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Title: Effects of Near Anaerobic Storage Conditions on Physiology and Flavor of Various Fruit Types and on Mortality of Apple Maggot (*Rhagoletis pomonella*)

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'Golden Delicious' and 'Granny Smith' apples, and 'Anjou' and 'Bartlett' pears were kept in static N_2 atmospheres at 0° and 20°C for 25 and 7 days, respectively. CO_2 accumulation, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities, accumulated headspace acetaldehyde and ethanol, tissue acetaldehyde and ethanol, and 1-aminocyclopropane-1-carboxylic acid (ACC) concentration increased with time in static N_2, but ethylene forming enzyme (EFE) decreased. When fruits were removed from anaerobic conditions and subsequently exposed to air at the same temperatures for 7 days, tissue ethanol slightly decreased, but tissue acetaldehyde increased (except in 'Golden Delicious' apples). 'Granny Smith' apples and 'Anjou' pears had no off-flavors or internal disorders when kept in N_2 at 0°C for up to 25 days.

Apple maggot (*Rhagoletis pomonella*) in mature 'Golden Delicious' apples kept in air at 0°C or in static N_2 at 0° and 20°C did not survive beyond 35, 24 and 7 days, respectively.

'Granny Smith' apples and 'Anjou' pears stored at 0°C in flowing streams (10 ml/min) of controlled atmospheres (CA) of 0.3 to 2.8% O_2 plus 1.4% CO_2 had delayed and suppressed ethylene production. Headspace ethanol was detectable only in fruits held in 0.3% O_2. After 24 days in storage, CA-stored fruits had higher PDC and ADH activities, tissue acetaldehyde and ethanol, and ACC concentration as O_2 decreased. The reverse was true for EFE activity. No off-flavors or internal disorders were detected.

'Blue Jay' blueberries, 'Amity' red raspberries, 'Marion' blackberries, and 'Italian' plums could be kept in static N_2 at 0°C for up to 9, 7, 4, and 5 days, respectively without developing off-flavors. A few days longer resulted in slight off-flavors which dissipated after transferring treated fruits to air for 3 days. In low-O_2 (0.3 to 4.3%) atmospheres at 0°C, headspace ethanol increased with time in storage and decreasing O_2 levels. Headspace ethanol was not detected in plums held in $\geq$1.7% O_2. Small fruits held in $\leq$1.3% O_2 and plums kept in $\leq$0.5% O_2 for 10 days developed off-flavors, but recovered from this undesired flavor after transfer to air.

Effects of Near Anaerobic Storage Conditions on Physiology and Flavor of Various Fruit Types and on Mortality of Apple Maggot (*Rhagoletis pomonella*)

by

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONT.)

TABLE OF CONTENTS (CONT.)

LIST OF FIGURES

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF FIGURES (CONT.)

LIST OF APPENDIX FIGURES

LIST OF APPENDIX FIGURES (CONT.)

LIST OF APPENDIX FIGURES (CONT.)

EFFECTS OF NEAR ANAEROBIC STORAGE CONDITIONS ON PHYSIOLOGY AND FLAVOR OF VARIOUS FRUIT TYPES AND ON MORTALITY OF APPLE MAGGOT (*RHAGOLETIS POMONELLA*)

CHAPTER I

INTRODUCTION

Since ethylene dibromide (EDB) was banned from most agricultural uses in 1984 by the United States Environmental Protection Agency (EPA), attempts have been made to develop procedures to control quarantined insects in harvested fruits and vegetables. Feasibility of any quarantine treatment depends not only on its performance from the standpoint of pest mortality, but also upon non-detrimental effects to the physiology and quality of the produce. Only procedures that kill the insect without damaging the commodity are worth considering.

Fruits and vegetables differ in their responses to storage atmospheres, depending on respiration rate, temperature, length of exposure and fruit anatomical characteristics influencing gas diffusion. Some may tolerate low O_2, high CO_2 or even anaerobic conditions for a short period without showing any physiological disorders. Normally, acetaldehyde and ethanol accumulate tremendously in fresh produce under anaerobic conditions, resulting in off-flavors. The commodity may recover from the undesired flavor after regaining access to O_2, provided both substances present in the tissue are lower than the toxic levels.

While there have been numerous studies dealing with fruit responses to low O_2 or high CO_2 over extended storage periods, there is very little information dealing with how long fruits can remain in anaerobic or extremely low O_2 conditions before developing irreversible losses in quality. This information is of value relative to problems in sealed film packaging, possible controlled atmosphere malfunctions, and in potential treatments to rid fruits of surviving quarantined insect pests. Indeed this latter interest is what prompted these studies.

Chapter II reviews the research literature directly related to this thesis problem. Since this thesis is written in the format for publication, each manuscript has a fairly extensive literature review. Thus, Chapter II will be abbreviated in order to avoid unnecessary repetition of information covered in later parts.

The first manuscript (Chapter III) examines responses of 'Golden Delicious' and 'Granny Smith' apples and 'Anjou' and 'Bartlett' pears to anaerobic conditions.

The second manuscript (Chapter IV) involves mortality of apple maggot eggs and larvae in infested 'Golden Delicious' apples held in air at 0°C or in static N_2 at 0° and 20°C.

The third manuscript (Chapter V) investigates the physiology and flavor of 'Granny Smith' apples and 'Anjou' pears as influenced by a range of low-O_2 atmospheres some of which may have potential as an insecticidal treatment.

The fourth manuscript (Chapter VI) evaluates anaerobic and low-O_2 effects on the physiology and flavor of 'Blue Jay' blueberries, 'Amity' red raspberries, 'Marion' blackberries and 'Italian' plums.

CHAPTER II

LITERATURE REVIEW

A. CONTROLLED ATMOSPHERE (CA) STORAGE

Controlled atmospheres mean removal or addition of gases resulting in an atmospheric composition surrounding the commodity that is different from that of the air (Kader, 1985). Usually this involves reduction of O_2 by adding N_2 and/or elevation of CO_2 concentrations. Benefits of CA storage include reduction of respiration rate, retardation of ripening, decay, aging and delay or prevention of development of physiological disorders (Lipton, 1975). However, CA can prevent desirable ripening, induce certain physiological disorders peculiar to CA , and cause an increase in decay when misused.

