Effects of Modified Atmospheres on Postharvest Pathogens of Fruits and Vegetables

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I. INTRODUCTION

Creating a controlled atmosphere (CA) or a modified atmosphere (MA) involves reducing oxygen and/or increasing carbon dioxide concentration. This modification can slow respiration and ethylene production, and delay ripening of fruits. When that is done, the physiological life of a commodity is lengthened (Dewey 1977, 1979; Dewey et al. 1969; Grier- son 1969). But conflicting results cloud assessment of CA's value in controlling decay in fruits and vegetables during and after storage.

Published reviews have mentioned the effects of CA on postharvest pathogens and the postharvest diseases they cause (Smith 1963, 1976; Harvey 1978; Tabak and Cooke 1968; Eckert and Sommer 1967; Tom- kins 1966b; Lougheed et al. 1978). Isenberg (1979) and Smock (1979) have provided reviews of the CA storage of vegetables and fruits, respectively. Burton (1974) has outlined biophysical principles relevant to CA storage of fruits and vegetables. Indexed reference lists on modified atmospheres contain valuable references on the subject (Murr et al. 1974; Kader et al. 1976; Kader and Morris 1977; Morris et al. 1971). This review covers the extensive literature on the effects of controlled atmospheres on postharvest pathogens and diseases.

II. METHODOLOGY

A. Atmosphere Maintenance

The creation and maintenance of test atmospheres of precise com- positions are crucial to critical studies. In early studies desired atmospheres for test organisms often were created in containers that were sealed until the end of the test. For most studies, such techniques have been found to be inadequate, yielding, as often as not, results subject to misinterpretation. In those closed systems, respiration of test commodi- ties usually caused a sharp increase in CO₂ concentration in the atmo- sphere, while O₂ was depleted. In experiments with fruit, the physiologically active gas ethylene (C₂H₄) may accumulate also along with certain volatile esters and aldehydes evolved by the fruit.
In modern test systems, gases are usually provided as a continuous flow at a rate sufficient to avoid appreciable accumulation of CO₂, C₂H₄, or other respiratory gases, or to prevent depletion of O₂. Mixed gases are sometimes obtained in pressure cylinders, from which desired flows can be metered using reducing valves and flow meters. Atmospheres may also be prepared by using cylinders of O₂, CO₂, and N₂ bought commercially without mixing before tests. When separate cylinders are used, each gas is metered in the proportions calculated to achieve the desired synthetic atmospheres (Claypool and Keefer 1942), and mixed before entered into test containers. Atmospheres low in O₂ are often obtained by diluting air with N₂. Although O₂ obtained by air dilution is satisfactory for many studies, it should be noted that CO₂ and other air contaminants are present in dilute quantities. Furthermore, gases from commercial sources may contain contaminants.

B. Disease Suppression

Disease suppression attributable to atmosphere modification has two effects: on the resistance of the host commodity and on the altered growth of the pathogen.

1. Commodity Maturity or Condition.—Fruits that go through pronounced ripening (apples, bananas, mangos, peaches, pears, papayas, etc.) usually are highly resistant to postharvest pathogens during most of their lives. Susceptibility increases as they approach maturity and proceed through the ripening process to senescence. Care must be taken to ensure that fruits are of about the same physiological age at harvest. Comparisons of surface or flesh color, penetrometer readings, or soluble solids measurements are generally satisfactory ways to compare uniformity of maturity. With climacteric-type fruits, it may be desirable to determine whether the fruits are truly pre-climacteric at the start of extended storage tests. Similarly, nonclimacteric fruits, leafy vegetables, stems, and roots or tubers undergo changes leading to senescence with a likely decrease in resistance to diseases. Lesions frequently develop much more rapidly in senescing fruit than in recently harvested fruits of proper maturity.

2. Inoculations.—Occasionally, tests can be conducted satisfactorily with uninoculated fruit. An experienced researcher can sometimes be reasonably certain that an acceptably uniform natural infection has occurred before harvest and is undeveloped, quiescent, or latent in the fruit. For example, pathogens causing Gloeosporium rots of pome fruits, Botrytis rots of strawberries and grapes, and anthracnose of various
subtropical and tropical fruits may heavily infect fruit before harvest but disease development occurs after harvest.

Fruits usually must be inoculated before being placed in chambers supplied with test gases. Inoculation methods vary and no one method is appropriate in all circumstances. Inoculation is often done by piercing the epidermis of the commodity with a needle contaminated with spores or cells of the fungal or bacterial pathogen. Uniformity of the inoculum may be improved by standardizing spore or cell concentrations using a hemocytometer. The inoculated commodity may be placed immediately in test chambers. To achieve greater uniformity of lesion size within treatments, however, fruits may be held in air at 20° to 25°C in high humidity for 12 to 24 hours to permit infection to occur before they are placed in chambers.

Once an atmosphere has been established, it should not be interrupted, and fruit should not be removed from a chamber until the end of the test. Tests at a particular atmosphere normally are ended before a lesion occupies a major portion of a fruit’s surface (generally less than 6 cm in diameter for apples, pears, or peaches). Lesion diameters can be measured and average radial growth rates are expressed as millimeters per day. Such data, however, suggest that growth has been linear when, in fact, it has really been sigmoidal with a lag phase before establishment of a rapid and steady rate of lesion development. Researchers occasionally have removed and weighed rotted tissue. Such fresh or dry weights would reflect, in a large measure, host tissue rather than mycelium. Similarly, measurement of the volume of the lesion does not indicate the density of fungal mycelium within the lesion.

Alternative methods have been suggested as possible indicators of the extent of pathogenic growth. Some have suggested respiration measurement (Okazaki and Sugama 1979), but most methods have involved chemical analyses of mycelial components in rotted host tissue. Chitin, a polymer of acetyl glucosamine, is a major component of mycelial walls of many fungi and is found in animals, but is generally not found in higher plant tissues. Chitin content of lesions can be used as a measure of mycelium within host tissue. In analyses, chitin is hydrolyzed to glucosamine and measured colorimetrically or chromatographically (Ride and Drysdale 1972; Wu and Stahmann 1975; Donald and Mirocha 1977).

Seitz et al. (1977, 1979) have suggested that the amount of ergosterol could be a measure of fungus growth in higher plant tissues. They have pointed out that ergosterol is the predominant sterol of most fungi and is either absent or sparse in most higher plants. Studies with Alternaria alternata (Fr.) Keissler, Aspergillus flavus Link, and A. amstelodami (Mangin) Thom & Church in cereal seeds led to the development of an
analytical procedure for high-pressure liquid chromatography. Extraction procedures for ergosterol are said to be simpler and less time-consuming than for chitin.

C. In vitro Studies

Studies of postharvest pathogens in culture provide a direct assessment of the effects of an atmosphere on the physiology of an organism. Eliminated are the indirect suppressive effects of host resistance, which may vary with maturity or condition of the host commodity (which, in turn, may be affected by the atmosphere).

Most studies reviewed herein employed common complex media such as potato-dextrose or malt-extract agar. Chemically defined media are required for many biochemical or physiological studies. Fungi have sometimes been observed to be more dramatically affected by atmospheric modification when grown on defined media instead of complex media (Brown 1922).

Solid media generally have been employed, but some researchers have used liquid media. Fungal growth curves resulting from various O₂ concentrations are often quite different in liquid than in solid media (Mitchell and Zentmyer 1970, 1971a,b; Wells and Uota 1970). Consequently, it is appropriate to review briefly some advantages and problems of solid versus liquid media.

1. Solid Media.—Growth of surface colonies of fungi on solid media has been the subject of many studies, of which only a few of the most pertinent have been included (Trinci 1971a,b; Pirt 1967; Righelato et al. 1968). Bull and Trinci (1977) covered the subject of filamentous fungi growth in solid versus liquid media in a recent review.

Trinci (1971a) and Carter and Bull (1971) studied in detail the growth on solid media of Penicillium chrysogenum Thom, Geotrichum lactis (= G. candidum Lk. ex Pers.), Rhizopus stolonifer (Ehrenb. ex Fr.) Lind., Mucor racemosus Fres., Absidia glauca Hagem, Aspergillus niger Van Tiegh., A. wentii Wehmer, and A. nidulans Eidam. Elongation of the hyphae occurred only at the apices. However, a much larger zone, including hyphal segments that have septa with yet unplugged pores, influenced growth by contributing protoplasm by mass transfer to the apex. Thus, the rate of extension was believed to be a function of the length of the terminal portion and the rate of duplication of protoplasm within the peripheral growth zone surrounding the colony behind the apices. Growth behind the peripheral area resulted in increased hyphal density, formation of fruiting structures, and bodies such as sclerotia but did not contribute to the colony's radial expansion.
Bull and Trinci (1977) pointed out that peripheral growth zones are much wider for colonies of fungi (8.5 mm for Rhizopus stolonifer) than for bacterial colonies (90 μm for Escherichia coli [Migula] Castellani and Chalmers). The wide growth zones result in radial growth rates that usually exceed the rate of diffusion of a chemical within the medium. Thus, secondary metabolites and other products formed at the center of the colony diffuse through the medium more slowly than the colony expands. The peripheral growth zone is thus advancing constantly into fresh, unexploited areas of the medium. Hence, exhaustion of nutrients and accumulation of toxic metabolites affect the central portion of the colony but not the periphery. Bacterial colonies, on the other hand, expand very slowly and secondary metabolites formed at the center of the colony diffuse through the medium and inhibit growth at the periphery.

In experiments involving effects of test atmospheres, hyphae of the peripheral zone are exposed to the atmosphere on or just below the agar surface. Hyphae submerged beneath the central portion of the colony, however, may be subject to anoxia and staling of the medium by secondary metabolites.

In tests of various atmospheres, measurements of colony diameters or radii presumably reflect peripheral growth accurately. However, measurements do not reflect differences in colony height, density, or of those growth processes involved in sporulation or formation of sclerotia. Presumably, methods proposed for estimating mycelia in host tissue could be adapted for growth measurements in solid media.

