and compositions were checked with an Orsat-type analyzer accurate to ± 0.1%. Concentrations of dissolved O₂ in the medium before and during growth were checked with an oxygen electrode. A flow rate of 8 ml per minute was sufficient to maintain a constant level of O₂ in the medium. Gases free of CO₂ were purged through a 25 cm Lithasorb (Fisher Scientific Co., Fairlawn, N. J.) column to insure removal of trace levels of CO₂.

Cultures were grown in controlled atmospheres in the dark for 24 hours at 21 C, at which time the ambient air checks were in the logarithmic phase of growth. In a separate test, pH throughout the 24-hour experimental period ranged between 6.5 and 6.9 in growing cultures. Culture turbidity was used as a measure of growth by the method of Trinci (14). The OD of growing cultures at 550 nm (OD 550) were correlated with cell numbers per ml by counting cells of cultures at several densities with a hemocytometer, and plotting cells per ml versus OD. Data were analyzed by linear regression analysis. Data presented in this paper are based on averages of three replications, each with four different isolates of *G. candidum*.

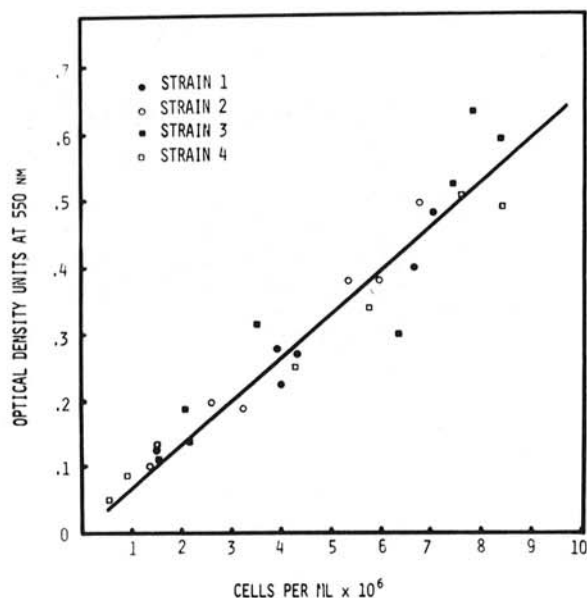


Fig. 1. Regression of optical density units at 550 nm on cell counts per milliliter of four strains of *Geotrichum candidum* grown in the dark for 16 hours at 21 C in a nutrient broth aerated by the passage of an air stream through the culture. Sample regression coefficient for data of all four strains is 15.1062.

Tomatoes (*Lycopersicon esculentum* Mill. 'Homestead') were selected, graded by color, and inoculated by methods previously described (11). After inoculation, 10 fruits of each color were placed in 114-liter steel chambers at 18.3 C, and the chambers were sealed with glass covers. Chambers were flushed with N₂ until the desired concentration of O₂ was obtained. CO₂ was added to the desired concentration. Thereafter, atmospheres were maintained from premixed gases, composed of the desired concentrations of O₂ and CO₂ with high-purity N₂ as the filler gas, in pressurized cylinders flowing at 5 liters per hour. After 7 days, tomatoes were examined for decay. One series of tests was designed to determine the effects of 3% O₂ with and without 5% CO₂, and another to determine the effects of 0.25% O₂ with 5% CO₂. Tests were replicated three times, and the data evaluated by analysis of variance and Duncan's multiple range test.

RESULTS.—Optical density units of *G. candidum* cultures at 550 nm were directly related to the number of cells per ml (Fig. 1). The sample regression coefficient for data of all four strains was 15.1062, that is, cell number per ml increased by 15.1062×10^6 with each increase in an OD unit. There were no significant differences among regression coefficients of each individual strain.