Although CA storage strikingly benefits 'McIntosh', 'Jonathan' and 'Yellow Newtown' apple cultivars, which develop low-temperature disorders (at 0° to -1°C) in regular cold storage, it also benefits most other cultivars. In CA rooms for apples, an atmosphere of 1.5 to 3.0% O_2, 1.0 to 8.0% CO_2 and the rest nitrogen is carefully maintained (Hardenburg, et al., 1986). Storage of 'Delicious', 'McIntosh', 'Spartan', 'Golden Delicious' and 'Idared' apples in low O_2 was successful under laboratory conditions (Anderson, 1967; Johnson and Ertan, 1983; Lange and Fica, 1982; Lau, 1983,1985; Lidster, et al., 1981). Occasionally, low O_2 can cause external discoloration, internal browning and scald. In Australia, exposure of 'Granny Smith' apples to an initial stress at 0.5% O_2 for 9 days, followed by storage in 1.5%

O_2 + 1.0% CO_2 resulted in better flesh firmness retention and decreased incidence of scald (Little, et al., 1982). Likewise, storage of 'McIntosh' apples initially in low O_2 (1.0%) for 2 to 5 months followed by the standard atmospheres reduced the risk of flesh browning (Lidster, et al., 1985).

Some of the benefits of CA compared to regular refrigerated storage of pears include better firmness retention, reduced scald, less chlorophyll breakdown and better acidity retention (Richardson, 1985). Low-O_2 (1.0%) storage of 'Anjou' pears resulted in not only better quality retention, but also decreased incidence of scald and stem-decay (Mellenthin, et al., 1980; Chen, et al., 1981).

Relatively less information is available on CA storage of small fruits and plums. According to Kader (1985) and others, blueberries , raspberries and blackberries should be stored in 5.0 to 10.0% O_2 + 15 to 20% CO_2 at 0° to 5°C, while plums should be kept in 1.0 to 2.0% O_2 + 0 to 5.0% CO_2 at 0° to 5°C.

B. ETHYLENE

Ethylene is a plant hormone that is involved in the regulation of growth, development and senescence (Abeles, 1973). Not all aspects of ethylene will be reviewed. Only those directly related to the thesis research are summarized.

Methionine is the precursor of ethylene in plant tissues (Lieberman, 1979). However, it was elucidated by Adams and Yang (1977, 1979) that S-adenosylmethionine (SAM) and 1-aminocyclo-

propane-1-carboxylic acid (ACC) are intermediates of ethylene biosynthesis. It is now well-established that the pathway is as follows:

methionine --> SAM --> ACC --> ethylene

Hoffman, et al. (1983) found that ACC could also be converted to 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC). Therefore, ethylene production, at least in climacteric fruits, can be controlled by synthesis of ACC, and by its conversion to ethylene by ethylene forming enzyme (EFE), and to MACC by malonyl CoA - ACC transferase (Mansour, et al., 1986) although the MACC conversion is of only minor consequence in fruits. The conversion of SAM to ACC, which is catalyzed by ACC synthase, is generally the rate-limiting reaction in most plant tissues (Yang, 1980) since application of ACC to various plant organs including root, stem, leaf, inflorescence and fruit, resulted in a marked increase in ethylene production (Cameron, et al., 1979). If ACC was applied to preclimacteric fruit tissues, however, only a relatively small increase in ethylene production occurred, compared with the increase which occurred during later stages of ripening. Thus, it appears that ethylene production in preclimacteric fruit is limited by lack of conversion of SAM to ACC (Yang, 1985). Both anaerobiosis and low O_2 inhibit the conversion of ACC to ethylene (Yang and Hoffman, 1984). In addition, low O_2 affects ATP synthesis which is needed for the conversion of methionine to SAM.

C. PYRUVATE DECARBOXYLASE (PDC) AND ALCOHOL DEHYDROGENASE (ADH)

When O_2 is limiting, reduced nicotinamide adenine dinucleotide (NADH) and pyruvate accumulate. Under this condition, anaerobic respiration takes place. PDC and ADH come into play to decarboxylate pyruvate to form acetaldehyde and to reduce acetaldehyde to ethanol, respectively. However, other compounds such as malate and glycerol also accumulate under anaerobiosis (Davies, 1980).

Pyruvate Decarboxylase

The pH optimum of partially purified PDC (EC 4.1.1.1) from 'Golden Delicious' apples was found to be between 6.1 and 6.4 (Bufler and Bangerth, 1982). Acetaldehyde at 5 mM and 33 mM inhibited apple PDC in a manometric test by 42 and 48%, respectively.

Alcohol Dehydrogenase

ADH (EC 1.1.1.1) activity markedly increased during ripening of bananas (Hyodo, et al., 1983) and tomatoes (Bicsak, et al., 1982). Extracts of apple, avocado, and banana fruits showed ADH activity in the presence of both NADH and reduced nicotinamide adenine dinucleotide phosphate -- NADPH (Rhodes, 1973). This was also true for grapes (Molina, 1986). In contrast, the only cofactor of tomato ADH was oxidized nicotinamide adenine dinucleotide --NAD^+ (Bicsak, et al., 1982). The pH optimum of ADH varied from commodity to commodity, and with substrates and cofactors. In the case of apples, ADH showed optimum activity at

pH 5.5 with acetaldehyde and NADPH as cofactor and at pH 5.5 to 6.0 with NADH as cofactor, whereas ADH assayed with ethanol and NAD^+ showed increasing activity in the pH range of 7.0 to 10.0, but no activity was observed with $NADP^+$ (oxidized form) as cofactor (Bartley and Hindley, 1980).

Molina, et al.(1986) found that the molecular weight values of grape ADH as determined by gel filtration, polyacrylamide gradient and SDS polyacrylamide gel electrophoresis were indicative of a dimeric enzyme constituted probably by two identical subunits of molecular weight of 45 ± 2 k Daltons. Inhibition studies showed that grape ADH was a metalloenzyme with SH groups essential for enzyme activity. Enzyme activity was rapidly destroyed during storage of berries at -20°C. However, the crude extract or purified enzyme could be stored at -20°C for several years without loss of activity in the presence of dithiothreitol and glycerol acting as protective agents.

D. ACETALDEHYDE AND ETHANOL

Acetaldehyde and ethanol are volatile compounds that contribute to flavor and aroma of many fruits. Usually, they are present in fruit tissue in very low concentrations, but relatively high amounts can be observed during fruit ripening (Nursten, 1970; Janes and Frenkel, 1978). Both substances increase tremendously in plant tissue under anaerobic conditions.