2. Liquid Media.—Problems associated with use of liquid media are exacerbated when tests involve atmosphere modifications. Difficulties with liquid media include (1) the low solubility of gases, (2) the growth habit of filamentous fungi, and (3) accumulation of secondary metabolites.

a. Solubility of Gases.—The solubility of gases in liquids follows definite patterns (Golding 1945; Brancato and Golding 1953), as follows:

(a) As indicated by Henry's law (Weast et al. 1964), the solubility of a gas that will dissolve in a liquid is proportional to the partial pressure of that gas over the liquid, and is independent of the partial pressures of other gases or of total pressure.

(b) Gases are more soluble at low than at high temperatures.

(c) In salt or sugar solutions, gases change in solubility in inverse proportion to the concentration of the solute, provided that the gases are not also soluble in the solute.
Finn (1954), Meynell and Meynell (1965), Carter and Bull (1969), Corman et al. (1957), Darby and Goddard (1950), Lockhart and Squires (1963), Roels et al. (1974), and Starks and Koffler (1949) have reviewed problems of O₂ supply and have pointed out that O₂ is relatively insoluble in water. Distilled water saturated with air at one atmosphere at 30°C contains only 240 µM/liter of dissolved O₂ (Harrison 1976). Atmospheres of 2% O₂ (16 mm Hg) or 1% O₂ (8 mm Hg) contain 24 µM/liter and 12 µM/liter, respectively. However, fungal respiration and growth are usually about equal between O₂ tensions of 160 mm and 20 mm Hg. The critical tension at which growth rates start to drop rapidly was found to be about 7 mm Hg (10.5 µM/liter) for Aspergillus nidulans (Carter and Bull 1969, 1971), although the critical tensions appeared to vary with the previous growth history of the fungus.

Commonly, 2% O₂ is used in MA tests with fruits and vegetables. Culture broths saturated with such an atmosphere may approach the critical dissolved O₂ tension for many fungi. If the rate of O₂ diffusion into the medium cannot meet respiratory demands of an organism, levels of dissolved O₂ in the medium may fall drastically. Inadequate diffusion causes the O₂ concentration of liquid media to be far below equilibrium with the partial pressure of the O₂ of the atmosphere. A lower-than-expected growth rate is a consequence. Thus, problems of O₂ diffusion in liquid media could result in apparent growth suppression at relatively high O₂ atmospheric pressures.

CO₂ dissolves in liquid media to form carbonic acid, which dissociates to form bicarbonate and carbonate ions as follows:

\[
\begin{align*}
\text{CO}_2 + \text{H}_2\text{O} & \rightleftharpoons \text{H}_2\text{CO}_3^- \\
\text{H}_2\text{CO}_3^- & \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \\
\text{HCO}_3^- & \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}
\end{align*}
\]

The concentration of bicarbonate ions, which affect growth, increases about 10-fold for every increase of one pH unit. Therefore, it is essential that cultures be buffered to avoid important pH shifts.

b. Fungal Growth Habit.—In liquid media, filamentous fungi clump under the usual conditions of a shake-flask culture. Spores added to such liquid media may be well dispersed initially but under conditions favorable to germination, clumping becomes noticeable after germ-tube protrusion. Germ tubes become elongated and, after additional growth, the resulting mycelium becomes clumped into masses of various sizes and shapes (Trinci 1971b). Researchers have long suspected that conditions of low O₂, or of anoxia, existed within such clumps because O₂ uptake
exceeds penetration of dissolved O₂. Bungay et al. (1969) obtained direct supporting evidence for that supposition by using an O₂ electrode 1.5 μ in diameter. The very low O₂ level within a 200 μ-thick bacterial film was characteristic of respiration limited by O₂ concentration. Therefore, mycelial clumping results in less growth than would otherwise be expected from the dissolved oxygen concentration of the fluid or from the O₂ partial pressure of the atmosphere.

c. Metabolite Accumulation.—Fungi produce secondary metabolites that accumulate in the media. Some of these either kill or otherwise affect the fungus. Bu’Lock (1975) reviewed the effects of secondary metabolites on growth and development. Secondary metabolites are dispersed thoroughly in a liquid when the medium is agitated for aeration. To prevent an excessive accumulation of these growth-suppressing products, fresh medium must be added constantly to replace an equal volume of old medium with its suspended cells. Single-celled organisms such as bacteria and yeasts are adapted to such a system. Unfortunately, the growth habit of filamentous fungi seriously complicates the establishment of a steady state. Chemostats for continuous culture of several filamentous fungi have been developed in the fermentation industry for the commercial production of antibiotics and other chemicals. Considerable effort has been devoted to studies of fungal growth kinetics and the problems of use of continuous cultures. These have been reviewed by Righelato (1975), Righelato and Pirt (1967), and Bull and Trinci (1977).

3. Anaerobic Conditions.—Tests designed to determine if an organism can grow in the absence of molecular O₂ may lead to erroneous results if O₂ contaminates the culture from unsuspected sources. Although serious problems are posed by the low solubility of O₂ in aqueous solutions and inadequate diffusion throughout the media and fungus structures, the removal of all molecular O₂ poses equally serious difficulties. Small amounts of O₂, unnoticed and unimportant in comparisons of O₂ levels in atmospheres for fruit or vegetable storage, may prevent the achievement of anaerobic conditions. Sources of contamination are varied. Media contain dissolved O₂ unless thoroughly purged. Air is introduced when flasks are opened to permit inoculation of cultures. Commercially supplied N₂ contains O₂ that can be removed by passing it through hot copper filings, bubbling it through alkaline pyrogallol, or both. Small amounts of O₂ may permeate rubber tubing. Furthermore, Claypool and Keefer’s (1942) flow meter system, widely used in postharvest laboratories, provides considerable contact with water that may contain dissolved O₂ (Bussel et al. 1968, 1969a,b, 1971).
III. ATMOSPHERIC EFFECTS IN VITRO

A. Oxygen Effects

1. General.—Respiration accounts for the greatest involvement of $O_2$ in aerobic organisms, where it serves as an electron acceptor during substrate oxidation. In addition to a tricarboxylic acid cycle, most fungi have an electron-transport system that closely resembles that of animals or higher plants. Fungal electron transport involves a flow of electrons from NADH$_2$ and substrate through a system that includes cytochromes b, c, and aa$_3$, and contains up to three energy-conserving sites (Bull and Trinci 1977). The cytochromes are hemoproteins found in mitochondrial membranes. Their principal biological function is to transport electrons, which they do by means of a reversible valency change of their prosthetic group, tetrapyrrolic chelate of iron ($Fe^{++} = Fe^{+++}$) (Bull and Trinci 1977; Harrison 1976; Lindenmayer 1965; Meynell and Meynell 1965).

In contrast with eukaryotic organisms such as fungi, bacteria have a great variety of cytochrome systems. Aside from the variety of cytochromes found in different species, it is not unusual for bacteria to have up to three different terminal cytochrome oxidases (Harrison 1976; Lindenmayer 1965). Other oxidative enzyme systems exist in cells and are probably affected by low-oxygen tensions, but their impact on growth suppression is less well known and normally involves much less $O_2$ than does the cytochrome system (Harrison 1976).

Bussel et al. (1969a) studied the effects of absence of $O_2$ on the ultrastructure of Rhizopus stolonifer sporangiospores. Spores were incubated for 3 hours with or without subsequent periods of anoxia. Of particular note was the condition of mitochondria, which were distributed randomly throughout the cell and exhibited well developed parallel cristae. After 72 hours of anoxia, mitochondria were few, small with indistinct cristae, and they were located at the periphery of the cytoplasm. The cytoplasm had become very mottled and had many vacuoles and vesicles. Lipid bodies were abundant. Upon returning the spores to air, mitochondria were again seen in the central portion of the cell. They had enlarged, developed prominent cristae, and otherwise resembled mitochondria of spores that had not been subjected to anoxia. In further studies, Bussel (1969b) showed that spores of R. stolonifer do not germinate if $O_2$ is rigorously excluded. Spores incubated 1, 2, or 3 hours, and spores given no such incubation, were insensitive to 72 hours of anoxia in liquid medium at 23° to 25°C. However, spores incubated for 4, 5, and 6 hours were very sensitive to 72 hours of anoxia; only 8, 0.2, and 0.09% were viable, respectively.

Research on the effects of $O_2$ at partial pressures above the 21% $O_2$ content in air has been reported by ZoBell and Hittle (1967) and re-
viewed by Haugaard (1968) and Harrison (1976). Toxicity to high O₂ tensions is widespread among animals, higher plants, and microorganisms. Among the latter, the susceptibility to high O₂ tensions varies, but probably no microorganism is completely insensitive to the toxic effects of O₂. Among anaerobic bacteria, which cannot grow except under strictly reduced conditions, any O₂ is toxic. Some aerobic bacteria and fungi can grow in atmospheres of near 100% O₂.

Haugaard (1968) attributed O₂ toxicity to such damaging events as the oxidation of sulphydryl groups in various compounds, such as glutathion, or of enzymes. He also suggested the possibility of peroxide formation, which can cause extensive destruction (particularly of lipids). Harrison (1976) mentioned work suggesting the possibility of free radical accumulation. Chance et al. (1965) reported on the toxic effects of high O₂ on intracellular oxidation states of reduced pyridine nucleotide in general, and on the energy-linked pathway for pyridine nucleotide reduction in particular. The belief that O₂ exerts its toxic effect through the formation of superoxide radicals (O₂⁻), which destroy some aspects of cell metabolism (Harrison 1976; Gregory and Fridovich 1973), has recently gained support.

Robb (1966) subjected 103 species of fungi to 10 atmospheres pressure of O₂ at 25°C for 7 or 14 days. Among postharvest disease fungi studied, Aspergillus flavus and A. niger grew after cultures were exposed to high O₂ for 14 days. Cladosporium herbarum. Link ex Fr., Colletotrichum dematium (Fr.) Grove, Trichoderma viride Pers. ex Fr., and Mucor racemosus survived 7 days. The following fungi were not able to withstand 7 days of high O₂ pressure: Phytophthora cactorum (Leb. & Cohn) Schroet., Rhizopus arrhizus Fischer, R. oryzae Went & Prin., R. stolonifer, Glomerella cingulata (Stonem.) Spauld. & Schr., Sclerotinia fructigena (Pers.) Schr., Alternaria tenuis (=A. alternata), Botrytis cinereae Pers. ex Fr., and Trichotheccium roseum Link. A lag before resumption of normal growth was frequently observed in fungi surviving the high O₂ treatments.

