Spore Germination and Mycelial Growth of Postharvest Pathogens under Hypobaric Pressure

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Contribution No. 123-E, 1976 Series, from the Agricultural Research Organization, The Volcani Center, P.O.B. 6, Bet Dagan, 50200, Israel.

Accepted for publication 4 June 1976.

ABSTRACT

APELBAUM, A., and R. BARKAI-GOLAN. 1977. Spore germination and mycelial growth of postharvest pathogens under hypobaric pressure. Phytopathology 67: 400-403.

The effect of hypobaric pressures used for storage of fruit and vegetables on common postharvest decay-causing pathogens was tested. Spore germination, mycelial growth, and sporulation of the tested fungi were inhibited under hypobaric pressure 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* after 5 days at 100, 50, and 25 mm Hg was inhibited by 5%-25%, 45%-80%, and 100%, respectively, and germination was retarded by 0, 40%-82%, and 100%. Delay in fungal sporulation was recorded under 50 and 25 mm Hg. Inhibition of *Geotrichum candidum* var. *citri-aurantii* was less pronounced under these conditions. Admitting O_2 to the cultures held at hypobaric pressure partially reversed the growth inhibition effect. In all cases, normal growth was resumed after cultures were transferred from hypobaric to atmospheric pressure. Growth inhibition in cultures held under 50 mm Hg at O_2 partial pressure of 0.008 atmospheres was more pronounced than that in cultures held at 760 mm Hg with the same O_2 tension. A possible mode of action of the hypobaric pressure on fungal growth is discussed.

Recent reports have revealed that storage life of fruits and vegetables is extended under hypobaric conditions (1, 4, 7, 10, 11, 13). This storage method consists of placing the commodity in a slowly moving stream of water-saturated air maintained at hypobaric pressure (4). Under these conditions, the commodity is degassed at a controlled atmosphere environment low in oxygen. As a consequence, storage life is extended because the ripening and senescence hormone, ethylene, escapes from the tissue while its production and action, like other metabolic processes, are greatly retarded (1, 4, 7, 11).

The prolongation of storage life of perishable fresh commodities at hypobaric conditions can be attributed to the retardation of both physiological and pathological deterioration. The effect of hypobaric conditions on physiological and biochemical processes in fresh produce has been studied extensively (1, 4, 7, 10, 11, 13). However, little attention has been given so far (14) to the responses of postharvest decay-causing organisms to these conditions. The reduction in pathological deterioration under hypobaric conditions could be the result of: (i) fruit being less prone to fungal attack; or (ii) a direct effect on fungal growth and development.

Inhibition of fungus spore germination and growth at low oxygen levels was demonstrated in the early studies of Brown (3). These results were supported by reports cited by Cochrane (5) and others (9, 12).

In modified atmosphere storage of the fruit of certain cultivars of apple, decay was effectively suppressed by 2.5% O_2. This effect evidently was a consequence of host condition rather than a direct effect on the fungi (8). Studies on the effect of low oxygen atmospheres on strawberry fruit showed that decay was reduced at oxygen concentrations of 0.50 percent or less (6). In vitro studies with strawberry decay fungi and other storage pathogens (6, 9, 12) demonstrated a direct effect of such environment on mycelial growth and sporulation.

In view of the above, how does hypobaric pressure reduce fresh produce pathological deterioration?

The aim of this work was to study the growth and survival of common postharvest decay-causing fungi cultures under hypobaric pressure in order to examine the possibility of a direct effect of such conditions on fungus growth and development.

MATERIALS AND METHODS

The effects of hypobaric pressure were tested on the following fungi: *Botrytis cinerea* Pers., isolated from a rotting strawberry; *Alternaria alternata* (Fr.) Keissler, isolated from tomatoes; and *Diplodia natalensis* P. E., *Penicillium digitatum* (Pers.) Sacc., and *Geotrichum candidum* var. *citri-aurantii* (Ferr.) R. Cif. & Cif., isolated from citrus fruits.

Petri dishes containing potato-dextrose agar (PDA) were seeded with fungal inocula transferred from an 8- to 10-day-old, single-spore culture on PDA at 23 C. Either a 10^4/ml spore suspension or the edge of a colony

containing both mycelium and spores was used.

