Effect of Oxygen on Carbon Monoxide Suppression of Postharvest Pathogens of Fruits

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ABSTRACT

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Botrytis cinerea, Monilinia fructicola, and *Penicillium expansum* grew well in an atmosphere containing only 4% oxygen. Large reductions in growth occurred only when oxygen dropped below 2%, a level that may result in fermentative respiration and injury to most fruits. Carbon monoxide (9–11%) strikingly suppressed fungal growth only if the atmosphere contained less than about 5 or 6% oxygen. Carbon monoxide may thus have utility as a fungistatic component of low oxygen modified atmospheres in transport or storage of fruits.

Modification of atmospheres is widely used to suppress respiration and extend the postharvest life of fruits that tolerate low oxygen (O_2) and elevated carbon dioxide (CO_2). At atmospheres commonly used, 2 or 3% O_2 and 5% CO_2, fungal decays may become serious during the fruits' extended postharvest life (2–4, 23, 26, 27).

Although elevated CO_2 has long been known to suppress microbial growth, its utility for that purpose is severely limited by the sensitivity of many host commodities. Striking fungal suppression is commonly observed at CO_2 concentrations of 15–20% or more, but few commodities tolerate those levels for more than a few days or weeks (13,22). CO_2 concentrations commonly used in controlled atmosphere storages (ie, 5%) suppress most fungi only modestly. The controlled atmosphere O_2 levels (ie, 2–3%) usually suppress fungi little, if at all (4,12,17,26). For example, Couey et al (8,9) showed that Botrytis rot in strawberries is adequately suppressed only when O_2 is lowered to about 0.5%. That level is dangerously close to the concentration at which anaerobic respiration results in off-flavors.

Clearly, an atmosphere containing a fungistatic gas would have advantages. A possible candidate is carbon monoxide (CO), which is slightly fungistatic in air and often highly fungistatic in reduced O_2 atmospheres (10,15,20). Currently, CO is sometimes included in the modified atmospheres (MA) of refrigerated vans or marine containers, usually to reduce physiologic difficulties, such as enzymatic browning of injured head lettuce (14,24,25). Such use has generated interest in the role of CO in suppressing postharvest diseases.

Although maximum fungal suppression by CO has been associated with low O_2 (ie, 2–4%) rather than with air (about 21% O_2) (10,15,20), no information is available on O_2 concentrations below 2% or between 4 and 21%. The effect of CO concentration on fungal suppression is also largely unexplored. The objective of this study, therefore, was to interrelate the roles of various O_2 and CO concentrations in suppressing fungal growth.

MATERIALS AND METHODS

The fungi studied are major postharvest pathogens of stone and pome fruits, namely *Botrytis cinerea* Pers. ex Fr., *Monilinia fructicola* (Wint.) Honey, and *Penicillium expansum* Lk. ex Thom. The fungi were originally isolated from rotting fruit and maintained on potato-dextrose agar (PDA) slants in a household refrigerator at 4 or 5 C. Their pathogenicity was verified by fruit inoculation before use in these studies.

Tests were conducted in constant-temperature rooms that varied ± 0.5 C from indicated mean temperatures. Temperatures were continuously recorded to ensure that mechanical malfunction had not caused unsuspected temperature fluctuations.

Gases used were commercial CO and nitrogen (N_2). Low O_2 was achieved by diluting air with N_2. Atmospheres are described in terms of the physiologically active gases O_2 and CO. The remaining portion of the atmosphere is N_2 with trace contaminants. Concentrations of CO did not exceed 11% because higher concentrations in air are potentially flammable (28). All gases were humidified and dispensed by capillary flow meters at a pressure of 50 cm of water, as described by Claypool and Keefer (7). Gas mixtures were initially monitored daily by gas-liquid chromatography to determine and verify concentrations of O_2, CO, and CO_2 in test chambers. Gas analyses were subsequently done periodically to ensure that respiration of cultures or fruit had not noticeably lowered O_2 or raised CO_2 and ethylene.

In vitro tests used PDA in a thin (about 2 mm) layer in petri dishes at 20 C, and growth was measured as radial extension of colonies. Inocula, each consisting of a 4-mm-diameter plug cut with a cork borer from the advancing edge of a culture several days old, were positioned at the center of test dishes. Lids were permanently raised by means of a bent sterile wire to facilitate maintenance of the cultures at the atmospheres of the test containers.