2. Low Oxygen at Normal Atmospheric Pressure.—The quantitative relations of postharvest fungi and O₂ supply vary considerably among species. The reduction of O₂ required for growth and/or spore-germination inhibition varies with the species. Table 10.1 includes a summary of the literature on the effects of various O₂ concentrations, and from them the following may be concluded:

Oxygen concentrations of about 1% or less are required to obtain appreciable reduction of growth, spore formation, and germination in many postharvest fungi. In most fungi, no growth occurs without molecular O₂ (Ashworth et al. 1969; Brown 1922; Brown and Kennedy
<table>
<thead>
<tr>
<th>Species</th>
<th>O₂ Concentration (%)</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternata</em> (Fr.) Keissler. Syn., <em>A. tenuis</em> Nees.</td>
<td>0.5</td>
<td>Growth reduced by 45% SM-FS at 15°C</td>
<td>Follstad 1966</td>
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<td></td>
<td>0</td>
<td>Growth absent SM-FS at 15°C</td>
<td>Follstad 1966</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Growth reduced by 69% and decreased linearly with decreasing O₂ below 4%. Sporulation inhibited at low O₂ LM-FS at 19°C</td>
<td></td>
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<tr>
<td></td>
<td>0</td>
<td>No growth LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td>Growth not affected SM-FS at 5.5° or 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Ascochyta caricae-papayae</em> Pat. Probable sexual state, <em>Mycosporella caricae</em> Syd.</td>
<td>2.3</td>
<td>Growth reduced by 20% SM-FS at 5.5° or 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> Van Tiegh.</td>
<td>Low</td>
<td>Good growth and spore germination SM-SS</td>
<td>Brancato and Golding 1953</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> Link</td>
<td>2</td>
<td>Growth and aflatoxin production low in cotton seed</td>
<td>Ashworth <em>et al</em>. 1969</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Good growth and aflatoxin production SM-FS at 20°C</td>
<td>Buchanan <em>et al</em>. (unpublished data)</td>
</tr>
<tr>
<td><em>Botryodiplodia theobromae</em> Pat.</td>
<td>2.3</td>
<td>Growth reduced by 15% SM-FS at 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Species</td>
<td>Effect Description</td>
<td>Reference</td>
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<tr>
<td>Botrytis cinerea Pers. ex Fr.</td>
<td>Growth reduced by 50%. Sporulation absent in 1% or less at 15°C</td>
<td>Follstad 1966</td>
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<tr>
<td>Sexual state, Botryotinia</td>
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<tr>
<td>fuckeliana (DeBary) Whetz.</td>
<td>Growth reduced by 55%. Sporulation inhibited at low O₂ at 19°C</td>
<td>Wells and Uota 1970</td>
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<td></td>
<td>Growth not affected at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
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<tr>
<td>Cladosporium herbarum Link ex Fr.</td>
<td>Growth reduced by 65%. Sporulation at low O₂ at 15°C</td>
<td>Follstad 1966</td>
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<td></td>
<td>Growth reduced by 50% at 19°C</td>
<td>Wells and Uota 1970</td>
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<tr>
<td>Colletotrichum gloeosporioides</td>
<td>Growth reduced ca. 10% at 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
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<td>(Penz.) Van Arx</td>
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<td>Sexual state, Glomerella cingulata</td>
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<td>(Stonem.) Spauld. &amp; Schr.</td>
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<tr>
<td>Fusarium roseum (Link) emend.</td>
<td>Growth reduced by 62% at 19°C</td>
<td>Wells and Uota 1970</td>
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<td>Snyd. &amp; Hans.</td>
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<tr>
<td>Geotrichum candidum Link ex Pers.</td>
<td>Growth as great as or greater than in air at 21°C</td>
<td>Wells and Spalding 1975</td>
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<td>Syn., Oospora lactis (Pres.) Sacc.</td>
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<td></td>
<td>Growth stimulated by 10% and 20% at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
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<td>Gloeosporium album Osterw.</td>
<td>Growth retarded at LM</td>
<td>Lockhart 1969</td>
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<tr>
<td>Syn., Phlyctaena vagabunda</td>
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<tr>
<td>Desm. Sexual state, Pezicula alba</td>
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<td>Guth.</td>
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<tr>
<td>Species</td>
<td>O₂ Concentration (%)</td>
<td>Remarks</td>
<td>Reference</td>
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<tr>
<td><em>Monilinia fructicola</em> (Wint.) Honey Syn., <em>Sclerotinia fructicola</em> (Wint.) Rehm.</td>
<td>Anoxia</td>
<td>Viability not lost after 24 or 48 hours, but after 72 hours only 50% grew when placed in air LM-FS at 23° to 25°C Growth reduced by 40% SM-FS at 5.5° and 12.5°C</td>
<td>Bussel et al. 1971</td>
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<td></td>
<td>2.3</td>
<td></td>
<td>El-Goorani and Sommer 1979</td>
</tr>
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<td><em>Penicillium digitatum</em> Sacc. and <em>P. italicum</em> Wehrm.</td>
<td>2.3</td>
<td>Growth slightly reduced SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Penicillium expansum</em> Link ex . Thom.</td>
<td>Low</td>
<td>Good germination and growth SM-SS Growth unaffected SM-FS at 5.5° and 12.5°C</td>
<td>Brancato and Golding 1953</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td></td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Phomopsis citri</em> Faw. Sexual state, <em>Diaporthe citri</em> (Faw.) Wolf</td>
<td>2.3</td>
<td>Growth not affected SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Phytophthora cactorum</em> (Leb. &amp; Cohn) Schroet.</td>
<td>21</td>
<td>Maximum growth LM-FS at 22° to 24°C Growth stimulated by 16% and 20% at 5.5° and 12.5°C SM-FS</td>
<td>Covey 1970</td>
</tr>
<tr>
<td></td>
<td>2.3</td>
<td></td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Phytophthora citrophthora</em> (R.E. Sm. &amp; Br.) Leonian</td>
<td>1.6</td>
<td>Growth occurred LM-FS at 25.5° to 27.5°C</td>
<td>Klotz et al. 1963</td>
</tr>
</tbody>
</table>
0.04  Zoospores did not germinate  
Klotz et al. 1963

5  Growth more rapid than in air  
SM-FS at 25°C  
Mitchell and Zentmyer 1971a

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>No germination</td>
<td>LM-FS</td>
</tr>
<tr>
<td>1.6</td>
<td>Good growth occurred</td>
<td>LM-FS at 25.5°C to 27.6°C</td>
</tr>
<tr>
<td>6</td>
<td>Growth greater than in air</td>
<td>SM-SS at 28°C</td>
</tr>
<tr>
<td>0.1</td>
<td>Growth very little</td>
<td>SM-SS at 28°C</td>
</tr>
<tr>
<td>21</td>
<td>Growth maximum</td>
<td>LM-FS at 25°C</td>
</tr>
</tbody>
</table>

Low O₂
1  Zoospores formed | SM-SS | Uppal 1924, 1926
Growth occurred | SM and LM-FS | Mitchell and Zentmyer 1971a
at 25°C

1.3  Growth reduced | LM-FS at 20° to 22°C | Brown and Kennedy 1966
4 and above | No growth reduction | LM-FS at 20° to 22°C | Brown and Kennedy 1966

0  No germination | LM-FS at 20°C | Wood-Baker 1955
2  Germination reduced 18% from air control | LM-FS at 20°C | Wood-Baker 1955
0.5  Growth reduced by 50%, No mature sporangia in 5 days | SM-FS at 15°C | Follstad 1966
0  Growth occurred | SM-FS at 15°C | Follstad 1966
2  Growth reduced 60 to 70% | LM-FS at 18°C | Wells 1967, 1968
0.25  No growth | LM-FS at 18°C | Wells 1967, 1968
0  No growth | LM-FS at 18°C | Wells 1967, 1968
Anoxia  Germinating sporangiospores inactivated but tolerated by non-germinating spores | Buckley et al. 1967
<table>
<thead>
<tr>
<th>Species</th>
<th>O\textsubscript{2} Concentration (%)</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thielaviopsis paradoxa (de Seynes)</td>
<td>4</td>
<td>Growth reduced by 15% LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td>Höhnel Sexual state, Ceratocystis paradoxa</td>
<td>0</td>
<td>Growth observed LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td>(Dade) Moreau.</td>
<td>2.3</td>
<td>Growth not affected SM-FS at 5.5°C and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Whetzelinia sclerotiorum (Lib.)</td>
<td>2.3</td>
<td>Growth not affected SM-FS at 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Korf &amp; Dumont Syn., Sclerotinia sclerotiorum</td>
<td>2.3</td>
<td>Growth reduced by 50% SM-FS at 5.5°C and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>(Lib.) Mass.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erwinia carotovora (Jones) Holland,</td>
<td>3</td>
<td>Growth ca. 50 to 60% of air decreased</td>
<td>Wells 1974</td>
</tr>
<tr>
<td>Erwinia atroseptica (Van Hall)</td>
<td></td>
<td>linearly to 0.25% LM-FS at 21°C</td>
<td></td>
</tr>
<tr>
<td>Jennison, and Pseudomonas fluourescens Migula</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1966; Follstad 1966; Wells and Uota 1970). However, some fungi were found to grow as rapidly in low O₂ atmospheres as in air. According to Hawker et al. (1960), a pressure as low as 0.01 atmosphere "... will suffice for many species of Penicillium growing on a solid medium." Follstad (1966) and Wells and Uota (1970) reported that Rhizopus stolonifer grew significantly at 0% O₂. Follstad (1966) added that gas chromatography detected a trace of O₂ in highly purified N₂, and that this amount possibly accounted for the measurable growth at 0% O₂.