Acetaldehyde

Acetaldehyde is a naturally occurring plant metabolite that results from decarboxylation of pyruvate. It accumulates during development of many physiological disorders (Smagula and Bramlage, 1977). However, its role in deterioration is not clear. It is believed that acetaldehyde is more cytotoxic than ethanol, but toxicity levels for each plant or plant part are not yet established. Interestingly enough, acetaldehyde accumulated in persimmon fruit in CO_2 enriched atmospheres played an important role in reducing fruit astringency (Matsuo, et al., 1976; Matsuo and Ito, 1977,1982; Pesis and Ben-Arie, 1984,1986). Application of acetaldehyde vapors enhanced sensory quality of grapes (Pesis and Frenkel, 1989), pears, tomatoes, and blueberries (Paz, et al., 1982). Applied acetaldehyde was effective as a fungicide for control of decay in raspberries, strawberries, and apples (Prasad and Stadelbacher, 1973,1974; Stadelbacher and Prasad, 1973) and an insecticide against green peach aphids in harvested lettuce and western flower thrips on strawberries (Aharoni, et al., 1979a,1979b; Stewart, et al., 1980).

Ethanol

Ethanol results from acetaldehyde reduction in the presence of NADH. The rate of ethanol production varied among plant cultivars and plant parts (Cossins and Beevers, 1963). Toxicity levels of ethanol were 60 mM in pea seedlings (Barclay and Crawford, 1981), and >100 mM in a variety of plant tissues (Jackson, et al., 1982). Internally produced ethanol was much

more toxic than that applied exogenously (Nagodawithana and Steinkraus, 1976). Crawford and Zochowski (1984) and Patterson and Nichols (1988) reported reduced toxicity of ethanol under a flow-through system. This may have been due to out-gassing of ethanol vapor, or metabolism of ethanol in aerobic flows.

E. APPLE MAGGOT

The review papers by AliNiazee (1986), AliNiazee and Penrose (1981), Fisher and AliNiazee (1984), Joos, et al.(1984) and Retan (1984) provide more information regarding the biology and control of apple maggot.

The apple maggot, *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) is native to the Northeastern United States and Canada. It was first discovered in the Pacific Northwest near Portland, Oregon in 1979. Apples are the main host for the apple maggot. Other hosts include hawthorn and crabapple. However, the apple maggot has been reported to attack cherries, plums, apricots, nectarines, peaches, blueberries, and pears.

Life Cycle

After mating, the female fly usually deposits a single egg beneath the skin of an apple or other host fruits. It then marks the fruit with a pheromone to keep other apple maggot females from laying eggs on the same fruit. However, it is commmon to find more than one egg per fruit, particularly when the insect population is high. The eggs hatch after 2 to 10 days, depending on temperatures. The larvae (maggots) feed in the fruit, passing

through 3 instars. After about 20 to 30 days in the fruit, the maggots drop to the ground where they bury themselves in the soil. There they change to the pupal stage, and spend the rest of the winter. The adults emerge from July through September. The timing of emergence may vary considerably, depending on the type of host available, temperature, elevation, soil type and rainfall. A few days after emerging, they are attracted to fruits, where they mate and the females lay eggs. There is one generation per year with a partial second generation in the warmer southern part of its range.

Fruit Damage

Injury to fruit varies from one variety to another. In soft-fleshed fruit, the egg-laying punctures become darkened and decayed; on firmer fruit, the punctures cause dimpling and distortion. Young larvae tunnel through the apple flesh, leaving small brown, irregular, thread-like trails. As the larvae grow, the tunnels enlarge and bacterial or fungal decay further destroys the fruit. Eventually, the fruit becomes soft and rotten.

Control

Several insecticides such as diazinon, dimethoate and phosmet are effective against the apple maggot if applied correctly at the proper times. Integrated pest management (IPM) is the ultimate goal in controlling the apple maggot. More research is needed before IPM can be successfully applied in commercial orchards.

Hence, it is important to maintain quarantine areas to prevent pest entry.

F. POSTHARVEST TREATMENTS FOR INSECT CONTROL

Ethylene dibromide (EDB) is a broad-spectrum fumigant effectively used to control major quarantined flies including Mexican fruit fly (*Anastrepha ludens*), Caribbean fruit fly (*A. suspensa*), Mediterranean fruit fly (*Dacus dorsalis*), and melon fly (*D. cucurbitae*) with little or no damage to commodities. It also is effective against apple maggot (*Rhagoletis pomonella*). In 1984, EDB was banned from most agricultural uses because of its carcinogenic activity when administered at high dose during chronic feeding studies conducted with rats and mice. Since then, attempts have been made to replace EDB fumigation with other quarantine treatments.

USDA (1984), and Mitchell and Kader (1985) outlined all approaches being considered as alternatives to EDB fumigation.

a) Treatments with other fumigants such as methyl bromide and phosphine.

b) Non-chemical treatments such as temperature manipulation, modified or controlled atmospheres, heat, microwave, irradiation and ultrasound.

c) Combinations of the above such as methyl bromide with modified atmospheres, methyl bromide with temperature manipulation and preconditioning fruit to reduce phytotoxic effects of methyl bromide.

All these approaches must be coupled with a rigorous integrated pest management program in order to produce fruits free from injuries and contamination caused by insects of quarantine importance.

Methyl bromide (MB), another effective fumigant, while less toxic to mammals, is more toxic to plants and subject to reevaluation by the United States Environmental Protection Agency. The toxicity varies among cultivars and orchards (Harvey and Harris, 1982). It also depends on dosage and temperature (O'Loughlin and Ireson, 1977). Other fumigants receiving more attention from entomologists are acetaldehyde (Aharoni, et al., 1979a,1979b, 1980; Stewart, et al., 1980) and ethyl formate (Stewart and Aharoni, 1983; Stewart and Mon, 1984). However, both fumigants have not been in commercial use, and are very hazardous to mammals.

Much has been said about the success of irradiation as a treatment against quarantined insects of economic importance (Burditt, 1982; Moy, 1977). Its adverse effects on quality of fresh produce seem to be forgotten in the frenzy to promote irradiation use (Sommer and Mitchell, 1986).