In vivo tests were done with Delicious apples inoculated with *B. cinerea.* Fruits grown near Watsonville, CA, were held in a commercial controlled atmosphere storage (2 or 3% O_2, 5% CO_2, at 0 C) for about 5½ mo. They were then moved to Davis and placed at 0 C in air until tests were initiated.

Conidia for fruit inoculation were obtained from 2-wk-old cultures of *B. cinerea* growing on V-8 juice agar in 300-ml flasks. Sterile distilled water, containing one drop of Tween-80 per 100 ml and glass beads, was added aseptically, and the flask was gently swirled to dislodge spores. The resulting spore suspension

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was transferred to a sterile screwcap tube, which was vigorously shaken to wet spores. The wet spores were pelleted by low-speed centrifugation (about 2,000 rpm). The liquid, with spores that had not been wetted and therefore not pelleted, was decanted and replaced with fresh Tween solution. Spores were washed twice by resuspension, centrifugation, and replacement of the liquid with fresh Tween solution. The concentration of the spore suspension was determined with the aid of a Bausch and Lomb Spectronic 20 colorimeter. Absorbancy at 490 nm was related to a curve previously established with the aid of a hemocytometer.

Fruits were washed briefly with a 0.5% solution of sodium hypochlorite before inoculation in two equatorial positions on opposing sides. The inoculation needle was dipped into a spore suspension (1.2×10^7 conidia per milliliter) before penetrating the skin to a depth of 2 mm. Fruits were incubated at 25 C for 24 hr to permit infection, exposed to moving air at 0 C to lower the core temperature to 5 C, and placed in 3.78-L jars that were at 5 C and fitted with two-hole rubber stoppers. The inlet tube went to the bottom of the jar, and the gas was exhausted from the top.

Ten apples were placed in jars through which gases were passed at the rate of approximately 120 cc/min. The flowing system prevented respiration-induced atmospheric changes.

Fruits were removed after 13 days. All 20 lesions on the 10 fruit were measured and the average of the longest and shortest diameters was recorded as the lesion size. In a few cases, fewer than 20 lesions were measured because infection had evidently not occurred and no lesions developed. Growth was expressed as the average lesion expansion in millimeters per day.

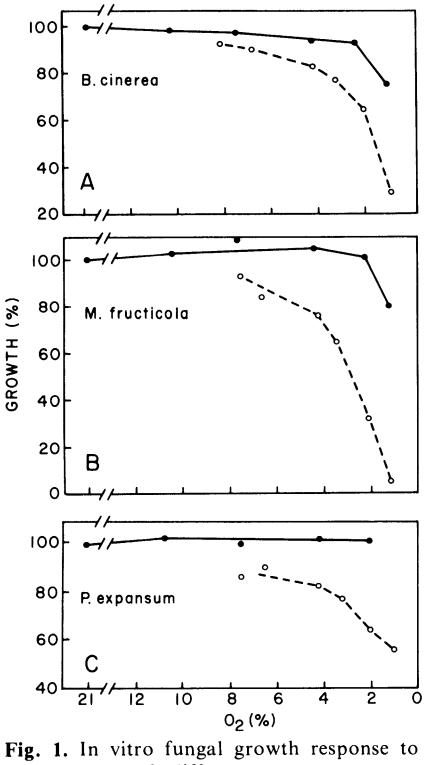

Fig. 1. In vitro fungal growth response to atmospheres of different oxygen concentrations alone (solid line) or with 10% carbon monoxide (dotted line) at 20 C.

RESULTS

O_2 levels below about 4% critically influenced the suppression of fungal growth in vitro by CO at 20 C (Fig. 1). Growth of *B. cinerea* in 2.2% O_2 without CO was about 94% that of the rate of growth in air (about 21% O_2). Addition of 10% CO to the 2.2% O_2 atmosphere resulted in growth 66% of that in air. The CO was thus responsible for a 28% reduction beyond the effects of low O_2 alone. At 1.1% O_2, growth was 75% of the air control; addition of 10% CO resulted in growth only 29% of the air control.

M. fructicola exhibited an even greater response to CO. Growth at 2.2% O_2 without added CO was essentially the same as in air. With addition of 10% CO, however, growth was only 32% of that in air. At 1.1% O_2, growth was 80% of that in air, but with CO added, the rate was only 4% of the growth in air.

P. expansum grew as well at 2.5% O_2 as in air. This finding agrees with those of others (17), who have previously demonstrated the ability of *P. expansum* to grow at low O_2 levels. CO in the atmosphere reduced growth to 62% of that in air.