These studies effectively demonstrated the ability of R. stolonifer sporangiospores to germinate at very low O₂ levels. Buckley et al. (1967) reported that holding R. stolonifer sporangiospores in anoxia not only prevented germination and growth but inactivated those that were germinating. Bussell et al. (1968, 1969b) showed that the viability of R. stolonifer spores was related to the stage of germination and length of exposure to anaerobiosis. In his studies, he purified the commercial N₂ (99.998% pure) of contaminating air by passing it twice through red-hot copper turnings before passing it through flow meters. Subsequently, the N₂ was bubbled twice through reduced cysteine hydrochloride and resazurin before being passed through the headspace of shaken flasks. The system was purged of O₂ by passage of O₂-free N₂ gas through the flow meters and culture flask. Glass or Tygon tubing was used throughout to minimize absorption of O₂, which can occur through rubber. Bussell et al. (1969a) showed that R. stolonifer underwent prominent ultrastructural changes under anoxia. For example, mitochondria were found scattered randomly throughout the cytoplasm in spores incubated aerobically for 3 hours, but they moved to the periphery of the cytoplasm when spores were exposed to anoxia.

It is clear from Table 10.1 that Phytophthora cactorum (El-Goorani and Sommer 1979), P. citrophthora (R.E. Sm. & Br.) Leonian (Mitchell and Zentmyer 1971a), and P. parasitica Dast. (Dukes and Apple 1965) have optimum growth rates at low O₂ concentrations when grown on agar, but have optimum growth rates at 21% O₂ in liquid medium (Covey 1970; Klotz et al. 1963). Mitchell and Zentmyer (1971a) reported that the reasons for this difference are unknown. Mycelium is more exposed to the atmosphere when grown on agar than in the submerged colony in the liquid medium. However, because only 15 ml of liquid medium per 250 ml flask was used in their study, the upper surface of the mat was always exposed to the atmosphere. Therefore, they believed that O₂ was probably not limiting. They suspected that inhibitory compounds accumulate at low O₂ levels and that these compounds cannot diffuse as rapidly through agar as through liquid.

Atmospheres low in O₂ permitted Geotrichum candidum to grow faster than in air when grown in liquid (Wells and Spalding 1975) or on solid media (El-Goorani and Sommer 1979).
3. Low Oxygen at Low Pressure (Hypobaric) Conditions.—Wu and Salunkhe (1972a) compared mycelial extension in potato-dextrose agar at 278 mm and 102 mm Hg with the normal atmospheric pressure of 646 mm Hg at their location. When air was withdrawn to produce the low pressure, the amount of available O₂ was effectively reduced. An atmosphere of 278 mm and 102 mm Hg would contain O₂ equivalent to 7.1% and 2.7% O₂, respectively, at normal atmospheric pressure. Generally, as pressures decreased, there was a slight suppression of colony size, amount of sporulation, or an increase in time for growth to be detected. When compared with 2.7% O₂ at their normal atmospheric pressure (646 mm Hg), growth and sporulation at 102 mm Hg were less and the time required for growth to appear was greater.

Borecka and Olak (1978) compared the growth and sporulation of *Penicillium expansum* Link ex Thom, *P. spinulosum* Thom, *P. diversum* Raper and Fennell, *Botrytis cinerea*, *Trichothecium roseum*, and *Rhizopus nigricans* (=*R. stolonifer*) at normal atmospheric pressure to that at 0.1 and 0.05 atmospheres. They reported that growth of *Penicillium expansum* at 0.1 atmosphere was reduced to about 85% of that in air, while at 0.05 atmosphere it was about 40% of the growth in air. Sporulation of the Penicillia was not affected by low pressure. *Rhizopus stolonifer* developed normally at 0.1 atmosphere, but it did not sporulate at 0.05 atmosphere. With *Botrytis cinerea* and *Trichothecium roseum*, no suppression was noted in growth at 0.1 atmosphere, but growth was reduced by one-half or more at 0.05 atmosphere. Although mycelial growth of *Botrytis cinerea* was normal at 0.1, no sclerotia were produced.

It has been possible to compare (Fig. 10.1) the growth of *Botrytis cinerea* at low pressures (Borecka and Olak 1978) to growth at normal barometric pressure in atmospheres of low O₂ composition (Couey *et al.* 1966; Follstad 1966; Sommer *et al.*, in press). Growth was remarkably similar at comparable O₂ levels regardless of barometric pressure. Therefore, the evidence suggests that suppression of *Botrytis cinerea* was due to a reduction of the partial pressure of O₂, and not to low total pressure. Definitive conclusions must await further comparisons with other post-harvest pathogens.

B. Carbon Dioxide Effects

Although CO₂ is low in air (ca. 0.03%), Hartman *et al.* (1972) showed that growth of *Verticillium albo-astrum* Reinke & Berth. was severely curtailed in a CO₂-free atmosphere in which glucose or glycerol was the sole carbon source. With increasing concentration, the role of CO₂ changes from stimulatory to inhibitory. While fungi can fix CO₂, it cannot be used as an exclusive source of carbon for metabolism (Burnett
Several enzymes have been implicated in CO₂ fixation in fungi (Bull and Trinci 1977). Similarly, elevated CO₂ evidently suppresses metabolic functions at multiple locations. Acidification of media by CO₂ was once considered to be an important cause for fungal suppression (Thornton 1934), but more recent evidence suggests that pH changes do not necessarily play a dominant role (Bull and Trinci 1977; Lwoff and Monod 1947).

