Effect of High-Carbon Dioxide and Low-Oxygen Controlled Atmospheres on Postharvest Decays of Apples

J. W. SITTON and M. E PATTERSON, Department of Horticulture and Landscape Architecture, Washington State University, Pullman 99164-6414

ABSTRACT

Sitton, J. W., and Patterson, M. E. 1992. Effect of high-carbon dioxide and low-oxygen controlled atmospheres on postharvest decays of apples. Plant Dis. 76:992-995.

Controlled atmosphere storage with carbon dioxide concentrations above 2.8% reduced the development of lesions incited by *Botrytis cinerea* (causing gray mold), *Penicillium expansum* (causing blue mold), and *Pezicula malicorticis* (causing bull's-eye rot) in McIntosh, Delicious, and Golden Delicious apples (*Malus domestica*) kept for 61 days at 0 C. Low-oxygen atmospheres were less effective for decay control. Older apples stored in air for 8 mo at 0 C prior to the CO_2 treatment had a higher incidence of brown skin discoloration (scald) when exposed to CO_2 concentrations above 8% than younger fruit stored in air at 0 C for 7 wk before receiving controlled atmosphere treatments. Apple firmness, soluble solids, and titratable acidity were not adversely affected by the high-CO_2 or low-O_2 atmosphere treatments.

In the Pacific Northwest, apples (*Malus domestica* Borkh.) are commercially stored at −0.5 to 0.5 C in a controlled atmosphere (CA) composed of 0.75–3.0% O_2 and less than 1.5% CO_2, with the balance N_2. This atmosphere retards fruit senescence and retains quality (7). Postharvest decays may occur during the storage (5,13). Prestorage applications of fungicides currently registered are quite effective (18), but many are being removed from the market. A high-CO_2 CA has reduced storage decay in avocado (14), blueberries (3), celery (11), muskmelons (15), and strawberries (5). A 10-day exposure of 20% CO_2-enriched air to Golden Delicious prior to storage at −1 C delayed softening and loss of acidity, as compared with fruit in continuous storage (4). However, under commercial conditions, fruits were injured by high-CO_2 prestorage treatments. This prompted changes in the duration times as well as in the CO_2 concentrations used, which reduced industry use. Disease control was not investigated.

Long-term CA treatments (low O_2) for apple storage retain fruit quality and reduce decay. Olsen (7) recommended 1.5–2.0% O_2 and <2.0% CO_2 for storage of apples in Washington. However, certain atmospheric compositions can have a direct effect on decay pathogens. Wells and Uota (17) showed that certain combinations of gases inhibited fungal growth. Sommer (13) recommended using long-term, high-level (>5%) CO_2 for tolerant commodities; however, apples are not currently stored under high CO_2.

Fungicides currently registered are quite effective in controlling most storage rots of pome fruits. However, some consumers are demanding fruit free of fungicide residue. Regulatory agencies have caused effective fungicides to be withdrawn from use for possible health and environmental reasons. Manipulation of atmospheric gases to control fungal growth is an attractive alternative. Unfortunately, high-CO_2 regimens can produce serious side effects (off-flavors, scald, internal and external injury), which has limited their use (2,7). Moreover, certain apple cultivars, such as McIntosh, appear to be sensitive to high CO_2 (2), whereas others, such as Delicious (9), may improve in quality during storage in CO_2.

We investigated the use of various combinations of CO_2 and O_2 to control three storage decays (blue mold, gray mold, and bull's-eye rot) on three apple cultivars (McIntosh, Delicious, and Golden Delicious) and evaluated associated changes in apple firmness, color, and titratable acidity.

MATERIALS AND METHODS

Fruit. McIntosh, Golden Delicious, and Delicious apples were harvested from Pullman, Wenatchee, and Yakima, Washington in September and October. They were cooled and stored in air at 0 C for either 7 wk or 8 mo prior to treatment. Apples were submerged in a 10% solution of 5% sodium hypochlorite for 1.5 min (1) to reduce surface populations of microbes, then rinsed in running tap water for 2 min, drained, and dried on sterile paper pulp trays on a laboratory bench at 23 C before inoculation and storage treatments.