The relation of various CO levels to growth was examined in tests in which O_2 was held at 2.1% and the temperature at 20 C (Fig. 2). Within the range of about 2–11% CO, the organisms exhibited the greatest reduction in growth rate as the CO concentration was increased to 6.5%. Benefits of concentrations beyond that were small.

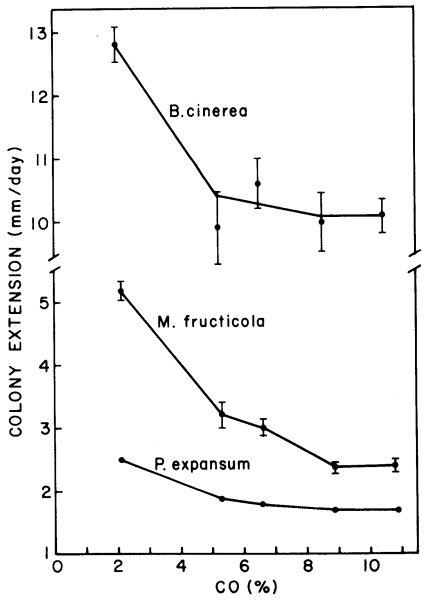

Fig. 2. In vitro fungal growth response to varying concentrations of carbon monoxide in the presence of 2.1% oxygen at 20 C.

Varying mixtures of O_2 and CO were tested with *B. cinerea*-inoculated apples (Fig. 3). CO suppression of lesion diameters was closely related to O_2 concentrations (Fig. 3A), as predicted from results in vitro (Fig. 1A). When O_2 remained constant at 2.1% and CO varied between 2 and 10.4%, lesions appeared to be suppressed linearly as concentrations increased (Fig. 3B).

DISCUSSION

Our results and previous findings (10,14–16,24,25,28) permit us to be cautiously optimistic that CO in storage or transit atmospheres may be commercially beneficial in the suppression of fungal rots. Woodruff (28) pointed out that if postharvest diseases are a limiting factor in a product's storage life, conventional MAs usually have marginal, if any, benefit. Consequently, any commodity that benefits physiologically from MA but suffers excessive disease losses during extended postharvest life might be a candidate for investigation. Particularly in transit vehicles, use of CO may partially compensate for less than ideal temperatures.

Assiduous attention to dangers of commodity freezing causes carriers to maintain disease-inducing temperatures of 4–5 C instead of the often preferred 0 C. On the other hand, chilling-sensitive

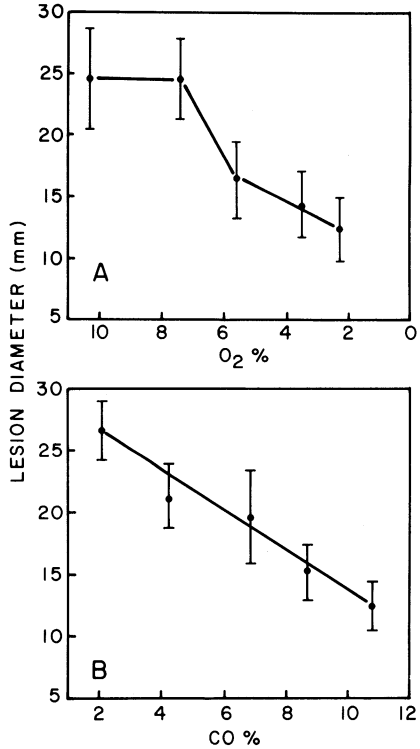

Fig. 3. Development of *Botrytis cinerea* in Delicious apples held in 5-C atmospheres: **(A)** Oxygen concentrations with 10.5% carbon monoxide. **(B)** Carbon monoxide concentrations with 2.1% oxygen.

commodities requiring temperatures of 10–13 C may show excessive rotting, which might be alleviated by use of an MA containing CO. Benefits might accrue from adding CO to MA storage of apples and pears to suppress *P. expansum* and *B. cinerea*. Added benefits would result if *Pezicula malicorticis* (Jacks.) Nannf. (= *Gloeosporium perennans* Zeller & Childs) and *P. alba* Guthrie (= *G. album* Osterw.) prove to be sensitive to CO because they are ordinarily insensitive to MA (1,17). Further, the suppression in commercial practice might be considerably greater than our results indicate because of the CO_2, which appears to have an additive effect with high CO and low O_2 in fungal suppression (10). On the other hand, evidence that CO, at concentrations used here, may mimic the effects of ethylene in stimulation of respiration and ripening (14,15) may limit use of CO for long-term storage of commodities that are highly sensitive to ethylene. The kiwi fruit (*Actinidae chinensis* Plan.), for example, is extremely sensitive to ethylene and ripens at 0 C within a few weeks. Although MA (2.5% O_2, 5% CO_2) slowed ripening, the 10% CO largely eliminated that beneficial effect of MA in tests not reported here.