The effect of hypobaric pressure was studied on both inocula and 2-day-old colonies cultured previously at atmospheric pressure. Petri dishes with the isolates to be tested were placed in 10-liter vacuum desiccators maintained at 23 C and continuously evacuated by means of a vacuum pump. Water-saturated air or pure oxygen was admitted to the desiccators through a Matheson Model 45 vacuum regulator, previously calibrated with a Hg manometer. This regulator maintained the selected vacuum. Incoming gases were humidified at the reduced pressure. The hypobaric pressure levels tested were 150, 100, 50, or 25 mm Hg. Cultures grown at atmospheric pressure (760 mm Hg) were used as controls. In some cases, fungi were held at atmospheric pressure and ventilated with a water-saturated air mixture having an oxygen tension of 0.008 atmospheres, which is similar to that at 50 mm Hg with air ventilation. Oxygen content in the desiccators was monitored and analyzed with a thermal conductivity gas chromatograph.

Germination was evaluated by incubating 0.25 ml of 10^4/ml spore suspension on PDA at 23 C and counting the colonies after 5 days as described elsewhere (2). Mycelial growth was determined by daily measurement of colony diameter for 8 days; sporulation was evaluated by microscopic examination. The data presented in this study are the means of nine replicated experiments.

RESULTS

Spore germination and mycelial growth of the fungi tested were inhibited when the inocula or 2-day-old colonies were exposed to hypobaric pressure. Inhibition of growth was exhibited by induction of a lag period in the growth curve, reduction in growth rate, and subsequent delay in sporulation. The degree of inhibition increased with the decrease in pressure below 150 mm Hg.

Reducing the pressure from 760 to 150 mm Hg did not alter the rate of growth and had no effect on spore germination (Fig. 1, 2). Further reduction in pressure, to 100 mm Hg, inhibited spore germination in the case of *P. digitatum* only (Fig. 1). Colony growth at 100 mm Hg was inhibited by 25% in cultures of *P. digitatum,* and by 20, 10, and 5% in *A. alternata, B. cinerea,* and *G. candidum* var. *citri-aurantii*, respectively (Fig. 2). No effect on sporulation was recorded at that pressure.

A marked reduction in both spore germination and mycelial growth was obtained when the pressure was reduced to 50 mm Hg, when 82%, 55%, and 40%

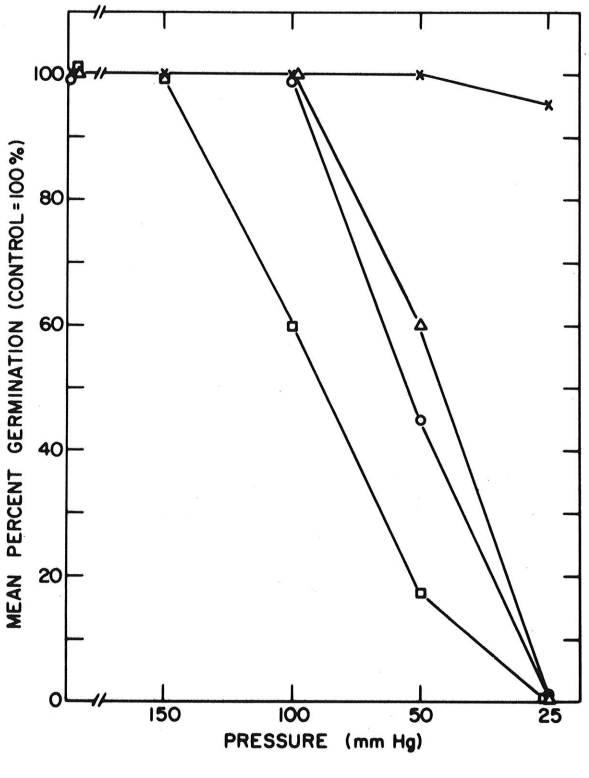

Fig. 1. Effect of hypobaric pressure on spore germination of *Penicillium digitatum* (□), *Botrytis cinerea* (○), *Alternaria alternata* (△), and *Geotrichum candidum* var. *citri-aurantii* (×). Samples (0.25 ml) of 10^4/ml spore suspension were spread on petri dishes containing PDA and placed in vacuum desiccators at various pressure levels and 23 C. Colony formation was determined on the 5th day after seeding. Results are means of nine replicated experiments.