Submerged growth of *G. candidum* after 24 hours in atmospheres of 1 or 3% O₂, with or without CO₂, was greater than growth in air (Fig. 2-A). Average OD 550 of cultures in air was 0.114 (1.7×10^6 cells/ml) compared with 0.193 (2.9×10^6 cells/ml) and 0.139 (2.1×10^6 cells/ml) for cultures grown in 1% O₂ with and without 3% CO₂, respectively. Similarly, OD 550 for cultures grown in 3% O₂ with and without 3% CO₂ were 0.225 (3.4

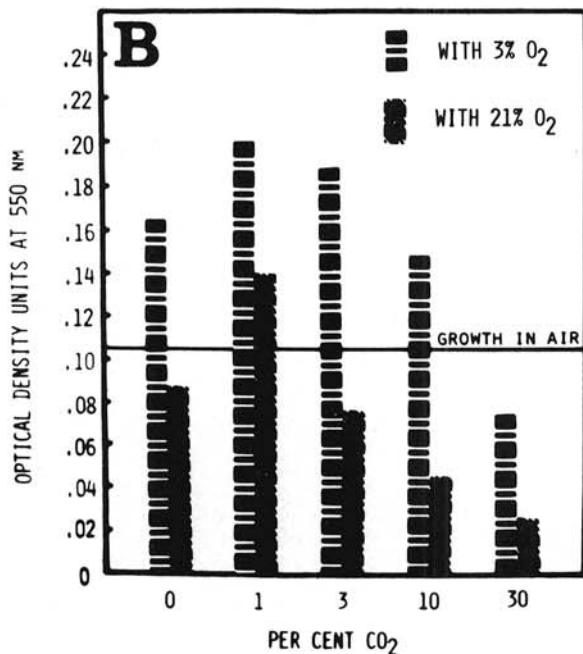
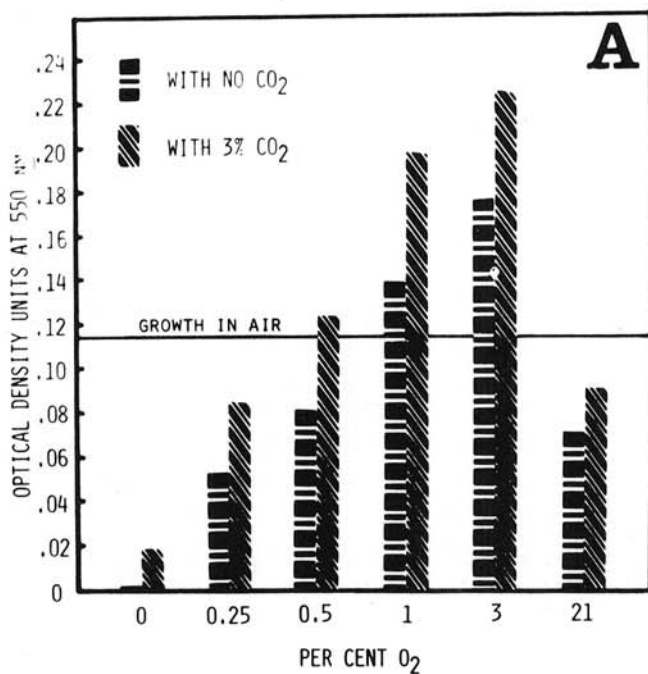


Fig. 2-(A, B). Growth of *Geotrichum candidum* in atmospheres containing: A) different concentrations of O₂ with or without 3% CO₂; and B) different concentrations of CO₂ plus 3% or 21% O₂. Horizontal line represents growth in normal atmosphere.

$\times 10^6$ cells/ml) and 0.178 (2.7×10^6 cells/ml), respectively. Growth in an atmosphere of 0.5% O₂ with 3% CO₂ was about equal to that in air.

Growth decreased linearly with decreasing O₂

TABLE 1. Decay of tomatoes inoculated with *Geotrichum candidum* at four stages of ripeness and held in air or a controlled atmosphere for 1 week at 18.3 C

Test series	Atmospheres ^y	Decay on tomatoes of indicated ripeness (%) ^z			
		Green	Breaking	Pink	Red
1	Air	12 a	9 a	2 a	55 c
	3% O ₂	32 b	12 a	32 b	82 d
	3% O ₂ with 5% CO ₂	38 b	12 a	33 b	98 e
2	Air	0 a	0 a	0 a	53 bc
	3% O ₂ with 5% CO ₂	40 b	33 ab	53 abc	83 d
	0.25% O ₂ with 5% CO ₂	0 a	0 a	0 a	0 a

^yBalance of atmospheres composed of N₂.