Low temperatures are effective in controlling Tephritid flies in infested fruits (Adsule, et al., 1984; Back and Pemberton, 1916; Benschoter, 1983,1984; Burditt and McAlister, 1982; Burditt and Balock, 1985; Chapman, 1933; Mason and McBride, 1934). Not all fruits can tolerate low temperatures. Some of them, particularly those of tropical or subtropical origin, may exhibit symptoms of

chilling injury. Besides, relatively longer times are needed for this treatment.

Hot water treatments combined with maturity selection were successfully used as a quarantine procedure against Mediterranean fruit fly for Hawaiian papayas (Couey and Hayes, 1986).

Much has been known about using controlled atmospheres to kill insects which infest grains and stored products (Ripp, et al., 1984; Shejbal, 1980). Information is scarcely available on CA storage effects on insects in horticultural commodities. Gaunce, et al.(1981), Klag (1985), and Soderstrom and Brandl (1985) critically reviewed the use of CA storage for quarantine control of insects on fresh fruits and vegetables. This technique has been applied to reduce the infestation of the apple rust mite and European red mite eggs on stored apples (Lidster, et al., 1984), green peach aphid on harvested head lettuce (Aharoni, et al., 1986), and western flower thrips on harvested strawberries (Aharoni, et al., 1981) with varying degrees of success. More recently, Smilanick and Fouse (1989) investigated the quality of nectarines stored in insecticidal low-O_2 atmospheres. Unfortunately, 0.5% O_2 caused physiological disorders and off-flavors in nectarine fruits stored at both 5° and 15°C.

CHAPTER III

Effects of Anaerobic Nitrogen Atmosphere on Physiology and Flavor of Apples and Pears

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ABSTRACT

'Golden Delicious' and 'Granny Smith' apples, and 'Anjou' and 'Bartlett' pears were kept in static N_2 atmospheres in sealed 4 liter glass jars at 0° or 20°C for 25 and 7 days, respectively. The changes of the parameters studied in fruits of all cultivars were almost the same, with the rate of change at 20°C being greater than that at 0°C. CO_2 accumulation, pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities, accumulated headspace acetaldehyde and ethanol, tissue acetaldehyde and ethanol, and 1-aminocyclopropane-1-carboxylic acid concentration increased with time in static N_2 , but ethylene forming enzyme activity decreased. When fruits were removed from anaerobic conditions and subsequently exposed to air at the same temperatures for 7 days, tissue ethanol slightly decreased, but tissue acetaldehyde increased (except in 'Golden Delicious' apples). This also resulted in partial reversal of off-flavors. 'Granny Smith' apples and 'Anjou' pears did not develop detectable

off-flavors when kept in N_2 at 0°C for up to 25 days. In all cases, no external or internal anaerobic injury symptoms were observed.

INTRODUCTION

Higher plants are obligate aerobes that can tolerate anaerobiosis for varying periods of time, but generally cannot survive strictly anaerobic conditions (Laing, 1941). Among the end products of anaerobic respiration, ethanol is most frequently measured as a parameter in studies related to anaerobic metabolism (Davies, 1980). Also classified as volatile compounds in fruit aroma, acetaldehyde and ethanol increase during aerobic fruit ripening (Nursten, 1970; Janes and Frenkel, 1978). However, both substances occur in only trace amounts under aerobic conditions. Under anaerobic conditions, acetaldehyde and ethanol along with pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) increase drastically (Ranson, 1953; Smagula and Bramlage, 1977; Laszlo and Lawrence, 1983). Plants tolerate relatively high levels of ethanol (Jackson, et al., 1982), and cultivars differ upon transfer from anaerobic to aerobic conditions in their ability to metabolize accumulated ethanol (Chang, et al., 1982). As early as 1925, Thomas found that ethanol accumulation was related to injuries in apples. Pome fruits subjected to anaerobiosis failed to ripen normally or exhibited physiological disorders (Meheriuk and Spencer, 1964; MacLean, et al., 1969). Long before the fruit is visibly injured, the taste will be impaired, and such fruit is unfit for sale (Fidler and Mann, 1972). When apples containing ethanol are removed from anaerobic conditions and kept in air, the ethanol level may decrease, causing the fruit to lose alcohol flavor (Fidler and North, 1971).

It has long been known that nitrogen atmosphere causes a cessation in ethylene production of pears (Hansen, 1942) and apples (Burg and Thimann, 1959), but that a surge of ethylene production occurs upon reexposure of the tissue to the air. These observations were interpreted to indicate that an intermediate accumulates during anaerobic incubation and is subsequently converted to ethylene upon exposure to oxygen. In 1979, Adams and Yang found 1-aminocyclopropane-1-carboxylic acid (ACC) to be an immediate precursor of ethylene synthesis by demonstrating the conversion of ACC trapped in apple tissue incubated in a nitrogen atmosphere to ethylene under aerobic condition. The ethylene forming enzyme (EFE) was first described to play an important role in this process. However, repeated attempts to isolate EFE have failed, and it is quite probable that several enzymes may be involved.

In recent years attempts have been made to develop postharvest treatments to control insects in harvested fruits and vegetables as an alternative to fumigation with ethylene dibromide (EDB), which was banned for most agricultural uses by the Environmental Protection Agency (EPA) in September 1984. Anaerobic treatments are well established to control insect pests only in grains and stored products (Williams, et al., 1980). However, prolonged exposure to anaerobiosis can cause damage to fresh produce. Success depends on how tolerant the produce is to such a condition. Generally, the extent of an adverse effect is a function of the intensity or concentration, the time of exposure

and temperature. Practical manipulation relies on whether or not the injury is reversible.

The purpose of this study was to evaluate responses of apples and pears in terms of physiology and flavor to various durations of exposure to anaerobic nitrogen atmospheres.