Inoculum preparation. Deionized water (100 ml) with two drops of Tween 20 (polyoxyethylene sorbitan monolaurate) were dispersed into beakers, autoclaved, and cooled to room temperature. Approximately 10 ml of sterile deionized water was added to 3-wk-old cultures of *Penicillium expansum* Link (isolates P-1 from Pullman, Washington, and P-2 [03/06/89-01] from Orondo, Washington), *Botrytis cinerea* Pers.:Fr. (isolates B-1 [08/24/89-02A; Morgan (6) conidial type A] from Walla Walla, Washington, and B-2 [03/24/89-06; Morgan (6) sclerotial type B] from Pullman), and *Pezicula malicorticis* (H. Jacks.) Nannf. (isolates N-1 [Duggan R-2] from Monitor, Washington, and N-2 [Duggan R-3] from Dryden, Washington). The cultures had been grown on Difco potato-dextrose agar in the laboratory at about 23 C. The conidia were suspended in the water by scraping the cultures with a sterile glass rod. The suspended conidia were transferred into sterile water and agitated. To determine the population of viable conidia, we removed an aliquot and transferred it to dilution bottles containing 0.1% water agar. One milliliter of each suspension was dispersed over 2% water agar in petri plates and incubated in the lab (23 C) for 3–5 days, and numbers of colonies were determined. The ranges of viable conidia per milliliter were: *B. cinerea* B-1, 3×10^4–3×10^5; *B. cinerea* B-2, 3×10^3–3×10^6; *P. expansum* P-1, 2×10^6–2.2×10^6, *P. expansum* P-2, 2.3×10^6–1×10^7; *P. malicorticis* N-1, 5×10^4–1×10^6; *P. malicorticis* N-2, 5.2×10^4–2×10^6. Fewer conidia were produced by cultures of *B. cinerea* type B than by *B. cinerea* type A, as in agreement with Morgan (6).

Inoculation and storage. The tip of a sterile wooden applicator stick (2 mm in diameter × 147 mm in length) was dipped into a spore suspension for 1 sec and then used to puncture (1–3 mm deep) the apple skin. The quantity of liquid on the stick was about one drop (0.06 ml). Each fruit was inoculated twice, on opposite sides. The apples were placed on paper pulp trays and transferred to 160-L Plexiglas storage chambers (9) sealed with lids floating in water moats to maintain the desired humidity and gas regimen. Each atmospheric chamber contained 10 apples from each of the three cultivars, inoculated with one of the isolates of the three pathogens. Nitrogen was manufactured with a nitrogen production system (model PSA-215, Kaldair Co., Houston, TX). Nitrogen, air, and CO_2 from cylinders were combined in precise mixtures and admitted to each chamber in a flow-through

Accepted for publication 4 May 1992.

system (200 μl/min) to produce the desired atmospheres. Chamber CO_2 atmospheres ranged from 0.5 to 17.1% CO_2, with 15.9–20.0% O_2 and the balance N_2. Oxygen atmospheres ranged from 0.5 to 20.2% O_2, with 0.2% CO_2 and the balance N_2. Outlet gas concentrations were verified with an automated system for measuring O_2 and CO_2 concentrations (8). Gases were manually mixed with microvalves. Atmospheres in the chambers were monitored 12 times daily with a microcomputer (HP 9816 series 200, Hewlett-Packard Co., Palo Alto, CA) in combination with an HP3054A automatic data acquisition-control system, which controlled both a 540A Servomex oxygen analyzer (Taylor Instruments Analytics Ltd., Crowborough, Sussex, England) and an infrared CO_2 analyzer (PIR-200, Horiba Instruments Inc., Irvine, CA). Fruits were exposed to the CA treatments for 32 or 61 days (McIntosh or Delicious and Golden Delicious, respectively) at 0 C.