^zMeans followed by no letter in common are significantly different, $P=0.05$. Each figure is the average of three tests with 10 fruits each.

concentrations below 3% O₂. At 0% O₂ there was no growth without CO₂ during the experimental period. In the presence of 3% CO₂ there was limited anaerobic growth. Growth in 21% O₂ was significantly less than growth in air. OD 550 for cultures in 21% O₂ with or without 3% CO₂ was 0.092 (1.4×10^6 cells/ml) and 0.071 (1.1×10^6 cells/ml), respectively.

The growth of *G. candidum*, at all concentrations of O₂, was stimulated by the presence of 3% CO₂ in the atmosphere (Fig. 2-A). Growth in the presence of 3% CO₂ was on the average 33% greater than that in the corresponding O₂ atmosphere alone.

High concentrations of CO₂ were inhibitory to the growth of *G. candidum* (Fig. 1-B). In the presence of 21% O₂, CO₂ concentrations of 3% and above were inhibitory. However, in the presence of 3% O₂, a concentration favoring the growth of *G. candidum*, inhibition of CO₂ occurred only at a concentration of 30% (OD 550 = 0.078, or 1.1×10^6 cells/ml).

In air, *G. candidum* produced decay in red tomatoes, but little or none in less mature fruit. (Table 1). However, in atmospheres of 3% O₂, with or without 5% CO₂, percent decay was significantly greater in green, pink, and red tomatoes, than in air. As in air, percent decay in red fruit in modified atmospheres was significantly greater than percentages in less mature fruit. Inoculated tomatoes held in 0.25% O₂ plus 5% CO₂ developed no decay. However, most sound fruit removed from 0.25% O₂ plus 5% CO₂ showed signs of injury after 1 week in air. Use of 5% CO₂ in the 3% O₂ atmosphere significantly increased the percentage decay of red tomatoes compared to percentages in 3% O₂ alone, but had no effect on decay of green, breaking, and pink tomatoes.

DISCUSSION.—*Geotrichum candidum* is a plant pathogen that is favored by low-O₂ atmospheres. Best O₂ concentrations tested for growth on artificial medium were 1 to 3%. In 21% O₂ there was an approximate 50% reduction of growth compared to that in 1 or 3% O₂. The phenomenon may be expressed in terms of O₂ inhibition or toxicity. Concentrations of O₂ higher than normal are toxic to some species of bacteria (5, 6). Oxygen toxicity with *G. candidum* might thus be expected at high-O₂ concentrations considering the significant inhibition of growth observed at 21% O₂.

Inhibition of growth also occurred in the presence of high CO₂ tensions and was evident at concentrations of 3% CO₂ and above with 21% O₂. In low-O₂ atmospheres,

however, CO₂ was inhibitory only at concentrations of 10% and above; concentrations that could injure tissue and adversely affect the market quality of most agricultural commodities.

Growth of *G. candidum* in 21% O₂ was significantly less than growth in air. Since air is composed of about 78% N₂, 21% O₂, 1% A, 0.03% CO₂, 0.01% H₂, and trace gases, one or more of these components might exert a significant effect on growth. Further work is required with trace levels of CO₂ and with the other gases to resolve this point.

In the present series of experiments, tomatoes were held in an atmosphere of 3% O₂ with 5% CO₂. Although 5% CO₂ was not included in the series of CO₂ concentrations used with 3% O₂ in in vitro studies, 5% CO₂ probably would have the same quantitative effect on growth of *G. candidum* as 3 or 10% CO₂.