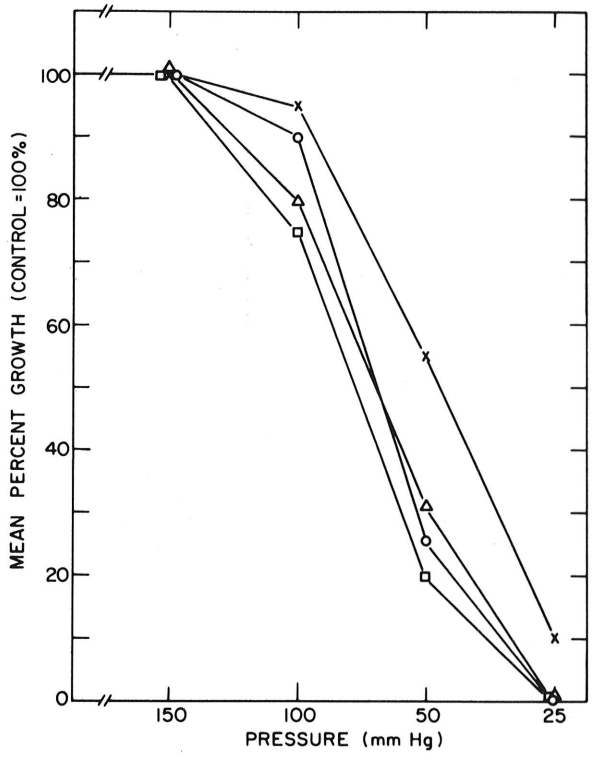

Fig. 2. Effect of hypobaric pressure on colony growth of *Penicillium digitatum* (□), *Botrytis cinerea* (○), *Alternaria alternata* (△), and *Geotrichum candidum* var. *citri-aurantii* (×). Petri dishes containing potato-dextrose agar were center-seeded with fungal inocula containing mycelium and spores. Seeded petri dishes were placed in vacuum desiccators at various pressure levels and 23 C. Growth (colony diameter) was recorded on the 5th day after seeding. Results are means of nine replicated experiments.

inhibition was observed in cultures of *P. digitatum, B. cinerea,* and *A. alternata*, respectively (Fig. 1). However, no effect on spore germination was obtained with *G. candidum* var. *citri-aurantii* at that pressure. Under 50 mm Hg, sporulation was delayed by 2 days in cultures of *P. digitatum* and by 3 days in cultures of *B. cinerea*. Sporulation of *A. alternata* was not affected at that pressure.

Further reduction of the pressure to 25 mm Hg resulted in a 100% inhibition of germination of *P. digitatum, B. cinerea,* and *A. alternata* but only a 5% inhibition in the case of *G. candidum* var. *citri-aurantii* (Fig. 1). Colony growth at that pressure was inhibited completely in all fungi tested except for *G. candidum* var. *citri-aurantii,* where 10% growth was recorded (Fig. 2). All cultures, however, resumed normal growth after being transferred from 25 mm Hg to atmospheric pressure.

Little or no measurable growth of *P. digitatum, B. cinerea, A. alternata,* and *D. natalensis* could be detected during the 3 days at 50 mm Hg with air ventilation (Fig. 3). *Geotrichum candidum* var. *citri-aurantii,* under these conditions, however, had made substantial growth during 3 days of treatment. Ventilating the cultures with pure O_2 partially reversed the growth inhibition (Fig. 3). Transferring the cultures from hypobaric to atmospheric pressure after the 3-day treatment period resulted in rapid radial growth; rates were similar to those of controls held continuously at atmospheric pressure. Extending the

TABLE 1. Effect of hypobaric pressure on growth of postharvest pathogens

Fungus	Growth (% of control) at: O_2 Tension = 0.008 atmosphere	
	50 mm Hg	760 mm Hg
Penicillium digitatum	30	55
Botrytis cinerea	37	60
Alternaria alternata	46	82
Diplodia natalensis	53	83
Geotrichum candidum var. *citri-aurantii*	75	100

Cultures were held for 8 days at 50 mm Hg with air ventilation (O_2 tension ≅ 0.008 atmospheres) or at 760 mm Hg with oxygen tension of 0.008 atmospheres.

Results are means of nine replicated experiments.