Results of tests do not permit visualization of the use of CO in MA as a substitute for existing disease control programs. At best, CO may permit longer storage or transport of commodities greater distances without losses to postharvest diseases. Such a benefit may be highly important, however, in commodities attacked by CO-sensitive organisms. Further, there is no suggestion that use of CO will eliminate the need for refrigeration when used with commodities customarily held at low temperature.

CO suppression of fungal growth is closely related to O_2 concentration. Curves (Fig. 1A and B) suggest that as O_2 levels approach zero, the effect of added CO will be a shifting of the fungal-growth-suppression curve to the left. The magnitude of the CO-induced growth suppression, as affected by O_2, was reasonably comparable whether in vitro (Fig. 1A) or in vivo (Fig. 3A). An important difference between in vitro and in vivo results occurred with regard to the effects of CO concentration. When in vitro (Fig. 2), the CO-suppression curve for *B. cinerea* leveled out with little added suppression as the CO concentration increased beyond 6.5%. When in vivo, however, the same fungus was increasingly affected as the CO concentration increased. The reason for the divergent results is not readily evident. The suppression of pathogenic growth and in vitro growth may differ fundamentally, but that seems unlikely.

Many hosts will tolerate the CO levels used in this study (10,14,16,25,28) if the O_2 levels do not fall too low. However, further studies are needed to test a range of CO and O_2 concentrations. Particularly needed are studies of the accumulation through anaerobic respiration of ethanol, acetaldehyde, and other products that lead to off-flavors. Any tendency for CO to exacerbate the effects of marginally low O_2 atmospheres, causing anaerobic respiration, might prove to be a serious drawback.

None of the methods for in vitro studies of the effects of various atmospheres on growth of fungi are wholly satisfactory. This is especially true in studies in which O_2 concentration is very critical and anoxia or near-anoxia must be carefully avoided.

Dry weight of total fungal matter produced is perhaps the best index of readily measurable growth. Ordinarily, however, liquid media is used to permit ready harvest of structures by filtration at the end of the test. Use of liquid media can create serious problems of aeration (5,11,18,19). Essentially, the fungus must obtain all molecular O_2 from that dissolved in the medium. Four conditions generally insure that dissolved O_2 at the point of absorption will not be in equilibrium with the atmosphere (5,19): the very low solubility of O_2 in media, the rheology of fungus cultures that limit the effectiveness of aeration by shaking (21), rapid O_2 consumption by growing fungi, and the clumping habit of filamentous fungi in liquid media. Formation of mycelial clumps or strands limits full access of interior cells to dissolved O_2 (6). A further concern with liquid media, unless continuous-flow conditions prevail, is the exhaustion of nutrients or the staling of the medium. These problems are not readily solvable with equipment generally available in research laboratories, but a partial solution has been reached with several fungus species by means of chemostats (5). Therefore, we believed that it was preferable in these studies to grow colonies on shallow, solid medium and use radial colony extension as the index of growth.