MATERIALS AND METHODS

'Granny Smith' apples and 'Anjou' pears were purchased from Naumes Inc., Medford, OR, whereas 'Golden Delicious' and 'Bartlett' pears were obtained from the OSU Lewis-Brown Experimental Farm, Corvallis, OR. Fruits were harvested at commercial maturity. Apples and pears were put in cardboard cartons lined with perforated polyethylene film, and kept in separate cold storages at 0° and -1°C, respectively, for about 3 to 4 weeks before being used in the experiment. About 1 kg of each fruit cultivar was placed in 4 liter wide-mouth glass jars, sealed, flushed with N_2 at a flow rate of 1.5 l/min for 25 min (until O_2 concentration was less than 0.05%), and stored at 20° or 0°C. Data collected at 1 to 3 day intervals included headspace CO_2 , acetaldehyde and ethanol; fruit flesh pyruvate decarboxylase and alcohol dehydrogenase activities as well as acetaldehyde and ethanol in the fruit tissue; 1-aminocyclopropane-1-carboxylic acid (ACC) concentration; off-flavor development and observations of other physiological disorders. Fruits were held anaerobically at 20° or 0°C for 7 or 25 days, respectively.

A 1 ml headspace sample was withdrawn by hypodermic plastic tuberculin syringe from the sealed jars. Carbon dioxide, oxygen and nitrogen were measured with a Carle Model 311 gas chromatograph equipped with a Molecular Sieve 5A column (2 m X 3 mm O.D., 60/80 mesh) and a HayeSep R column (2 m X 3 mm O.D., 80/100 mesh) at 55°C and a thermal conductivity detector. Helium carrier gas flow was 30 ml/min. Acetaldehyde and ethanol

concentrations were measured with the same gas chromatograph equipped with a Porapak Q column (2 m X 3mm, 80/100 mesh) at 130°C and a flame ionization detector. Compressed air flow was 300 ml/min, and hydrogen flow was 30 ml/min. Peak areas were quantified with a Shimadzu C-R3A digital integrator and calibrated with standard curves prepared from authentic acetaldehyde and ethanol. Since the boiling point of acetaldehyde is 21°C (Windholz, et al., 1983), it is important to make standards up in the cold room. The values reported are the means of three jars (replications).

PDC and ADH activities were measured at 20°C by following the oxidation of NADH at 340 nm in a Bausch & Lomb 2000 spectrophotometer according to the method of Chang, et al. (1982) with some modifications. Crude extracts in each replication were obtained by homogenizing three 1-g flesh plugs (one from each fruit in the same jar). Crude extracts used in assaying the PDC activity were buffered with Tris-maleate solutions of which optimal pH's were determined in preliminary experiments to be 5.8 for apples, and 7.0 for pears. The corresponding optimal pH's of Tris-maleate buffer used in measuring ADH activity in apple and pear crude extracts were similarly determined to be 6.3 and 7.9. The enzyme activities were expressed as International Units, in which a unit is equivalent to 1.0 umol NADH oxidized/ min/ mg protein. Protein concentration was estimated with the Bio-Rad protein assay kit. Acetaldehyde and ethanol concentrations in the fruit tissue were determined enzymatically by the method of

Beutler (1983a, 1983b). The assays are based on the principle that acetaldehyde is quantitatively oxidized by nicotinamide adenine dinucleotide (NAD^+) to acetate in the presence of aldehyde dehydrogenase (AlDH), whereas ethanol is first oxidized by NAD^+ in the presence of ADH under alkaline conditions, and then the resulting acetaldehyde is further oxidized to acetate by AlDH. The amount of NADH formed is stoichiometric with the amount of acetaldehyde, but that formed in the above coupled reactions is stoichiometric with half the amount of ethanol. NADH is measured by means of its absorbance at 340 nm. Values of PDC and ADH activities were averaged from 3 replications.

EFE activity and ACC concentration were analyzed according to Blankenship and Richardson (1986). A replication consisted of 3 fruits from the same jar. For each fruit, two plugs of flesh were cut with a 1.1 cm I.D. cork borer and trimmed to 4 discs of 5 mm thickness. For each replication, 6 discs weighing about 3 g were vacuum infiltrated (90 sec, water aspirator) with 0.35 M mannitol as the control. The other 6 discs were treated with 0.5 mM ACC in 0.35 M mannitol. The excess liquid was drained, the discs blotted dry, and a partial vacuum applied for 10 sec to free trapped ethylene. The discs were then placed in a 25 ml Erlenmeyer flask, sealed with serum caps, and incubated for 30 min at 20°C. Then, 1 ml atmospheric gas samples were withdrawn and ethylene was measured in a Carle Model 311 gas chromatograph equipped with a Porapak Q column at 55°C and a flame ionization detector. Control discs (0.35 M mannitol-treated) ethylene was substracted from that

produced by ACC-treated discs to calculate EFE activity, expressed as nl ethylene/g fresh wt/h. For ACC determination, 3 g of fruit flesh tissue was homogenized in 6 ml 9% perchloric acid and the homogenate was filtered through a Whatman #1 filter paper into a scintillation vial. The extract was then stored at -20°C until it was used. An aliquot equivalent to 1 g fresh wt of tissue was passed through a 2 ml Dowex-50W (50 - 100 mesh) cation exchange column, washed with water, and ACC eluted from the column with 5 ml of 2 N NH_4OH. The NH_4OH was removed under vacuum. To each 14 ml vial containing the sample, 800 ul distilled water, and 100 ul of 100 uM mercuric chloride were added. The vials were sealed with a rubber serum cap and 100 ul of a solution of 5% NaOCl and saturated NaOH (2:1) was added. The vials were shaken intermittently for 2 min. A 1 ml gas sample was withdrawn from the vial and the concentration of ACC converted to ethylene was measured as described earlier. The values of EFE activity and ACC concentration reported are the means of 3 replications.

Off-flavor development was determined by a taste panel of 10 trained evaluators using an arbitrary hedonic scale of 0 to 8 with 0 = none, 2 = slight, 4 = moderate, 6 = strong and 8 = severe. A score of 1 was arbitrarily chosen as a level of off-flavors that might be regarded as undesirable by the taste panel. The off-flavor score is the mean of 10 replications.

Data of most parameters except headspace analysis were collected immediately after removing the fruits from the

anaerobic condition or after holding the fruits in air at the same temperatures for 7 additional days.

RESULTS

Carbon Dioxide Accumulation

CO_2 accumulation of 'Golden Delicious' apples in static N_2 at 0°C increased steadily from 2.3% on day 4 to 12.8% on day 25, whereas at 20°C, CO_2 increased much more rapidly from 4.6% on day 1 to 25.8% on day 7 (Fig. III.1). The CO_2 accumulation rates of 'Bartlett' pears under static N_2 at both temperatures were almost the same as those of 'Golden Delicious' apples (Fig. III.4). CO_2 accumulation of 'Anjou' pears in anaerobic conditions was slightly lower than that of 'Golden Delicious' apples or 'Bartlett' pears (Fig. III.3). 'Granny Smith' apples showed the lowest CO_2 accumulation in anaerobiosis with the values at 0°C increasing from 1.2% CO_2 on day 4 to 6.9% CO_2 on day 25, and at 20°C from 3.3% CO_2 on day 1 to 16.2% CO_2 on day 7 (Fig. III.2).