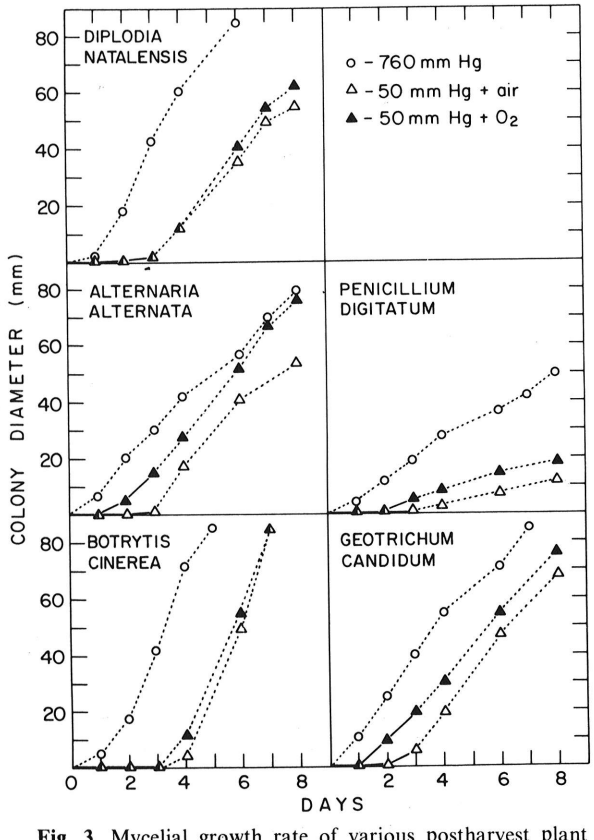

Fig. 3. Mycelial growth rate of various postharvest plant pathogenic fungi at 50 mm Hg with air or O_2 ventilation. Petri dishes containing potato-dextrose agar were center-seeded with fungal inocula and placed for 3 days in vacuum desiccators at 50 mm Hg (———). After 3 days at 50 mm Hg, the cultures were transferred to atmospheric pressure (760 mm Hg) for 5 days (···). Colony diameter was measured daily. Results are means of nine replicated experiments.

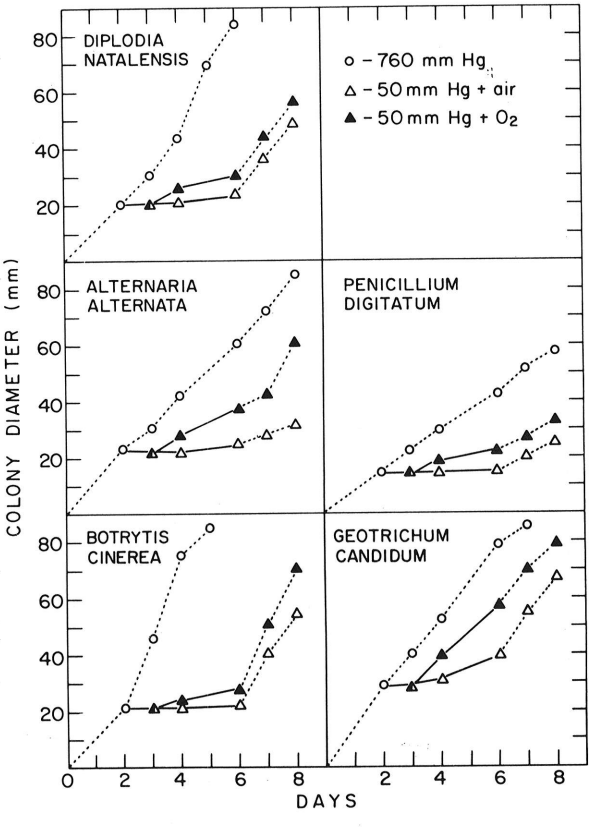

Fig. 4. Effect of hypobaric pressure on mature fungus colonies. Petri dishes containing 48-hr-old fungal colonies grown on potato-dextrose agar at atmospheric pressure (···) were placed for 4 days in desiccators held at 50 mm Hg (———) and returned to atmospheric pressure (760 mm Hg) for 2 days (···). Colony diameter was measured daily. Results are means of nine replicated experiments.

duration of the reduced pressure treatment from 3 to 8 days had almost no further pronounced effect on the lag period and, after a 3- to 4-day lag period, slow but steady rates of growth were observed in all fungi at 50 mm Hg.

When cultures were held at atmospheric pressure (760 mm Hg) but with oxygen partial pressure of 0.008 atmospheres (which is similar to the O_2 prevailing at 50 mm Hg with air ventilation), mycelial growth recorded was higher than that observed at 50 mm Hg and ranged from 55% to 100% of the controls on the 8th day of treatment (Table 1).

Transferring 2-day-old colonies, cultured at 760 mm Hg, to a pressure of 50 mm Hg with air ventilation, resulted in a marked fungal growth retardation (Fig. 4). Almost no measurable growth of *P. digitatum, B. cinerea, A. alternata,* or *D. natalensis* was recorded for the entire 4-day treatment period at the reduced pressure, whereas substantial growth of *G. candidum* var. *citri-aurantii* was recorded after a 2-day lag period. Admission of O_2 to the cultures held at the hypobaric pressure partially reversed growth inhibition. Normal growth rate was resumed with all fungi tested upon returning cultures to atmospheric pressure (Fig. 4).