The amount of CO₂ required to inhibit growth and/or spore-germination varies with the species (Cochrane 1958; Cochrane et al. 1963). Table 10.2 summarizes the reported effects of various concentrations; from it, the information suggests that high levels of CO₂ are required to slow growth and/or germination of fruit-rotting fungi. Some effects of CO₂ on bacterial growth are reported by Coyne (1933), and Lwoff and Monod (1947) provided an early review of the role of CO₂ in microbial growth.
<table>
<thead>
<tr>
<th>Species</th>
<th>CO₂ Concentration (%)</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alternaria alternata</em> (Fr.) Keissler.</td>
<td>20</td>
<td>Growth reduced by about 50% LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td>Syn., <em>A. tenuis</em> Nees</td>
<td>32</td>
<td>Spore germination inhibited LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Growth not reduced SM-FS at 19°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Growth reduced by 65% and 15% at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Alternaria solani</em> (Ell. and G. Martin) Sor.</td>
<td>&gt;0.5</td>
<td>Growth inhibition increased with increasing concentration</td>
<td>Klaus 1941</td>
</tr>
<tr>
<td><em>Ascochyta caricae-papayae</em> Pat.</td>
<td>5</td>
<td>Growth not reduced SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Probable sexual state, <em>Mycosporella caricae</em> Syd.</td>
<td>18</td>
<td>Growth reduced by about 70% SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> Van Tiegh.</td>
<td>0</td>
<td>Spores germinated very poorly</td>
<td>Rippel and Bortels 1927</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>Spore germination stimulated LM-SS</td>
<td>Vakil <em>et al.</em> 1961</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Spore germination inhibited SM-SS</td>
<td>Vakil <em>et al.</em> 1961</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Growth inhibited SM-SS (gases changed daily)</td>
<td>Brancato and Golding 1953</td>
</tr>
<tr>
<td><em>Aspergillus flavus</em> Link</td>
<td>&lt;80</td>
<td>Growth and sporulation not visibly affected. Peanuts—FS at 15°C</td>
<td>Landers <em>et al.</em> 1967</td>
</tr>
<tr>
<td>Pathogen</td>
<td>Dosage</td>
<td>Effect</td>
<td>Temperature</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td>-------------</td>
</tr>
<tr>
<td><em>Botryodiplodia theobromae</em> Pat.</td>
<td>20 to 80</td>
<td>Aflatoxin production suppressed. Peanuts—FS at 15°C</td>
<td>Landers <em>et al.</em> 1967</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Growth unaffected</td>
<td>SM-FS at 12.5°C</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Growth reduced by 25%</td>
<td>SM-FS at 12.5°C</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em> Pers. ex Fr. Sexual state, <em>Botryotinia fuckeliana</em> (DeBary) Whetzel.</td>
<td>10 and 20</td>
<td>Germination of conidia suppressed</td>
<td>Brown 1922</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Germination prevented</td>
<td>LM-SS at 15°C to 18°C</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Growth suppressed</td>
<td>LM-SS at 15°C to 18°C</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Growth suppressed almost completely</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Growth suppressed by about 50%</td>
<td>LM-FS at 19°C</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Growth unaffected</td>
<td>SM-FS at 5.5°C and 12.5°C</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Growth suppressed 86% at 5.5°C and 69% at 12.5°C</td>
<td>SM-FS</td>
</tr>
<tr>
<td><em>Cladosporium herbarum</em> Link ex Fr.</td>
<td>20</td>
<td>Growth reduced by 50%</td>
<td>LM-FS at 19°C</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Spore germination inhibited by about 90%</td>
<td>LM-FS at 19°C</td>
</tr>
<tr>
<td><em>Colletotrichum gloeosporioides</em> (Penz.) Van Arx Sexual state, <em>Glomerella cingulata</em> (Stonem.) Spauld. &amp; Schr.</td>
<td>5</td>
<td>Growth reduced by 10%</td>
<td>SM-FS at 12.5°C</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Growth reduced by 77%</td>
<td>SM-FS at 12.5°C</td>
</tr>
<tr>
<td>Species</td>
<td>CO₂ Concentration (%)</td>
<td>Remarks</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-----------------------</td>
<td>----------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Colletotrichum lindemuthianum</td>
<td></td>
<td>Little response to CO₂ treatment</td>
<td>Brooks et al. 1936</td>
</tr>
<tr>
<td>(Sacc. &amp; Magn.) Boriosi and Cav.</td>
<td></td>
<td>Beans and SM-SS</td>
<td></td>
</tr>
<tr>
<td>Dothiorella gregaria Sacc.</td>
<td>18</td>
<td>Growth reduced 85% and 28% at 5.5°C and 12.5°C, respectively</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>20</td>
<td>Growth reduced 15 to 21% SM-FS at 25°C</td>
<td>Durbin 1959</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>Spores germinated strongly SM-SS</td>
<td>Brown 1922</td>
</tr>
<tr>
<td>Fusarium roseum (Link) emend.</td>
<td>10</td>
<td>Growth stimulated LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td>Snyd. &amp; Hans.</td>
<td>45</td>
<td>Growth inhibited about 50% LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Spore germination stimulated LM-FS at 19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td>Geotrichum candidum Link ex Pers.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syn., Oospora lactis (Pres.) Sacc.</td>
<td>5</td>
<td>Growth unaffected SM-FS at 5.5°C and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Monilinia fructicola (Wint.) Honey</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syn., Sclerotinia fructicola (Wint.) Rehm.</td>
<td>10 to 20</td>
<td>Spore germination suppressed by high CO₂ LM-SS at 15° to 18°C</td>
<td>Brown 1922</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Growth inhibited LM and SM</td>
<td>Thornton 1934</td>
</tr>
<tr>
<td></td>
<td>Species/Mixture</td>
<td>Phenomenon</td>
<td>Temperature</td>
</tr>
<tr>
<td>---</td>
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<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>5</td>
<td>Growth stimulated</td>
<td>SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>18</td>
<td>Growth reduced by 65%</td>
<td>SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td></td>
<td><em>Mucor sp.</em></td>
<td>Spore germination suppressed by high CO₂</td>
<td>LM-SS at 15° to 18°C</td>
</tr>
<tr>
<td>10</td>
<td>Spore germination inhibited</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>Spore germination totally inhibited, Spores not killed by 3-month exposure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Growth stimulated</td>
<td>SM-FS at 22°C</td>
<td>Swinburne 1974</td>
</tr>
<tr>
<td>10</td>
<td>Growth suppressed</td>
<td>SM-FS at 22°C</td>
<td>Swinburne 1974</td>
</tr>
<tr>
<td></td>
<td><em>Nectria galligena Bers.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Growth unaffected</td>
<td>SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>18</td>
<td>Growth suppressed by 70% and 37% at 5.5° and 12.5°C, respectively</td>
<td>SM-FS</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium digitatum Sacc. and P. italicum Wehmer</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Growth unaffected</td>
<td>SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>18</td>
<td>Growth suppressed 55 to 65%</td>
<td>SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium expansum Link ex Thom</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Growth unaffected</td>
<td>SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>18</td>
<td>Growth suppressed 55 to 65%</td>
<td>SM-FS at 5.5° and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium glaucum Link</em></td>
<td>Growth suppressed</td>
<td>SM-SS at 15°C</td>
</tr>
<tr>
<td></td>
<td><em>Phomopsis citri Faw. Sexual state, Diaporthe citri (Faw.) Wolf</em></td>
<td>Growth unaffected</td>
<td>SM-FS at 5.5° and 12.5°C</td>
</tr>
<tr>
<td>Species</td>
<td>CO₂ Concentration (%)</td>
<td>Remarks</td>
<td>Reference</td>
</tr>
<tr>
<td>---------</td>
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<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>Phomopsis vexans (Sacc. and Syd.) Harter</td>
<td>18</td>
<td>Growth suppressed 65% (5.5°C) and 15% (12.5°C) SM-FS at 5.5°C and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Growth completely suppressed at high CO₂</td>
<td>Brooks et al. 1936</td>
</tr>
<tr>
<td>Phytophthora cactorum (Leb. &amp; Cohn) Schroet.</td>
<td>5</td>
<td>Growth inhibited 25% SM-FS at 25°C</td>
<td>Durbin 1959</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Growth unaffected SM-FS at 5.5°C and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Growth suppressed 70 to 80% SM-FS at 5.5°C and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Phytophthora citrophthora (R.E. Sm. &amp; Br.) Leonian</td>
<td>15</td>
<td>Growth reduced about 30% LM and SM-FS at 25°C</td>
<td>Mitchell and Zentmyer 1971a</td>
</tr>
<tr>
<td>Phytophthora parasitica Dast. Syn., P. nicotianae var. parasitica (Dast.) Waterh.</td>
<td>15</td>
<td>Growth unaffected SM-SS at 28°C</td>
<td>Dukes and Apple 1965</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>Growth completely suppressed. Mycelia not killed after 7 days SM-SS at 28°C</td>
<td>Dukes and Apple 1965</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>Growth suppressed by ½ LM and SM-FS at 25°C</td>
<td>Mitchell and Zentmyer 1971a</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Growth suppressed by 40% SM-FS at 5.5°C and 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Pathogen</td>
<td>Effect</td>
<td>Temperature(s)</td>
<td>Source(s)</td>
</tr>
<tr>
<td>-------------------------------</td>
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<tr>
<td><strong>Phytophthora spp.</strong></td>
<td>Sporangia and oospore formation reduced with increasing CO₂ when O₂ was 1.5 or 20% LM and SM-FS at 22°C to 25°C</td>
<td></td>
<td>Mitchell and Zentmyer 1971b</td>
</tr>
<tr>
<td><strong>Rhizoctonia solani Kuhn</strong></td>
<td>Growth suppressed 31 to 80% SM-FS at 25°C</td>
<td>25°C</td>
<td>Durbin 1959</td>
</tr>
<tr>
<td></td>
<td>Growth suppressed by high CO₂</td>
<td></td>
<td>Brooks <em>et al.</em> 1936</td>
</tr>
<tr>
<td></td>
<td>Growth moderately suppressed in soil FS</td>
<td>19°C</td>
<td>Papavizas and Davey 1962a.b</td>
</tr>
<tr>
<td></td>
<td>Growth suppressed drastically in soil FS</td>
<td>12.5°C</td>
<td>Papavizas and Davey 1962a.b</td>
</tr>
<tr>
<td><strong>Rhizopus stolonifer (Ehrenb. ex Fr.)</strong>&lt;br&gt; Lind. Syn., R. nigricans Ehren.</td>
<td>Germination suppressed by high CO₂ LM-SS at 15°C to 18°C</td>
<td></td>
<td>Brown 1922</td>
</tr>
<tr>
<td></td>
<td>Growth nearly completely suppressed</td>
<td></td>
<td>Brooks <em>et al.</em> 1932</td>
</tr>
<tr>
<td></td>
<td>Growth reduced by about 50% LM-FS at 19°C</td>
<td>19°C</td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td></td>
<td>Spore germination suppressed ca. 90% LM-FS at 19°C</td>
<td></td>
<td>Wells and Uota 1970</td>
</tr>
<tr>
<td></td>
<td>Growth suppressed by 93% (5.5°C) and 75% (12.5°C) SM-FS</td>
<td>12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td><strong>Sclerotinia minor Jagger</strong></td>
<td>Growth suppressed 85% SM-FS at 21°C</td>
<td></td>
<td>Louvet and Bilit 1964</td>
</tr>
<tr>
<td><strong>Sclerotium rolfsii Sacc.</strong></td>
<td>Growth suppressed 55% SM-FS at 25°C</td>
<td></td>
<td>Durbin 1959</td>
</tr>
<tr>
<td></td>
<td>Growth suppressed by high CO₂</td>
<td></td>
<td>Brooks <em>et al.</em> 1936</td>
</tr>
<tr>
<td>Species</td>
<td>CO₂ Concentration (%)</td>
<td>Remarks</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
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</tr>
<tr>
<td>Thielaviopsis paradoxa (de Seynes) Höhnel Sexual state, Ceratocystis paradoxa (Dade) Moreau.</td>
<td>18</td>
<td>Growth suppressed by 55% SM-FS at 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Whetzelinia sclerotiorum (Lib.) Korf &amp; Dumont Syn., Sclerotinia sclerotiorum (Lib.) Mass.</td>
<td>18</td>
<td>Growth reduced by 80% SM-FS at 12.5°C</td>
<td>El-Goorani and Sommer 1979</td>
</tr>
<tr>
<td>Erwinia carotovora (Jones) Holland, Erwinia atroseptica (Van Hall) Jennison, Pseudomonas fluorescens Migula</td>
<td>30 0</td>
<td>Growth reduced by 15 to 30% LM-FS at 21°C Erwinia spp. did not grow at any concentration of O₂ within 24 hours LM-FS at 21°C</td>
<td>Wells 1974</td>
</tr>
</tbody>
</table>
C. Low Oxygen with High Carbon Dioxide

Several investigators studied the effect of low \( O_2 \) in the presence of high \( CO_2 \) concentrations on suppression of growth of postharvest pathogens. Organisms studied included the following: *Botrytis allii* Munn, *Rhizopus stolonifer*, *Penicillium expansum* (Littlefield et al. 1966), *Gloeosporium album* Osterw. (Lockhart 1967), *Alternaria tenuis*, *Botrytis cinerea*, *Sclerotinia sclerotiorum* (= *Whetzelinia sclerotiorum* (Lib.) Korf & Dumont) (Adair 1971), *Erwinia* spp., and *Pseudomonas fluorescens* Migula (Wells 1974). On the other hand, others have observed that atmospheres of low \( O_2 \) and high \( CO_2 \) have little effect in slowing the mycelial growth of *Rhizoctonia solani* Kuhn, *Fusarium roseum* (Link) emend. Snyd. & Hans. (Adair 1971), and *Penicillium alba* Guth. (Bompeix 1978a). In fact, Wells and Spalding (1975) found that in an atmosphere of 3% \( O_2 \) with 3% \( CO_2 \), the average growth of *Geotrichum candidum* in 24 hours was about twice that in air. They added that at concentrations of 3% or more, \( CO_2 \) repressed growth of *G. candidum* in the presence of 21% \( O_2 \). In a low \( O_2 \) atmosphere, however, 30% \( CO_2 \) was necessary to repress growth.

Yackel et al. (1971) showed that the effect of CA (10.5% \( CO_2 + 2\% O_2 \)) on growth and development of 7 spoilage molds (*Mucor hiemalis* Wehmer, *Rhizopus oryzae*, *Aspergillus niger*, *Gloeosporium herbarum*, *Penicillium expansum*, *Alternaria* sp., and *Fusarium bulbigenum* Cooke & Masse) is quite variable and, for the most part, is temperature related.