Observations on the effect of controlled atmospheres on the in vitro growth of *G. candidum* are consistent with those on the effects in vivo. The resistance of green, breaking, and pink tomatoes to infection by *G. candidum* decreased significantly in low-O₂ atmospheres. This loss of resistance suggests that either the physiological resistance to disease of the tomato is altered by storage in low-O₂ atmospheres, or as suggested by the data presented herein, that the effect is due to low O₂-stimulation of the metabolic activity of the fungus.

LITERATURE CITED

- BROOKS, C., E. V. MILLER, C. O. BRATLEY, J. S. COOLEY, P. V. MOOK, and H. B. JOHNSON. 1932. Effect of solid and gaseous carbon dioxide upon transit diseases of certain fruits and vegetables. U. S. Dept. Agric. Tech. Bull. 318. 60 p.
- BURTON, C. L., and W. R. WRIGHT. 1969. Sour rot disease of peaches on the market. Plant Dis. Rep. 53:580-582.
- CEPONIS, M. J. 1966. Occurrence of *Geotrichum candidum* in Western melons on the New York market. Plant Dis. Rep. 50:222-224.
- COCHRANE, V. W. 1958. Physiology of fungi. John Wiley and Sons, New York. 524 p.
- DALTON, H., and J. R. POSTGATE. 1967. Inhibition of growth of *Azotobacter* by oxygen. J. Gen. Microbiol. 48:v-vi.
- DOWNEY, R. J. 1966. Nitrate reductase and respiratory

- adaptation in *Bacillus stearothermophilus*. *J. Bact.* 93:634-641.
7. EL-TOBSHY, Z. M., and J. B. SINCLAIR. 1969. Pathological relationship between plant and animal isolates of *Geotrichum candidum* in avian egg. *Phytopathology* 59:532-535.
 8. HARVEY, J. M. 1967. Modified atmospheres in transport. Pages 193-194 in *United Fresh Fruit and Vegetable Assoc. Yearbook*. United Fruit and Vegetable Assoc., Washington, D. C. 352 p.
 9. LOCKHART, C. L. 1967. Influence of controlled atmospheres on the growth of *Gloeosporium album* in vitro. *Can. J. Plant. Sci.* 47:649-651.
 10. LOCKHART, C. L. 1968. Influence of various carbon dioxide and oxygen concentrations on the growth of *Fusarium oxysporum* in vitro. *Can. J. Plant Sci.* 48:451-453.
 11. PARSONS, C. S., and D. H. SPALDING. 1972. Influence of a controlled atmosphere, temperature, and ripeness on bacterial soft rot of tomatoes. *J. Am. Soc. Hortic. Sci.* 97:297-299.
 12. POOLE, R. F. 1922. A new fruit rot of tomatoes. *Bot. Gaz.* 74:210-214.
 13. STOVER, R. H., and S. R. FREIBERG. 1958. Effect of carbon dioxide on multiplication of *Fusarium* in the soil. *Nature* 181:788-789.
 14. TRINCI, A. P. J. 1972. Culture turbidity as a measure of mould growth. *Trans. Br. Mycol. Soc.* 58:467-473.
 15. WELLS, J. M. 1974. Growth of *Erwinia carotovora*, *E. atroseptica*, and *Pseudomonas fluorescens* in low oxygen and high carbon dioxide atmospheres. *Phytopathology* 64:1012-1015.
 16. WELLS, J. M., and M. UOTA. 1970. Germination and growth of five fungi in low-oxygen and high-carbon dioxide atmospheres. *Phytopathology* 60:50-53.
 17. WILSON, J. W., and O. A. PLUNKETT. 1965. *The fungous diseases of man*. University of California Press., Berkeley. 428 p.
 18. WIMPENNY, J. W. T. 1969. Oxygen and carbon dioxide as regulators of microbial growth and metabolism. Pages 161-198 in *17th Annu. Sympos., Soc. Gen. Microbiol.* 1969.
 19. WRIGHT, W. R., N. A. SMITH, and L. BERAHA. 1964. Sour rot of carrots. *Plant Dis. Rep.* 48:837-838.