PDC and ADH Activities

The initial values before anaerobic treatment for PDC activity in 'Golden Delicious' apples, 'Granny Smith' apples, 'Anjou' pears, and 'Bartlett' pears were 0.10, 0.09, 0.07 and 0.29 units. The respective values for initial ADH activities were 0.14, 0.03, 0.06 and 0.15 units. PDC and ADH activities in fruits kept in air at 0°C for 25 days and at 20°C for 7 days were essentially the same as the initial values, i.e. about 0.1 unit (data not shown). ADH activity in anaerobiosis was higher than PDC activity in the fruits of all cultivars tested (Fig. III.5 - III.8) with the rate at 20°C being much greater than that at 0°C. Only at 0°C did an increase in PDC activity as influenced by anoxia appear

to parallel that in ADH activity. Regardless of cultivars, PDC activity in fruits held at 20°C in static N_2 for 7 days was about 1.3 times the PDC activity in fruits held in N_2 at 0°C for 25 days. ADH activity under anaerobic conditions at 20°C was found to be about 2.4 times the activity at 0°C. 'Granny Smith' apples at 0° and 20°C had lowest PDC and ADH activities, and 'Bartlett' pears showed highest enzyme activities. When fruits were removed from anaerobic treatment and subsequently kept in air at the same temperatures for 7 days, the enzyme activities remained unchanged (Appendix Fig. A.1 - Fig. A.4).

Headspace Acetaldehyde and Ethanol

Slight differences in headspace acetaldehyde and ethanol were noted among 'Golden Delicious' apples, 'Anjou' pears and 'Bartlett' pears (Fig. III.9, III.11, III.12). Headspace acetaldehyde in N_2 at 20°C was in the range of 2 to 9 ug/l, compared with headspace ethanol, which ranged from 37 to 203 ug/l. At 0°C, headspace acetaldehyde was below detection limits (0.5 ug/l), and ethanol was in the range of 1 to 2 ug/l. 'Granny Smith' apples had lowest headspace acetaldehyde and ethanol among fruits of the cultivars studied (Fig. III.10). In all cases, a difference in headspace ethanol under anaerobic conditions at 0° and 20°C was about two orders of magnitude.

Tissue Acetaldehyde and Ethanol

Initial values of tissue acetaldehyde in 'Golden Delicious' and 'Granny Smith' apples, and 'Anjou' and 'Bartlett' pears were 4, 2, 2 and 3 ug/g fresh wt, respectively. The respective values of initial tissue ethanol were 62, 34 , 49 and 72 ug/g fresh wt. After 25 days in air at 0°C, tissue acetaldehyde and ethanol in fruits of all cultivars remained unchanged, whereas tissue ethanol in fruits at 20°C increased by about 39% (data not shown). Under anaerobic conditions, tissue acetaldehyde and ethanol of fruits at 20°C increased at a more rapid rate than those at 0°C (Fig. III.13 - III.16). The level of acetaldehyde was much lower than that of ethanol, and the difference was about two orders of magnitude at both temperatures. Since tissue acetaldehyde and ethanol were quite similar although slight differences in change rates existed among cultivars, 'Golden Delicious' apples were chosen as a representative. After fruits were removed from static N_2 and subsequently held in air at 0°C for 7 days, tissue acetaldehyde decreased by about 12% (averaged over 8 time intervals), whereas tissue ethanol decreased by about 21% (Fig. III.13). An appreciable decrease occurred only when fruits were held in static N_2 for 4 to 10 days. Thereafter, a slight decrease was detected. A similar pattern of decreases in acetaldehyde and ethanol was also noted at 20°C. However, tissue acetaldehyde in 'Granny Smith' apples, 'Anjou' pears and 'Bartlett' pears under static N_2 at 0° and 20°C seemed to increase slightly even after 7-day exposure to air (Fig. III.14 - III.16).

ACC Concentration and EFE Activities

Although ACC levels of fruits in anaerobic conditions at both 0° and 20°C tended to increase with time in static N_2 (Fig. III.17 - III.20), and EFE activity tended to decrease (Fig. III.21 - III.24), the values were not consistent enough to be conclusive. Neither were the values after 7-day exposure to air at the same temperatures.

Off-flavor Development

At 0°C, 'Golden Delicious' apples did not develop detectable off-flavors when held in static N_2 for up to 20 days (Fig. III.25) In contrast, the fruits at 20°C started to develop off-flavors on day 3 in static N_2. From day 2 on, partial reversal (10 to 71%) in off-flavors was obtained when the fruits were transferred to air, and scores were in the range of 0.2 to 3.4 (technically none to moderate off-flavors). 'Granny Smith' apples and 'Anjou' pears did not develop off-flavors at all when kept in static N_2 at 0°C for up to 25 days (Fig. III.26, III.27). They could be held in N_2 at 20°C for 4 days without off-flavors being organoleptically detected. 'Bartlett' pears showed a similar pattern in off-flavor development when compared with 'Golden Delicious' apples (Fig. III.28), except that 'Bartlett' pears developed off-flavors faster than 'Golden Delicious' apples.

DISCUSSION

In controlled or modified atmosphere storage at near optimum temperatures, the maximum CO_2 concentration that pome fruits can tolerate is about 2% (Kader, et al., 1989). However, short-term (2 to 3 weeks) high CO_2 (12-15%) treatment has been used to quickly slow fruit respiration and may improve storage quality of fruit (Meheriuk, 1979; Mellenthin and Olsen, 1978; Tietjen and Hudson, 1984; Wang and Mellenthin, 1975). Under sustained anaerobic conditions in closed containers, fruits normally show CO_2 injury, particularly in extended storage at high temperatures. That was not the case in our short-term experiment. Neither external nor internal CO_2 injuries were observed in treated apples and pears. Cultivars and individual fruits have been reported to vary in susceptibility to CO_2 injuries because of anatomical differences influencing gas diffusion characteristics rather than biochemical differences (Bussel and Maxie, 1966; Porritt, et al., 1982).