When \( O_2 \) concentrations were 1% or 2%, and thereby limiting to growth, \( CO_2 \) at low levels stimulated growth and/or germination of *Gloeosporium album* (Lockhart 1967), *Alternaria tenuis*, *Botrytis cinerea*, *Gloeosporium herbarum*, *Rhizopus stolonifer*, and *Fusarium roseum* (Wells and Uota 1970). Mitchell and Zentmyer (1970, 1971a) found that growth of *Phytophthora* spp. on solid media was stimulated by the addition of 5% \( CO_2 \) when the \( O_2 \) level was 1%. However, \( CO_2 \) concentrations above 5% reduced growth of most isolates in liquid or solid media.

IV. ATMOSPHERIC EFFECTS ON POSTHARVEST DISEASES

Pathogenic growth of bacteria and fungi may be affected by MA through altered physiology of the organism. An important influence on disease development is the physiological condition of the host commodity. Commonly, fruits are resistant to most postharvest pathogens until the initiation of ripening. Similarly, tissues other than fruits may be very resistant until they become senescent. Consequently, MA-induced delay of ripening or senescence to thereby maintain host resistance may be highly important in disease suppression.
A. Low Oxygen

Several investigators have studied the effect of low O\(_2\) concentrations on postharvest decay during storage of fruits and vegetables. Decay of fresh strawberries caused by *Botrytis cinerea* was decreased by lowering the O\(_2\) concentration to 0.5% or less. Berries were evaluated after 5 days at 3\(^\circ\)C in the low O\(_2\) atmosphere and after an additional 2 days at 15\(^\circ\)C in air (Couey *et al.* 1966). Wells (1967, 1968) reported that decayed areas on strawberries inoculated with *Rhizopus stolonifer*, and held at 15\(^\circ\)C in 1% O\(_2\), averaged half the size of those on strawberries held in air. The least decay occurred under 0% O\(_2\). However, strawberries developed off-flavors at O\(_2\) concentrations that reduced decay (Couey *et al.* 1966; Tomalin and Robinson 1971a, b).

Decay of cranberries caused by *Botrytis cinerea*, *Pullularia pullulans* (de Bary) Berk., and *Rhizopus* sp. was decreased by storage in an atmosphere of N\(_2\) rather than in air (Lockhart *et al.* 1971). A marked increase in the amount of physiological breakdown of cranberries caused by storage in N\(_2\), however, precluded that type of storage for fresh fruit.

Lockhart and Eaves (1967) found that tomatoes rotted more when held in atmospheres of 100% N\(_2\) at 10\(^\circ\)C than in those containing 2.5% O\(_2\). *Rhizopus* spp., *Fusarium* spp., *Alternaria tenuis*, and bacteria were the major rot organisms. Lockhart and Eaves added that tomatoes from air and 2.5% O\(_2\) had excellent flavor, but those from 100% N\(_2\) had a strong off-flavor. Parsons *et al.* (1970) found that decay of tomatoes by *Rhizopus* spp. and *Alternaria* spp. was significantly less after storage in a 3% O\(_2\) atmosphere than in air.

Aharoni and Lattar (1972) found that atmospheres containing low concentrations of O\(_2\) (2.5%, 5%) greatly reduced rotting of 'Shamouti' oranges caused chiefly by *Alternaria citri* Ell. & Pierce, *Penicillium digitatum* Sacc., *P. italicum* Wehrm., and *Diplodia natalensis* P. Evans. They added that under these conditions the peel of developing fruits harvested before October became injured; this did not occur on fruits harvested in October or later. Mature fruits harvested from October to March showed very little rot under low O\(_2\) atmospheres, and rot that was present was caused by *Penicillium digitatum*, *P. italicum*, and *Diplodia natalensis*. The developing fruit harvested earlier were largely rotted by *Alternaria citri*.

Lipton (1972) reported that atmospheres of 2% O\(_2\) at 10\(^\circ\)C inhibited the growth of aerial mycelium by 50% more than air on radishes with lesions of downy mildew (*Peronospora parasitica* Pers. ex Fr.).

Several investigators have studied the effect of low O\(_2\) concentrations on the bacterial soft rots of vegetables. Lipton (1965) observed that O\(_2\) atmospheres in the range of 1 to 21% had little effect on soft rot (*Erwinia carotovora* [Jones] Holland) of asparagus spears stored for 17
days at 6°C. Atmospheres lacking O₂ caused severe injury. It was shown that ‘White Rose’ potatoes did not tolerate low O₂ concentrations (1%, 0.5%) when held at temperatures used in transcontinental shipments (Lipton 1967). These atmospheres prevented suberization and periderm formation below skinned areas or small wounds that permitted un-checked bacterial action at 15°C and 20°C. He found that even 5% O₂ may be undesirable in practice because the thinner periderm at this O₂ concentration favors mechanical injury and subsequent decay. Lipton also reported (1972) serious bacterial soft rot in radishes stored 8 days or 15 days in 0.25% O₂ at 10°C and no reduction of bacterial soft rot of lettuce by low O₂ (Lipton 1968).

B. High Carbon Dioxide

Brown (1922) suggested that, within CO₂ concentrations permissible in practice, the gas was not as important as the lowering of temperature in reducing the fungal growth. Brooks et al. (1932, 1936) and Brooks and McColloch (1937) studied effects on some transit and storage diseases of exposing fruits and vegetables to high CO₂ levels (20 to 30%) for short periods as a possible substitute for precooling. Diseases in most cases were noticeably suppressed. Host tolerance to CO₂ varied considerably and the suppression of diseases also varied greatly, presumably as a consequence of differences among pathogens. Ryall (1935) obtained similar results in studies with prunes.

With strawberry fruits, Brooks et al. (1932) examined the effect of CO₂ after inoculation with Botrytis cinerea and Rhizopus stolonifer. They found that 10 to 13% CO₂ had little effect upon growth, but that 23% almost stopped growth of both organisms, and 37% stopped growth entirely. Similar results were obtained by Smith (1957) and Couey and Wells (1970). Sommer et al. (1973) pointed out that the effectiveness of high CO₂ (5 to 15%) is striking in suppressing gray mold rot at 5°C or above. At lower temperatures (2.2°, 0°C), the benefits of CO₂ were essentially undetectable during the 7-day simulated transit and marketing period, because little fungus developed. Brooks et al. (1932) concluded that to inhibit strawberry rots satisfactorily, concentration of CO₂ should be approximately 25%. Couey and Wells (1970) detected off-flavors caused by CO₂ only at 30% CO₂ when fruits were held at 15°C. No off-flavors were detected at either 10% or 20% CO₂ at 10°C. Botrytis rot was reduced to about one-third and Rhizopus rot to about half of that in air.

Brooks et al. (1932) and Luvisi and Sommer (1960) found that major reductions in peach decay caused by Monilinia fructicola (Wint.) Honey and Rhizopus stolonifer could be achieved only at high CO₂ levels, which have been shown to harm fruit quality.
Orange rots were studied by Brooks et al. (1932), who found that high CO₂ concentrations reduced rotting caused by *Penicillium digitatum* and *P. italicum*. High CO₂ concentrations were not effective against orange rots caused by *Diplodia natalensis* (Brooks et al. 1932; Aharoni and Lattar 1972) or *Phomopsis* sp. (Brooks et al. 1932).

High concentrations of CO₂ were found to be effective in suppressing certain organisms in other fruits and vegetables: *Monilinia fructicola* and *Penicillium expansum* on apricots (Brooks et al. 1936), *Sclerotinia sclerotiorum* and *Rhizoctonia solani* on carrots (Brooks et al. 1932, 1936), *Sclerotium rolfsii* Sacc. and *R. solani* on beans (Brooks et al. 1932), *Rhizopus* sp. on asparagus (Brooks et al. 1936), *Gloeosporium perennans* Zeller & Child and *G. album* on apples (Dullum and Rasmussen 1951; Talvia 1960), and *Botrytis cinerea* on cabbage (Androsova 1962).

On the other hand, high concentrations of CO₂ were found to be ineffective in suppressing other organisms on other fruit and vegetable hosts: *Colletotrichum lindemuthianum* (Sacc. & Magn.) Borioši & Cav. on beans (Brooks et al. 1932), *Alternaria solani* Jones & Court on potato tubers (Klaus 1941), *Fusarium* spp. on tomatoes (Eaves and Lockhart 1961), *Nectria galligena* Bers. on apples (Swinburne 1970a,b, 1974), *Fusarium* spp. on gladiolus corms (Magie 1971), and *Phomopsis* spp. (presumably *P. citri* Faw.) on grapefruit (Hatton et al. 1972).

High concentrations of CO₂ caused severe decay of potato tubers inoculated or infected with *Fusarium* sp. (Workman and Twomey 1969, 1970), *Fusarium roseum* var. *sambucinum* (Fuckel) Snyder & Hansen (Workman et al. 1976; Nielsen 1964; Cameron 1978), *Erwinia carotovora* (Nielsen 1964; Cameron 1978), and *Erwinia aroidae* (Townsend) Holland (Nielsen 1964). Workman et al. (1976) reported that increasing CO₂ during storage at 0°C and 5°C increased sugar content and membrane permeability. They added that a significant and high correlation was obtained among the rate of Erwinia rot, membrane permeability, and sucrose content of the tubers.

C. Low Oxygen with High Carbon Dioxide

Many reports related primarily to horticultural or physiological responses of commodities have indicated that postharvest losses of fruits and vegetables due to unspecified decays were reduced during storage in atmospheres of low O₂ and high CO₂ rather than in air (Kader et al. 1975; Lockhart et al. 1969; Zagoryanshii 1933; Anon. 1940; Ostrowski et al. 1958; Eaves et al. 1964; Smith 1957; Eaves 1964; Eaves and Lockhart 1961; Furlong 1946; Hassan 1966; Huelin and Tindale 1947; Edney 1973; Tomkins 1966a; Yackel et al. 1971; Van den Berg and Lentz 1973;
Martin and Cerny 1956). However, some investigators noticed that unspecified rots have sometimes been more extensive in commodities held in CA than in air (Weichmann 1973; Biale 1953; Scholz et al. 1960; Tomkins 1963; Rygg and Wells 1962; Grierson et al. 1966).