DISCUSSION

The retardation of fungal development under hypobaric pressure can be attributed to the reduction in the partial O_2 tension prevailing at the reduced pressure. Under water saturation conditions, O_2 tension decreases with the decrease in total pressure; thus, at 760, 150, 100, 50, and 25 mm Hg, the O_2 partial pressure is approximately 0.20, 0.035, 0.020, 0.008, and 0.001 atmospheres, respectively. Fungal respiration depends upon dissolved oxygen which, in turn, is proportional to the O_2 tension in the gas phase (5). Indeed, ventilating the cultures under the reduced atmospheric pressure with O_2 resulted in reversal of fungal growth inhibition. Although the admission of O_2 to the system operated at 50 mm Hg raised the O_2 partial pressure to 0.04 atmospheres, which in turn should have facilitated normal growth (3, 9, 12), only partial reversal of the growth inhibition was observed in the present study. Furthermore, mycelial growth rate under atmospheric pressure (760 mm Hg) and 0.008 atmospheres O_2 was significantly higher than at the same O_2 tension at a pressure of 50 mm Hg. This suggests that growth inhibition under low-pressure conditions may be due, at least in part, to effects other than the reduction of O_2 tension.

A reduction in atmospheric pressure causes a reduction in the CO_2 partial pressure as well. Evidence for the physiological role of CO_2 in the growth of fungi has long been observed in many fungi (5). Carbon dioxide is fixed in lactic, fumaric, citric, and other acids of the Krebs cycle which are later utilized for energy and growth. This type of energy utilization may be significant for some fungi when oxygen is limiting for growth (12). The additive effect of simultaneous reduction of O_2 and CO_2 tension and perhaps the acceleration of the escape of volatile compounds from within the tissue may account for the pronounced inhibition of fungal growth under subatmospheric pressure.

The fact that all fungi exposed to hypobaric pressure resumed normal growth after being transferred to atmospheric pressure suggests that no irreversible damage to the fungi was caused by the treatment. This indicates a fungistatic and not a fungicidal effect.

The results support previous findings (14) and suggest that if hypobaric pressure storage is to be used for control of common postharvest decay fungi, pressures below 50 mm Hg would be required. The host-pathogen response, however, may differ somewhat from the response of the pathogens in culture.

LITERATURE CITED

1. BANGERTH, F. 1974. Hypobaric storage of vegetables. Acta Hortic. 38:23-28.
2. BARKAI-GOLAN, R., R. S. KAHAN, and R. PADOVA. 1969. Symergistic effects of gamma radiation and heat on the development of Penicillium digitatum in vitro and in stored citrus fruit. Phytopathology 59:922-924.
3. BROWN, W. 1922. On the germination and growth of fungi at various concentrations of oxygen and carbon dioxide. Ann. Bot. 36:257-283.
4. BURG, S. P., and E. A. BURG. 1966. Fruit storage at subatmospheric pressures. Science 153:314-315.
5. COCHRANE, V. W. 1958. Physiology of fungi. John Wiley & Sons, New York. 524 p.
6. COUEY, H. M., M. N. FOLLSTAD, and M. UOTA. 1966. Low oxygen atmospheres for control of post-harvest decay of fresh strawberries. Phytopathology 56:1339-1341.
7. DILLEY, D. R. 1972. Hypobaric storage–a new concept for preservation of perishables. Pages 82-89 *in* 102nd Annu. Rep., Mich. State Hortic. Soc. 196 p.
8. ECKERT, J. W., and N. F. SOMMER. 1967. Control of diseases of fruits and vegetables by postharvest treatment. Annu. Rev. Phytopathol. 5:391-432.
9. 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.
10. SALUNKHE, D. K., and M. T. WU. 1973. Effects of subatmospheric pressure storage on ripening and associated chemical changes of certain deciduous fruits. J. Am. Soc. Hortic. Sci. 98:113-116.
11. TOLLE, W. E. 1969. Hypobaric storage of mature green tomatoes. U. S. Dep. Agric., Mkt. Res. Rep. 842. 9 p.
12. WELLS, J. M., and M. UOTA. 1970. Germination and growth of five fungi in low-oxygen and high carbon dioxide atmosphere. Phytopathology 60:50-53.
13. WU, M. T., S. J. JADHAV, and D. K. SALUNKHE. 1972. Effects of subatmospheric pressure on ripening of tomato fruits. J. Food Sci. 37:952-955.
14. WU, M. T., and D. K. SALUNKHE. 1972. Fungistatic effects of subatmospheric pressures. Experientia 28:866-867.