LITERATURE CITED

1. Bompeix, G. 1978. The comparative development of *Pezicula alba* and *P. malicorticis* on apples in vitro (air and controlled atmosphere). Phytopathol. Z. 91:97-109.
2. Brooks, C., Bratley, C. O., and McColloch, L. P. 1936. Transit and storage diseases of fruits and vegetables as affected by initial carbon dioxide treatments. U.S. Dep. Agric. Technol. Bull. 519. 24 pp.
3. Brooks, C., Miller, E. V., Bratley, C. O., Cooley, J. S., Mook, P. V., and Johnson, H. B. 1932. Effect of solid and gaseous carbon dioxide upon transit diseases of certain fruits and vegetables. U.S. Dep. Agric. Technol. Bull. 318. 6 pp.
4. Brown, W. 1922. On the germination and growth of fungi at various temperatures and in various concentrations of oxygen and of carbon dioxide. Ann. Bot. 36:257-283.
5. Bull, A. J., and Trinci, A. P. J. 1973. The physiology and metabolic control of fungal growth. Adv. Microbiol. Physiol. 15:1-84.
6. Bungay, H. R., Whalen, W. J., and Sanders, W. M. 1969. Microprobe techniques for determining diffusivities and respiration rates in microbial slime systems. Biotechnol. Bioeng. 11:765-772.
7. Claypool, L. L., and Keefer, R. M. 1942. A colorimetric method for CO_2 determination in respiration studies. Proc. Am. Soc. Hortic. Sci. 40:177-186.
8. Couey, H. M., Follstad, M. N., and Uota, M. 1966. Low-oxygen atmospheres for control of postharvest decay of fresh strawberries. Phytopathology 56:1339-1341.
9. Couey, H. M., and Wells, J. M. 1970. Low-oxygen or high-carbon dioxide atmospheres to control postharvest decay of strawberries. Phytopathology 60:47-49.
10. El-Goorani, M. A., and Sommer, N. F. 1979. Suppression of postharvest plant pathogenic fungi by carbon monoxide. Phytopathology 69:834-838.
11. Finn, R. K. 1954. Agitation-aeration in the laboratory and in industry. Bacteriol. Rev. 18:254.
12. Follstad, M. N. 1966. Mycelial growth rate and sporulation of *Alternaria tenuis, Botrytis cinerea, Cladosporium herbarum,* and *Rhizopus stolonifer* in low-oxygen atmospheres. Phytopathology 56:1098-1099.
13. Isenberg, F. M. R. 1979. Controlled atmosphere storage of vegetables. Hortic. Rev. 1:337-394.
14. Kader, A. A., Brecht, P. E., Woodruff, R., and Morris, L. L. 1973. Influence of carbon monoxide, carbon dioxide, and oxygen levels on brown stain, respiration rate, and visual quality of lettuce. J. Am. Soc. Hortic. Sci. 98:485-488.
15. Kader, A. A., Chastagner, G. A., Morris, L. L., and Ogawa, J. M. 1978. Effects of carbon monoxide on decay, physiological responses, ripening and composition of tomato fruits. J. Am. Soc. Hort. Sci. 103:665-670.
16. Kader, A. A., and Morris, L. L. 1977. Relative tolerance of fruits and vegetables to elevated CO_2 and reduced O_2 levels. Pages 260-265 in: D. H. Dewey, ed. Controlled Atmospheres for the Storage and Transport of Perishable Agricultural Commodities. Hortic. Rep. 28, Mich. State Univ., East Lansing, MI.
17. Lockhart, C. L. 1969. Effect of CA storage on storage rot pathogens. Proc. Nat. CA Res. Conf., Mich. State Univ., 27-28 Jan. 1969. pp. 113-121.
18. Lockhart, W. R., and Squires, R. W. 1963. Aeration in the laboratory. Adv. Appl. Microbiol. 5:157.
19. Meynell, G. C., and Meynell, E. 1965. Theory and Practice in Experimental Bacteriology. Cambridge Univ. Press. 287 pp.
20. Ogawa, J. M., Chastagner, G. M., and Kader, A. A. 1978. Effect of storage environment on development of postharvest diseases of temperate fruit. (Abstr.) 3rd Int. Congr. Plant Pathol., Munich, 16-23 August, 1978. p. 270.
21. Roels, J. A., Van Den Berg, J., and Voncken, R. M. 1974. The rheology of mycelial broths. Biotechnol. Bioeng. 16:181-208.
22. Smock, R. M. 1979. Controlled atmosphere storage of fruits. Hortic. Rev. 1:301-336.
23. Spalding, D. H., and Reeder, W. F. 1975. Low-oxygen high-carbon dioxide controlled atmosphere storage for control of anthracnose and chilling injury of avocados. Phytopathology 65:458-460.
24. Stewart, J. K., and Uota, M. 1972. Carbon dioxide injury to lettuce as influenced by carbon monoxide and oxygen levels. HortScience 7(2):189-190.
25. Stewart, J. K., and Uota, M. 1976. Postharvest effect of modified levels of carbon monoxide, carbon dioxide, and oxygen on disorders and appearance of head lettuce. J. Am. Soc. Hortic. Sci. 101:382-384.
26. Tabak, H. H., and Cooke, W. B. 1968. The effects of gaseous environments on the growth and metabolism of fungi. Bot. Rev. 34:126-252.
27. Valley, G. 1928. The effect of carbon dioxide on bacteria. Q. Rev. Biol. 3:209.
28. Woodruff, R. E. 1977. Use of carbon monoxide in modified atmospheres for fruits and vegetables in transit. Pages 52-54 in: D. H. Dewey, ed. Controlled Atmospheres for the Storage and Transport of Perishable Agricultural Commodities. Hortic. Rep. 28, Mich. State Univ., East Lansing.