1. Pome Fruits.—Blue mold rot caused by *Penicillium expansum* has been retarded when apples were stored in low O₂-high CO₂ (Nyhlén and Nilsson 1960; Lin 1948; Borecka 1976; Nilsson et al. 1956). Gloeosporium rot in apples was sometimes retarded by storage in CA (Edney 1956; Schulz 1974; Bompeix 1978a,b; Montgomery 1958; Nyhlén and Johansson 1964; Nilsson et al. 1956; Edney 1964). Edney (1956) found that if samples stored in CA were heavily infected, either naturally or by inoculation, development of Gloeosporium rot was similar to what would occur in air. However, it has been shown that apples do not respond to storage conditions in the same manner every year. The reason for this conflicting performance has not been established. However, according to Edney (1964), it is possible that, in some years, 5% CO₂ may have a slightly harmful effect on the fruit at low levels of O₂. Further, Gloeosporium rot may be caused by both *Gloeosporium album* and *G. perennans*. Some workers have suggested that there is considerable difference between the two organisms in their response to atmospheres of low O₂-high CO₂. Montgomery (1958) and Olsson (1965) observed that *Gloeosporium album* tended to predominate in air storage and that *G. perennans* tended to cause the most rotting in CA. Montgomery (1958) suggested that *Gloeosporium album* develops lesions more slowly and is therefore more susceptible to the suppression caused by low O₂-high CO₂ atmospheres, which may not be reached until several days after storage rooms are closed. Apple scab lesions caused by *Venturia inaequalis* (Cooke) Wint. are suppressed on fruits in CA storage (Julien and Phillips 1963; Phillips and Julien 1966; Phillips et al. 1959).

Several investigators found that pre-storage treatment of certain apple and pear cultivars with high CO₂ (12 to 15%) in the presence of low O₂ (3 to 5%) delayed softening (Bramlage et al. 1977; Lau and Looney 1978; Mellenthin and Wang 1977; Couey and Olsen 1975). The treatment extended for about 2 weeks at 0° to 3°C before establishment of normal CA. Bramlage et al. (1977) concluded, however, that such treatment did not merit commercial application to ‘McIntosh’ despite the very serious problem of excessive softening and injury during and following its CA storage. Couey and Wright (1977) found that a prestorage CO₂ treatment of ‘d’Anjou’ pears reduced stem decay.

2. Stone Fruits.—The effect of storing peaches and nectarines in CA was studied by Smith and Anderson (1975). They pointed out that the atmosphere surrounding the fruits during storage apparently had no
significant effect on decay development caused by Monilinia fructicola, Botrytis cinerea, Penicillium expansum, and Alternaria spp.

3. Citrus Fruits.—Harding (1969) reported that storage in CA (3% or 5% O₂—about 2% CO₂) was deleterious to citrus fruits (lemons, grapefruit, and Valencia oranges). Decay by Penicillium digitatum and P. italicum was increased in all three fruits by such storage. Moreover, sporulation in these molds was not controlled. Besides the tendency to increase fungal decay and rind breakdown, the low O₂ atmosphere (3.0 to 3.5%) caused a fermented odor and flavor. McGlasson and Eaks (1972) found that Penicillium digitatum rot was hardly affected by storage in 3% O₂-5% CO₂. Stem-end decay (mainly Fusarium sp.) was significantly lower in air treatment than in the CA treatment.

4. Avocados.—CA storage of fruit of cold-sensitive avocados in 2% O₂-10% CO₂ prevented development of both anthracnose (Colletotrichum gloeosporioides Penz.) and chilling injury at 7.2°C. The suppression of decay development appears to be on delay of fruit ripening and maintenance of resistance rather than on the metabolism of the fungus. It was concluded that the action of the CA in slowing the rate of softening would serve to keep the fungus dormant (Reeder and Hatton 1970; Spalding and Reeder 1972, 1975).

5. Other Fruits.—Anderson et al. (1963) showed no benefit to cranberries from MA in relation to unspecified decays or breakdown. Ceponis and Cappellini (1979) reported a reduction of blueberry fruit decays by MA. Diplodia rot of mangos was not controlled by MA. Hatton and Reeder (1969) found that papayas stored in 1% O₂ + 5% CO₂ at 12.5°C for 21 days were less infected with anthracnose (Colletotrichum gloeosporioides) and Diplodia natalensis than were papayas stored in air.

6. Vegetables.—Rots of tomatoes caused by Botrytis cinerea, Rhizopus spp., Penicillium spp., Sclerotinia sclerotiorum, and Colletotrichum coccodes (Wallroth) Hughes were reduced by CA storage (3% O₂ and 3% CO₂). Although Alternaria tenuis was retarded slightly in CA, it was still a problem since it developed rapidly on CA-stored tomatoes when they were transferred to the ripening room (Lockhart 1969; Eaves and Lockhart 1961). Tomatoes inoculated with Erwinia carotovora (Jones) Holland and held 6 days at 12.5°C kept better in a CA with 3% O₂ and 5% CO₂ than in air (Parsons and Spalding 1972). On the other hand, Fusarium rot (Lockhart 1969; Eaves and Lockhart 1961) and Geotrichum candidum rot of tomato fruits were stimulated by 3% O₂ + 3% CO₂ and 3% O₂ with or without 5% CO₂ atmospheres, respectively (Wells and Spalding 1975).
Adair (1971) found that the fungi important in postharvest decay of cabbage (Botrytis cinerea and Sclerotinia sclerotiorum) were apparently controlled in 1.4% O₂ + 4.7% CO₂, but Fusarium roseum was an active pathogen under these conditions and invaded cabbage leaf tissue in atmospheres of 0.8% O₂.

Nielsen (1968) found that in potato tubers aerated with humidified air, little decay developed during a 3-day test period. By contrast, when potatoes were placed in sealed containers, O₂ was depleted, CO₂ exceeded 30%, and soft rot developed in nearly all tubers. Lund and Wyatt (1972) and Lund and Nicholls (1970) found that the most extensive potato tuber rots caused by Erwinia carotovora occurred in anaerobic conditions. Accumulation of CO₂ due to tuber respiration did not affect the production of rots significantly. In addition to Erwinia carotovora, pectolytic Clostridia spp. could be recovered from the spreading rots.

D. Low Pressure (Hypobaric) Storage

Recently, much research interest has been devoted to the possibilities of storage or transport of fruit and vegetables under hypobaric conditions (Burg 1967; Dilley 1977; Salunkhe and Wu 1973; Spalding and Reeder 1977). Lougheed et al. (1978) prefer the term low-pressure storage (LPS), because the term is less medicinal in implication and is readily abbreviated. This storage method consists of placing the commodity in a slowly moving stream of humidified air maintained at low pressure. Under these conditions, the commodity is partially degassed at a controlled-atmosphere environment low in O₂. As air is bled continually into the low-pressure chamber from the exterior and then expelled, a slow-moving flowing system is created. As a consequence, respiratory gases which would otherwise accumulate are constantly removed. The storage life is extended because the ripening hormone, ethylene, escapes from the tissue, while its production and action, like other metabolic processes, are greatly retarded (Apelbaum and Barkai-Golan 1977; Lougheed et al. 1978). Several investigators suggested that LPS may suppress postharvest pathogens (Burg 1973; Wu and Salunkhe 1972a,b; Adams et al. 1976; Spalding and Reeder 1976; Tolle 1969; Borecka 1976; Apelbaum and Barkai-Golan 1977; Borecka and Olak 1978). Wu and Salunkhe (1972a) found that the growth and sporulation of several postharvest storage fungi, such as Penicillium expansum, Rhizopus stolonifer, Aspergillus niger, Botrytis allii, and Alternaria spp., were retarded when grown at a pressure of 278 mm Hg; suppression was more pronounced at 102 mm Hg. In these tests, potato-dextrose agar at pH 5.6 was inoculated at the center of petri dishes with suspended spores before the dishes were placed in storage at several pressures. Growth at 102 mm Hg,
the lowest used, was compared with growth under normal atmospheric pressure in which the \( O_2 \) level was maintained at 2.7%. Thus, similar \( O_2 \) supplies were provided in the two atmospheres. Relative to 2.7% \( O_2 \) at normal atmospheric pressure, all fungi tested grew sooner, sporulated earlier, and showed greater growth at 102 mm Hg. As a consequence of these results, the authors concluded that "... the inhibition of growth by sub-atmospheric pressure is due not only to lower oxygen concentration but also lower pressure exerted on fungi."

Apelbaum and Barkai-Golan (1977) found that spore germination, mycelial growth, and sporulation of tested fungi were inhibited under LPS at 23°C. Inhibition increased with the decrease in pressure below 150 mm Hg. Mycelial growth of *Penicillium digitatum*, *Alternaria alternata*, *Botrytis cinerea*, and *Diplodia natalensis* was inhibited 5 to 25%, 45 to 80%, and 100% after 5 days at 100, 50, and 25 mm Hg, respectively. Delay in fungal sporulation was recorded under 50 mm and 25 mm Hg. Inhibition of *Geotrichum candidum* was less pronounced under these conditions. Borecka and Olak (1978), in a similar study, inoculated 2% malt extract agar in petri dishes with the following fungi: *Penicillium expansum*, *P. spinulosum*, *P. diversum*, *Botrytis cinerea*, *Trichothecium roseum*, and *Rhizopus niger* (=*R. stolonifer*). Incubation was at 15°C for 10 days in air at 0.1 and 0.05 atmospheres pressure, which have the same partial pressures of \( O_2 \) as do 2% and 1% \( O_2 \) at normal atmospheric pressure, respectively. A comparison was made with CA at normal atmospheric pressure. *Penicillium* spp. were significantly suppressed at both LPS atmospheres, with suppression at 0.05 greater than at 0.1 atmospheres. On the other hand, *Botrytis cinerea* grew equally well at 0.1 atmosphere as in air, but sclerotia were absent. At 0.05 atmosphere growth was reduced to about 55% that of growth in air (ca. 21% \( O_2 \)).