After the CA treatment, fruits were rated for lesion diameters and quality changes (skin darkening, firmness, soluble solids, and titratable acidity). Fruit skin darkening was determined with a reflection meter (model 670, Photovolt Corp., New York, NY) equipped with a tri-green filter (peak 416 nm). A white porcelain plate was used for 100% reflectance, and a black box was used for 0%. For soluble solids and titratable acidity, fruit firmness was measured with a Topping mechanical penetrometer (16). Each apple was ground with a juicer. Soluble solids for each sample were measured with an AO Abbe Mark III refractometer and titratable acids determined with a Metrohm 672 titrator (Brinkman Inst. Inc., Westbury, NY).

Sample sizes were 10 fruits per analysis. The data were analyzed with SAS-GLM and Fisher's protected LSD (12) or comparison of standard deviations. The lesion-development experiments were split plots, and the storage (quality) experiments were whole plots. The experiment was duplicated in crop years 1989–1990 and 1990–1991.

RESULTS

Disease suppression in high CO_2. In all combinations of cultivars and pathogens, lesion development decreased with increased CO_2 atmospheres (Table 1). With most cultivar–pathogen combinations, CO_2 as low as 2.8–2.9% provided a significant reduction in lesion size. With certain cultivar–pathogen combinations, CO_2 at 9.4–12.8% completely prevented lesion formation. *B. cinerea* appeared to be the most sensitive for high CO_2 combinations, whereas *P. expansum* was the least sensitive. The latter was not completely inhibited, even at the highest CO_2 level. Strain differences were also evident within the three pathogens. *B. cinerea* (conidial, type A) grew faster than the sclerotial type B, but the stains behaved similarly in the various CA atmospheres. With *P. malicorticis*, lesion development was significantly reduced at 2.5% CO_2 for certain combinations of strains and cultivars but not others. In contrast, 12.8% CO_2 prevented lesion development with all combinations.

Disease suppression in low O_2.

Table 1. Lesion development of *Botrytis cinerea* (B-1, B-2), *Penicillium expansum* (P-1, P-2), and *Pezicula malicorticis* (N-1, N-2) in wound-inoculated McIntosh, Delicious, and Golden Delicious apples that were stored in various carbon dioxide regimens at 0 C for 61 days[z]

Cultivar	$CO_2 + O_2$ (%)	Lesion diameter (mm)					
		B-1	B-2	P-1	P-2	N-1	N-2
McIntosh	0.5 + 19.3	65.4 a	20.8 a	46.5 a	47.2 a	8.4 a	9.6 a
	2.8 + 17.3	55.6 b	14.3 a	37.6 b	38.6 b	8.7 a	6.4 b
	4.4 + 16.8	25.1 c	1.6 b	31.8 c	31.8 c	7.4 a	4.9 b
	8.0 + 16.0	8.0 d	0.5 b	21.9 d	23.8 d	3.8 b	1.4 c
	9.4 + 16.1	0.8 d	0 b	17.0 e	16.9 e	0.8 c	0.5 c
	12.0 + 16.1	0 d	0 b	12.7 f	12.1 f	0.5 c	0 c
	LSD ($P = 0.05$)	7.98	7.28	1.53	1.84	1.66	1.66
Delicious	0.6 + 19.3	85.1 a	47.2 a	34.4 a	40.3 a	9.3 a	9.2 a
	2.9 + 17.0	58.4 b	38.8 b	24.9 b	30.0 b	7.8 b	6.9 b
	4.9 + 16.4	31.2 c	12.3 c	16.7 c	21.2 c	6.2 c	4.4 c
	8.7 + 15.4	7.8 d	3.6 d	10.1 d	12.4 d	3.5 d	1.8 d
	10.9 + 16.3	0.1 e	0 d	7.3 e	7.9 e	0.5 e	0.6 e
	12.8 + 15.4	0 e	0 d	2.0 f	2.8 f	0 e	0 e
	LSD ($P = 0.05$)	3.85	7.82	1.76	1.53	0.87	0.99
Golden Delicious	0.6 + 19.3	88.1 a	67.9 a	38.7 a	44.3 a	9.3 ab	11.5 a
	2.9 + 17.0	62.8 b	50.3 b	26.0 b	32.5 b	10.3 a	9.0 b
	4.9 + 16.4	32.5 c	25.6 c	19.3 c	22.7 c	8.2 b	4.8 c
	8.7 + 15.4	7.1 d	1.5 d	12.8 d	13.6 d	3.9 c	1.5 d
	10.9 + 16.3	0.4 e	0.5 d	7.6 e	9.0 e	1.9 d	1.5 d
	12.8 + 15.4	0 e	0 d	3.6 e	2.3 f	0 e	0 e
	LSD ($P = 0.05$)	3.87	4.43	3.96	2.32	1.11	0.92