PDC and ADH activities reported in this paper were based on oxidation of added NADH at 340 nm (Chang, et al., 1982; Chang, et al., 1983). Existing NADH in the crude enzyme extract might also account for differences in the activities. Also NADPH may be present in the extract, and this has been reported to be a cofactor of this reaction (Molina et al., 1986; Rhodes, 1973). In citrus fruit, maturation affected PDC and ADH activities (Bruemmer, 1985). It is a well-known principle that the enzyme activity is higher at a higher temperature provided the temperature is in the biological range.

Headspace ethanol was proportional to tissue ethanol (North and Cockburn, 1975). In our experiment, both the difference between headspace ethanol at 0° and 20°C and that between tissue ethanol at the same temperatures were about two orders of magnitude. Headspace concentration depended on gas diffusion through barriers which varied among cultivars (Solomos, 1985). The barriers to gas diffusion in fruit involve the skin, the intercellular spaces of the flesh, and the cell wall and membrane.

Acetaldehyde and ethanol usually occur in only trace amounts in plant tissues due to the relatively low activity of pyruvate decarboxylase, but should pyruvate accumulate, then acetaldehyde and ethanol may begin to accumulate (Beevers, 1960). Ethanol accumulation in the flesh of harvested apple fruits was related to the cultivar, the season, level of O_2 in storage and other treatments influencing the physiological age of the fruits (Blanpied, et al., 1968). Although the initial values of acetaldehyde and ethanol varied among cultivars, the values were within the ranges reported by Fidler (1968), Janes and Frenkel (1978), North and Cockburn (1975), and Thomas (1925, 1928). Under anaerobic conditions, ethanol concentration increased with time of exposure and increasing temperature (Saltveit and Ballinger, 1983a ,1983b). High CO_2 affected acetaldehyde production in persimmon fruit (Pesis and Ben-Arie, 1984), but had no effect on ethanol accumulation in grapes (Saltveit and Ballinger, 1983). In contrast, ethanol concentration in sweet potato roots was higher in CO_2 than in N_2 atmospheres (Chang, et al., 1983).

Ethanol which accumulated during anaerobiosis could be metabolized when plant tissues gained better access to O_2 (Cossins and Beevers, 1963; Knee and Hatfield, 1981). Acetaldehyde could also be metabolized by plant tissues (Fidler, 1968). When apples containing ethanol were kept in air at warm temperatures , some of the ethanol was lost by diffusion, but most of it was metabolized (Fidler and North, 1971). Fruits of different cultivars might have different metabolic rates and diffusitivity of acetaldehyde and ethanol. In some cases, tissue acetaldehyde increased when fruits previously held in anaerobic conditions were exposed to air. This could be explained as a function of reversible ADH activity during ethanol oxidation (Patterson and Nichols, 1988). In addition, fruits held at a high temperature lost more ethanol than fruits held at a low temperature (Nichols and Patterson, 1987).

No internal or external anaerobic injury symptoms were noticed in anaerobiosis under static system at both 0° and 20°C. This was also the case for 'Delicious' apples in a flow-through system (Patterson and Nichols, 1988) in which ethanol toxicity was reduced (Crawford and Zochowski, 1984). However, in a severe case fruits exhibited off-flavors which might disappear even though there was only a slight decrease in acetaldehyde or ethanol after exposing fruits to air. This indicated that there must be compounds other than acetaldehyde and ethanol that caused off-flavors. No attempt has been made to distinguish the compounds responsible for this undesirable flavor, but it would make a very interesting follow-up study.

When ACC was applied to various plant organs (with the exception of preclimacteric fruits and flowers) from a number of plant species, a marked increase in ethylene production was observed (Cameron, et al., 1979). Apples and pears stored in 1% O_2 atmosphere accumulated a relatively high amount of ACC (Bufler and Streif, 1986; Blankenship and Richardson, 1986). Anaerobic stress not only blocks conversion of ACC to ethylene, but also stimulates ACC synthesis (Yang, 1980). CO_2 inhibited ethylene production through the conversion of ACC to ethylene and the formation of ACC (Cheverry, et al., 1988; Zhen-guo, et al., 1983). In tomato fruit, ethanol was found to inhibit ACC conversion to ethylene (Saltveit, 1989; Saltveit and Mencarelli, 1988). Based on the aforementioned findings by others, conclusions about mechanisms would be difficult to draw from our results on changes in ACC and EFE activity of apples and pears under anaerobic conditions. More replications may be needed so that data would be more consistent and informative.