Couey et al. (1966) determined growth of *Botrytis cinerea* on potato-dextrose agar at 15°C at normal barometric pressure but at a low partial pressure of \( O_2 \). They reported that in atmospheres containing 1, 0.5, and 0.25% \( O_2 \), growth was 83, 38, and 1.4% of that in air, respectively. These results, at normal atmosphere at 15°C, can be compared to results of Borecka and Olak (1978) who grew *Botrytis cinerea* at the same temperature and similar partial pressures of \( O_2 \) achieved by LPS conditions. The results between the two are remarkably similar (Fig. 10.1).

The retardation of fungal development *in vitro* under LPS can be attributed to the reduction in partial \( O_2 \) tension prevailing at the reduced pressure. However, it was found that mycelial growth rates under normal atmospheric pressure were significantly higher than at subatmospheric pressures (Wu and Salunkhe 1972b; Apelbaum and Barkai-Golan 1977). Thus, it has been suggested that growth inhibition under low-pressure conditions may be partially due to effects other than the reduction of \( O_2 \).
E. Carbon Monoxide

Recently, CO has been used as an added component to CA or MA, primarily as a discoloration inhibitor, especially during transit of lettuce (Kader et al. 1973, 1977; Stewart 1978; Stewart and Uota 1976; Stewart et al. 1970). Boarini and Buonocore (1973) reported that 1% CO added to 2 to 3% O₂ to 3% CO₂ during storage of endive at 0°C for periods of up to 39 days reduced bacterial activity, wilting, and growth of the flower stem. Kader et al. (1977) observed less decay (diseases not specified) in CO-treated tomato and pepper lots than in those held in air. Woodruff (1977) reported that CO (5 to 20%) was effective in controlling unspecific decays of several fruits and vegetables, but presented no data.

Kader et al. (1978) found that 5 to 10% CO retarded growth of _Botrytis cinerea in vitro_ and rot incidence and severity in tomatoes. Suppression was greater when CO was added to an atmosphere containing 4% O₂ instead of air.

El-Goorani and Sommer (1979) studied _in vitro_ and _in vivo_ effects of atmospheres enriched with 9% CO on 18 postharvest pathogens held at 5.5° or 12.5°C. Test fungi differed greatly in response to CO. The mean percentage of growth in air plus CO ranged from 20 to 100% of that in air alone. The effect of CO was generally much greater if the atmosphere was low in O₂. The mean percentage of growth in 9% CO plus 2.3% O₂ was 4.8 to 89.5% that of air. Suppression was sometimes increased when CO₂ (5% or 18%) was added. The test fungi most sensitive to CO were _Monilinia fructicola_, _Penicillium expansum_, _P. italicum_, _P. digitatum_, and _Whetzelinia sclerotiorum_. Disease development was similarly suppressed. In comparison with air, CO added to 2.3% O₂ + 5% CO₂ reduced rot development by 80 to 90% in strawberries (_B. cinerea_), apples (_P. expansum_), lemons (_W. sclerotiorum_), and oranges (_P. italicum_ and _P. digitatum_) that were inoculated and then incubated for 11 to 23 days at 5.5° or 12.5°C. No phytotoxicity was observed. Occasional off-flavors appeared to be associated with O₂ and CO₂ modification rather than CO addition. CO combined with low O₂ effectively reduced decay in sweet cherries (Ogawa et al. 1978) and peaches inoculated with _Monilinia fructicola_. Freshly harvested pistachio nuts inoculated with _Aspergillus flavus_ and having high moisture (67% on a dry-weight basis) remained bright and free of fungus growth after 18 days at 20°C when exposed to 3% O₂ plus 10% CO. Colonization did occur in air or in MA without CO. The fungus also grew in 10% CO + 3% O₂, duration and temperature as above.

Burg and Burg (1969) and Kader et al. (1977) have shown CO to mimic the biological effects of C₂H₄. Solomos and Laties (1973) showed that CO hastened the climacteric and ripening of avocados and bananas. Similar results were observed with tomato fruits (Morris and Kader 1976).
Commercial use of MA containing CO may, therefore, be restricted to commodities relatively insensitive to \( \text{C}_2\text{H}_4 \) at storage or transport temperatures.

V. EFFECTS OF MODIFIED ATMOSPHERES ON MYCOTOXIN ACCUMULATION

Mycotoxins are toxic secondary metabolites of fungi. They are of special concern if they are also carcinogenic and, therefore, any amount is objectionable. In fresh fruits and vegetables the greatest concern has been for the mycotoxin patulin, a suspected carcinogen, which is produced by several species of *Aspergillus* and *Penicillium*, including the blue mold organism of deciduous fruit, *P. expansum*.

Because *P. expansum* is sometimes prevalent in apples for processing, the presence of patulin in juice or other apple products is of concern. Unlike with fresh fruits, consumers of apple products are unable to visually avoid the blue mold disease lesions. Accumulation of patulin in deciduous fruits has been studied by Buchanan *et al.* 1974, Sommer *et al.* 1974, Sommer and Buchanan 1978, and Wilson and Nuovo 1973.

The effect of MA on fungus growth and patulin production was tested by Lovett *et al.* (1975), Orth (1976), and Sommer *et al.* (1977). With atmospheres commonly used for apples (2 to 3% \( \text{O}_2 \) - 5% \( \text{CO}_2 \)), growth of *P. expansum* was only modestly suppressed. Patulin accumulated in apples stored in MA, but in air at the same temperature (0°C) levels were from 5- to 60-fold greater.

Aflatoxin, produced by *Aspergillus flavus* and *A. parasiticus* Speare, is a potent carcinogen of test animals. Although the elaborating fungi are not common postharvest pathogens of fresh fruit or vegetables, they pose a more serious threat to commodities to be processed by drying or to various tree nuts (almonds, Brazil nuts, cashews, filberts, macadamias, pecans, pistachios, and walnuts).

Studies with figs for drying showed that fruits on the tree were resistant to *A. flavus* until they became completely ripe. As fruits dried on the tree or ground considerable aflatoxin was produced before fruits were sufficiently dry to prevent further fungal growth (Buchanan *et al.* 1975). Pistachios supported growth of *A. flavus* when it was inoculated into the mesocarp of developing fruits (Sommer *et al.* 1976). In California, the incidence of aflatoxin in almonds and walnuts is generally associated with insect activity in the mature nuts, particularly that of the navel orangeworm, *Myelois venipars* Dyar. (Sommer and Buchanan 1978).

MA's effects on *A. flavus* and aflatoxin accumulation were studied by Landers *et al.* (1967), Wilson and Jay (1976), El-Goorani and Sommer
(1979), Shih and Marth (1973), and Buchanan et al. (unpublished data). In general, the effects of MA on storage of freshly harvested or only partially-dried nuts have been as follows: levels of 2% O\textsubscript{2} or higher are only modestly suppressive of *A. flavus* growth and aflatoxin production; CO\textsubscript{2} concentrations of 20% or higher suppressed fungal growth and aflatoxin accumulation considerably, but much less suppression was observed in 10% CO\textsubscript{2} or less. The possible adverse effects of MA on high moisture nuts are not known. Presumably taste, odor, or onset of rancidity might be affected.

Possible utilization of CO in atmospheres to inhibit aflatoxin accumulation was suggested by El-Goorani and Sommer (1979) and Buchanan *et al.* (unpublished data). When accompanied by low O\textsubscript{2} (2 to 3%), CO (ca. 10%) suppressed fungal growth beyond the effects of low O\textsubscript{2} and greatly suppressed aflatoxin accumulation in high moisture pistachios. However, atmospheres approaching those commonly used for fresh fruits and vegetables slowed but did not stop growth of *A. flavus*, even with the addition of CO. Aflatoxin generally could be detected given sufficient time.

VI. SUMMARY AND CONCLUSIONS

The rate of postharvest disease development in fruits and vegetables depends upon the pathogenic powers of the bacterium or fungus when arrayed against the resistance of the host commodity. The environment may influence the antagonists differently. Low temperatures, for example, may drastically suppress the pathogen’s activity. At the same time the physiological activity of the commodity is suppressed. Often the result is an extension of the period before the normal resistance of the fruit or vegetable host is lost. Similarly, elevated CO\textsubscript{2} in atmospheres may suppress the fungus pathogen and simultaneously delay ripening of fruit to thereby retain a greater resistance to postharvest diseases. It is essential to delay the onset of senescence in the host as long as possible.

Lowering the O\textsubscript{2} composition of the atmosphere from about the 21% of air to the 2 to 3% common in modified atmospheres evidently suppresses pathogen activity very little. Postharvest pathogens tested have usually been strikingly suppressed only after the O\textsubscript{2} had been lowered to less than 1%, a level at which commodities risk injury.

Levels of CO\textsubscript{2} commonly are restricted to little more than about 5% in extended storage for fear that higher concentrations would lead to injury of the commodity. The CO\textsubscript{2}'s effect is suppression of the fruit or vegetable's respiration. The growth of pathogens *in vitro* is generally suppressed moderately in an atmosphere of 5% CO\textsubscript{2}. Fruits or vegetables may tolerate high CO\textsubscript{2} levels for short periods. CO\textsubscript{2} at 10 to 20% has been
used successfully with strawberries to suppress *Botrytis cinerea* and with sweet cherries to suppress *Monilinia fructicola* and *Botrytis cinerea* during transit periods of up to 8 to 10 days.

MA may increase disease incidence. Examples where MA worsens the disease problem can be found in potato tubers or various root crops which have highly active wound-healing processes involving periderm formation. Atmospheres low in O₂ slow wound healing to thereby give pathogens increased time to establish infections and colonize the wound while it is still highly susceptible.

The effect of MA on phytoalexin production evidently has been less well studied. However, it is likely that phytoalexin formation would be slowed by MA as a consequence of lowered metabolic activity of the commodity.

It is unrealistic, where serious postharvest diseases are encountered, to expect MA to substitute for other disease control measures. Fungistatic gases, such as CO₉, suppress but do not completely arrest fungal growth. However, MA can be an important adjunct to chemical controls by reducing the disease pressure in the fruit or vegetable during storage or transport. The delay in ripening of fruits and senescence of vegetables by MA may be especially effective against those weak pathogens which attack mostly ripe or senescing fruits and vegetables to sometimes cause serious losses.

**VII. LITERATURE CITED**


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