[z] Different letters within columns indicate significant differences according to Fisher's protected LSD ($P = 0.05$).

Table 2. Lesion development of *Botrytis cinerea* (B-1, B-2), *Penicillium expansum* (P-1, P-2), and *Pezicula malicorticis* (N-1, N-2) in wound-inoculated McIntosh, Delicious, and Golden Delicious apples that were stored in various oxygen regimens with 0.2% CO_2 at 0 C for 61 days[z]

Cultivar	O_2 (%)	Lesion diameter (mm)					
		B-1	B-2	P-1	P-2	N-1	N-2
McIntosh	19.8	65.4 b	20.8 b	46.5 a	47.2 a	8.4 c	9.6 bc
	3.6	84.1 a	32.6 a	45.2 ab	48.7 a	12.6 a	11.5 a
	2.0	66.7 b	35.2 a	41.4 bc	41.7 b	10.3 b	9.9 b
	1.6	64.9 b	36.1 a	41.2 c	40.2 b	10.9 b	9.6 bc
	1.2	41.3 c	37.4 a	31.8 d	35.6 c	10.2 b	8.7 cd
	0.7	32.1 d	19.3 b	24.1 e	24.3 d	9.6 bc	8.4 d
	0.5	22.2 e	10.0 b	19.2 f	19.3 e	8.6 c	7.8 d
	LSD ($P = 0.05$)	6.91	10.87	3.83	3.28	1.31	1.18
Delicious	19.3	85.1 a	47.2 bc	34.4 a	40.3 a	9.3 abc	9.2 b
	4.2	90.5 a	30.5 d	32.9 b	37.9 b	10.4 a	11.0 a
	2.3	91.1 a	57.4 a	26.9 b	26.9 c	9.4 ab	7.8 cd
	2.1	90.2 a	53.6 ab	25.4 bc	28.1 c	8.7 bc	7.6 d
	1.5	75.1 b	53.8 ab	25.8 bc	27.7 c	8.7 bc	9.0 bc
	1.1	57.5 c	41.6 c	24.4 cd	24.4 d	8.1 c	8.8 bcd
	0.7	28.3 d	24.2 d	22.7 d	24.3 d	8.6 bc	7.7 d
	LSD ($P = 0.05$)	7.94	8.50	2.31	2.25	1.19	1.27
Golden Delicious	19.3	88.1 a	67.9 ab	38.7 a	44.3 a	9.3 bc	11.5 b
	4.2	90.4 a	71.4 a	35.2 b	44.4 a	11.4 a	12.8 a
	2.3	76.0 b	65.8 b	27.4 c	31.5 bc	9.7 b	7.7 c
	2.1	79.6 b	61.3 c	23.2 d	32.1 b	8.6 bcd	7.8 c
	1.5	70.0 c	65.3 b	27.2 c	29.5 bc	7.9 d	8.4 c
	1.1	58.0 d	59.0 c	25.1 cd	29.0 c	7.8 d	8.2 c
	0.7	34.0 e	32.5 d	22.7 d	24.3 d	8.3 cd	8.3 c
	LSD ($P = 0.05$)	5.47	3.99	3.16	2.81	1.11	1.02

[z] Different letters within columns indicate significant differences according to Fisher's protected LSD ($P = 0.05$).

Reduced O_2 concentrations were not nearly as effective in preventing lesion development as increased CO_2 (Table 2), but lesion development, caused by the three test fungi, appeared to be inhibited at O_2 concentrations below 2.3% O_2. However, with certain cultivar-pathogen combinations, lesion development was greater in reduced O_2 (3.6–4.2%) than in ambient air. The two *B. cinerea* isolates responded differently to the O_2 treatments. With type A, lesions developed rapidly on fruit stored in air, but when fruits were stored in the 0.5% O_2, the lesions were 33–38% of the controls. With type B, lesion development was slower than type A for apples stored in air, but the effect of 0.5% O_2 was 48–51% of the air control. With *P. expansum*, lesion development in the 0.5% O_2 atmosphere was reduced to 40–65% of the control. In contrast, lesion development of *P. malicorticis* was more rapid in 3.6–4.2% O_2 than in air but was significantly reduced in 0.5–0.7% O_2. Lesion development trends among the fungi on the three apple cultivars responded similarly to reduced O_2 concentrations.

Quality after storage. With fruit stored in air at 0 C for 8 mo before CA treatment, high-CO_2 atmospheres increased the incidence of scaldlike skin darkening, depending on the cultivar. Significant skin darkening occurred with McIntosh stored for 32 days in >11.3% CO_2 or with Golden Delicious stored for 61 days in >3.7% CO_2 concentrations (Table 3). In contrast, Delicious fruit stored for 61 days in CA with up to 14.1% CO_2 was not significantly darker than that stored in air at 0 C. Exposure to low O_2 levels produced no significant reduction in reflectance (skin darkening) on any of the cultivars. With fruit stored in air for 7 wk before being subjected to CA, skin darkening in the three cultivars due to high CO_2 atmospheres was not significant.

Table 3. Reflectance[x] of McIntosh, Delicious, and Golden Delicious apples that had been stored in various carbon dioxide regimens for 32, 61, and 61 days, respectively[y,z]

	Reflectance (%)		
$CO_2 + O_2$ (%)	McIntosh	Delicious	Golden Delicious
0.2 + 20.2	34.2 a	...	...
0.3 + 19.9	...	10.1 ab	47.6 a
2.5 + 16.9	32.2 a	...	...
3.7 + 18.1	...	10.7 ab	40.1 b
4.7 + 16.3	31.1 a	...	...
6.1 + 17.9	...	11.2 a	41.2 b
7.4 + 17.3	...	10.9 ab	32.6 c
9.9 + 16.3	...	9.5 ab	20.7 d
11.3 + 15.0	18.4 b	...	...
14.1 + 16.0	...	9.0 b	22.0 d
17.1 + 14.5	17.3 b	...	...
LSD ($P = 0.05$)	3.64	2.10	6.06

[x] Darkening or chlorophyll retention, measured with a Photovolt meter.

[y] The apples had been stored at 0 C in air for 8 mo prior to the controlled atmosphere treatments.

[z] Different letters within a column indicate significant differences according to Fisher's protected LSD ($P = 0.05$).

Fruit firmness was not affected by high CO_2 (Fig. 1A) or low O_2 (Fig. 1B), although Delicious increased slightly in firmness in elevated-CO_2 storage. Soluble solids were not significantly affected by the CO_2 or O_2 treatment; the titratable acidity of Delicious and Golden Delicious apples, however, appeared somewhat higher at the higher CO_2 levels, with a slight peak at 8.7% CO_2, but the changes were not significant. Reduced oxygen concentrations did not appreciably affect titratable acidity.

DISCUSSION

Given the results presented herein, we conclude that high carbon dioxide can be a very effective fungistatic agent in stored apples. However, excessively high concentrations of CO_2 adversely affected the appearance quality of Golden Delicious and McIntosh stored for 8 mo prior to the CA treatment. The use of high CO_2 atmospheres in apple storages may be a promising means of reducing decay without the use of chemical fungicides. The atmospheric effect was fungistatic rather than fungicidal, because the fungi resumed growth when the fruits were removed from the CO_2 or O_2 treatment (Sitton and Patterson, *unpublished*). Thus, apples from high CO_2 must be kept in optimum cold storage to preserve marketability. Low-O_2 storage appeared to have limited fungistatic properties and is less effective than CO_2. Poor CA management (i.e., O_2 above 3%) may favor the development of diseases caused by postharvest decay organisms. This work substantiates the in vitro work of Wells and Uota (17).

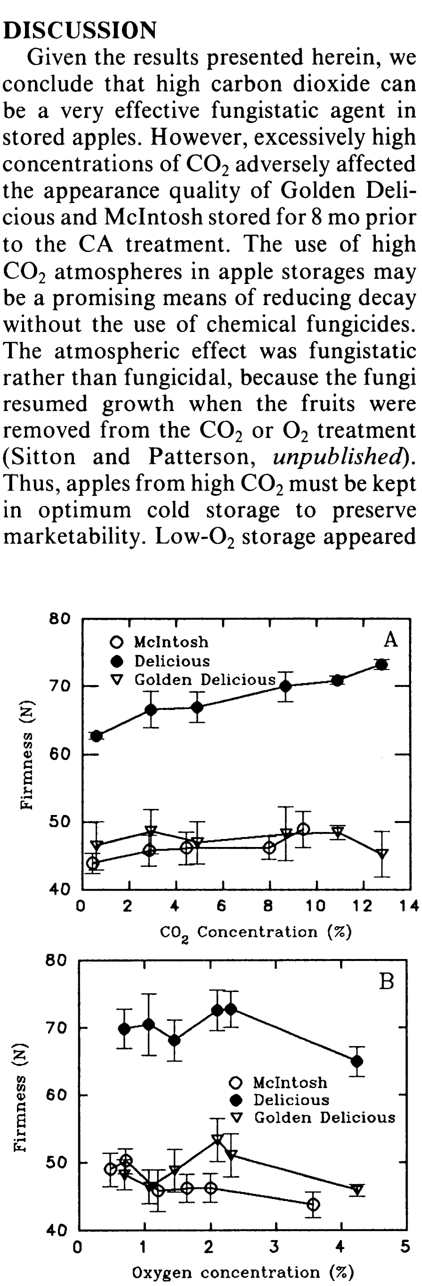

Fig. 1. Firmness (N = newtons) of McIntosh, Delicious, and Golden Delicious apples stored for 61 days at 0 C in various carbon dioxide **(A)** and oxygen **(B)** regimens. (Bar = 1 standard deviation).

Apple quality parameters, including firmness, soluble solids, and titratable acidity, were not compromised by either high CO_2 or low O_2 atmospheres. Certain atmospheres led to improved firmness but promoted skin darkening among fruits stored for 8 mo before treatment. The apparent reversal from scald inhibition to the predisposition of fruit to scald in physiologically older apples associated with high CO_2 levels confirms earlier work with Rome apples (10). With physiologically younger (7 wk postharvest) apples, even the highest CO_2 concentrations did not cause skin darkening. So, high CO_2 CA treatments could be used in freshly harvested apples to control postharvest diseases.

Careful manipulation of O_2 and CO_2 atmospheres has a significant impact in reducing rates of decay. This work further confirms the work of Brooks et al (2), Ceponis at al (3), Couey and Wells (5), Reyes (11), Sommer (13), Spalding and Reader (14), and Stewart (15), who recommended using combinations of low O_2 or high CO_2 to reduce decay. This study does not support the recommendation to keep CO_2 below 2% (7). More work is needed on the optimum concentrations of O_2 and CO_2 for disease control, particularly with regard to cultivar and fruit age. Other factors, such as tree fertility, may also be significant. The effect of timing the CA application, of higher temperatures, and of alternations between commercial CA with pulses of high CO_2 should also be investigated. Identification of the role that fruit age plays in scald development in high CO_2 regimens requires further investigation.

ACKNOWLEDGMENTS

We thank the Auvil Fruit Company for support of this work through a grant for the controlled atmosphere facility; M. A. Evans, Statistical Program, Washington State University, for statistical assistance; F. A. Younce, A. Jones, and D. R. Retan for technical assistance; and John Timpson of Timpson Orchards for the Kaldair nitrogen generator. H/LA Paper 91-16. Project 0766, College of Agriculture and Home Economics Research Center, Washington State University, Pullman.

LITERATURE CITED

1. Baker, K. F., and Heald, F. D. 1932. Some problems concerning blue mold in relation to cleaning and packing of apples. Phytopathology 22:879-898.
2. Brooks, C., Bratley, C. O., and McColloch, L. P. 1936. Transit and storage diseases of fruits and vegetables as affected by initial carbon dioxide treatments. U.S. Dep. Agric. Tech. Bull. 519.
3. Ceponis, M. J., and Cappellini, R. A. 1985. Reducing decay in fresh blueberries with controlled atmospheres. HortScience 20:228-229.
4. Couey, H. M., and Olsen, K. L. 1975. Storage response of 'Golden Delicious' apples after high-

carbon dioxide treatment. J. Amer. Soc. Hortic. Sci. 100:148-150.

5. Couey, H. M., and Wells, J. M. 1970. Low-oxygen or high-carbon dioxide atmospheres to control postharvest decay of strawberries. Phytopathology 60:47-49.
6. Morgan, D. J. 1971. Numerical taxonomic studies of the genus *Botrytis*. II. Other *Botrytis* taxa. Trans. Br. Mycol. Soc. 56:327-335.
7. Olsen, K. L. 1980. Rapid CA and low oxygen storage of apples—A new concept for long term storage of Golden Delicious and more effective storage of Red Delicious apples. Proc. Wash. State Hortic. Assoc. 76:121-125.
8. Patterson, M. E, and Apel, G. W. 1984. A computer operated controlled atmosphere research facility. HortScience (Abstr.) 19:551.
9. Patterson, M. E, and Nichols, W. C. 1988. Metabolic response of 'Delicious' apples to carbon dioxide in anoxic and low oxygen environments. HortScience 23:866-868.
10. Patterson, M. E, and Workman, M. 1962. The influence of oxygen and carbon dioxide on the development of apple scald. Am. Soc. Hortic. Sci. 80:130-136.
11. Reyes, A. A. 1988. Suppression of *Sclerotinia sclerotiorum* and watery soft rot of celery by controlled atmosphere storage. Plant Dis. 72:790-792.
12. SAS Institute. 1986. SAS system for linear models. SAS Institute, Cary, NC. 210 pp.
13. Sommer, N. F. 1985. Role of controlled environments in suppression of post harvest diseases. Can. J. Plant Pathol. 7:331-339.
14. Spalding, D. H., and Reeder, W. F. 1975. Low-oxygen high-carbon dioxide controlled atmosphere storage for control of anthracnose and chilling injury of avocados. Phytopathology 65:458-460.
15. Stewart, J. K. 1979. Decay of muskmelons stored in controlled atmospheres. Scientia Hortic. 11:69-74.
16. Topping, A. J. 1981. A recording laboratory penetrometer for fruit. J. Agric. Eng. Res. 26:179-183.
17. Wells, J. M., and Uota, M. 1970. Germination and growth of five fungi in low-oxygen and high-carbon dioxide atmospheres. Phytopathology 60:50-53.
18. Wilson, E. E., and Ogawa, J. M. 1979. Fungal, bacterial, and certain nonparasitic diseases of fruit and nut crops in California. Division of Agricultural Sciences, University of California, Berkeley. 190 pp.