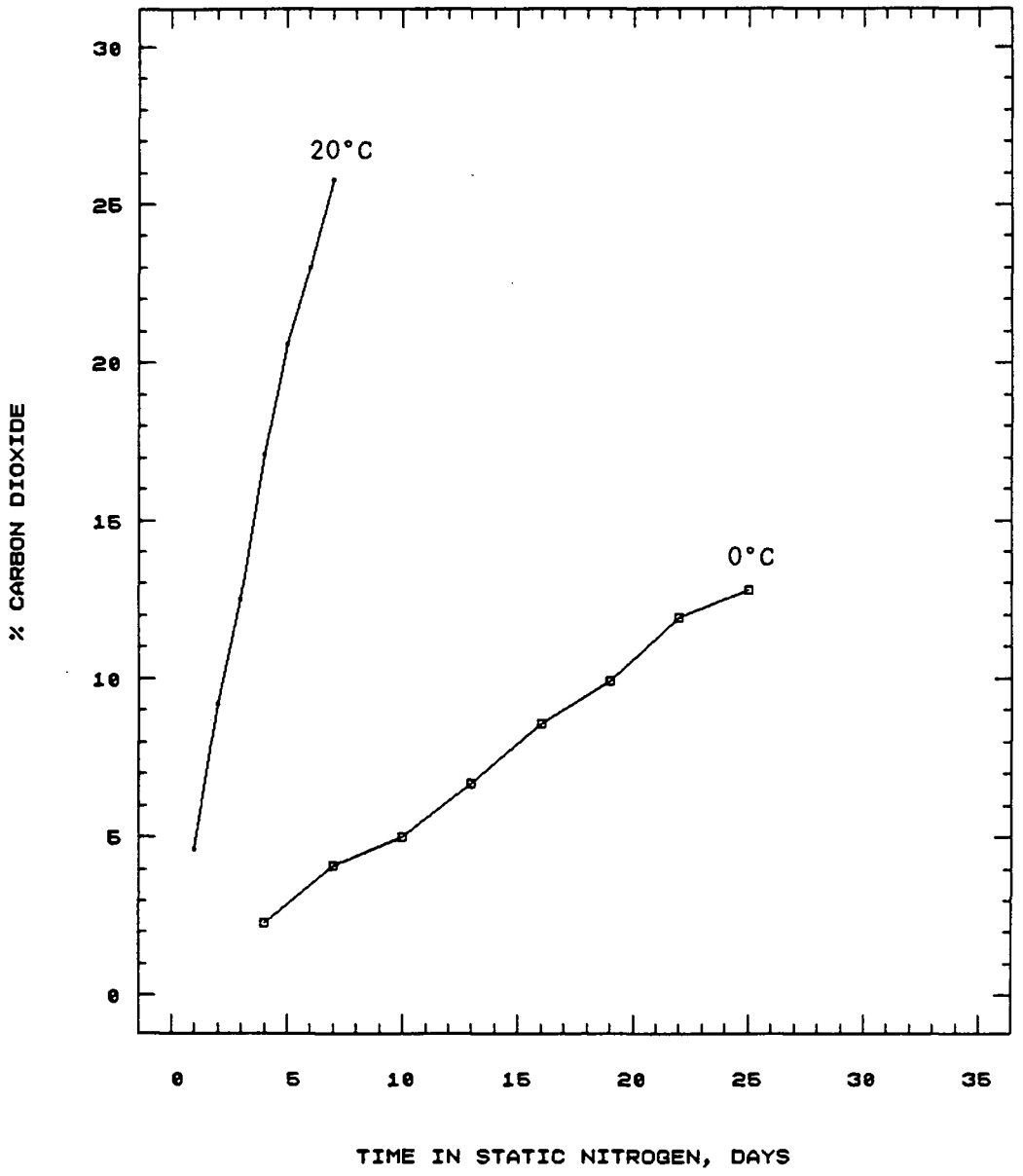

Fig. III.1. CO_2 accumulation of 'Golden Delicious' apples in static N_2 at 0° and 20°C. Each point represents the mean of 3 observations.

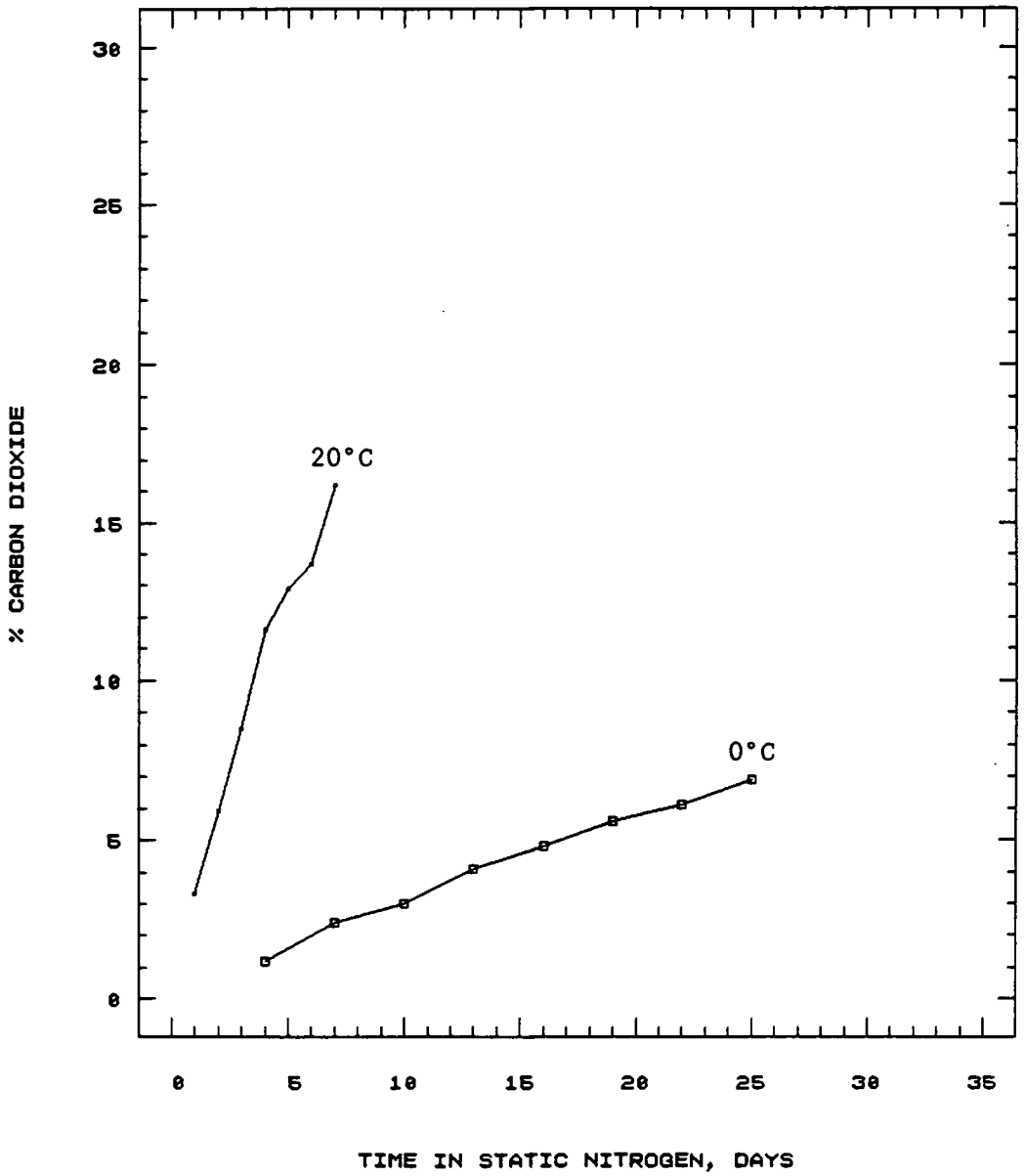

Fig. III.2. CO_2 accumulation of 'Granny Smith' apples in static N_2 at 0° and 20°C. Each point represents the mean of 3 observations.

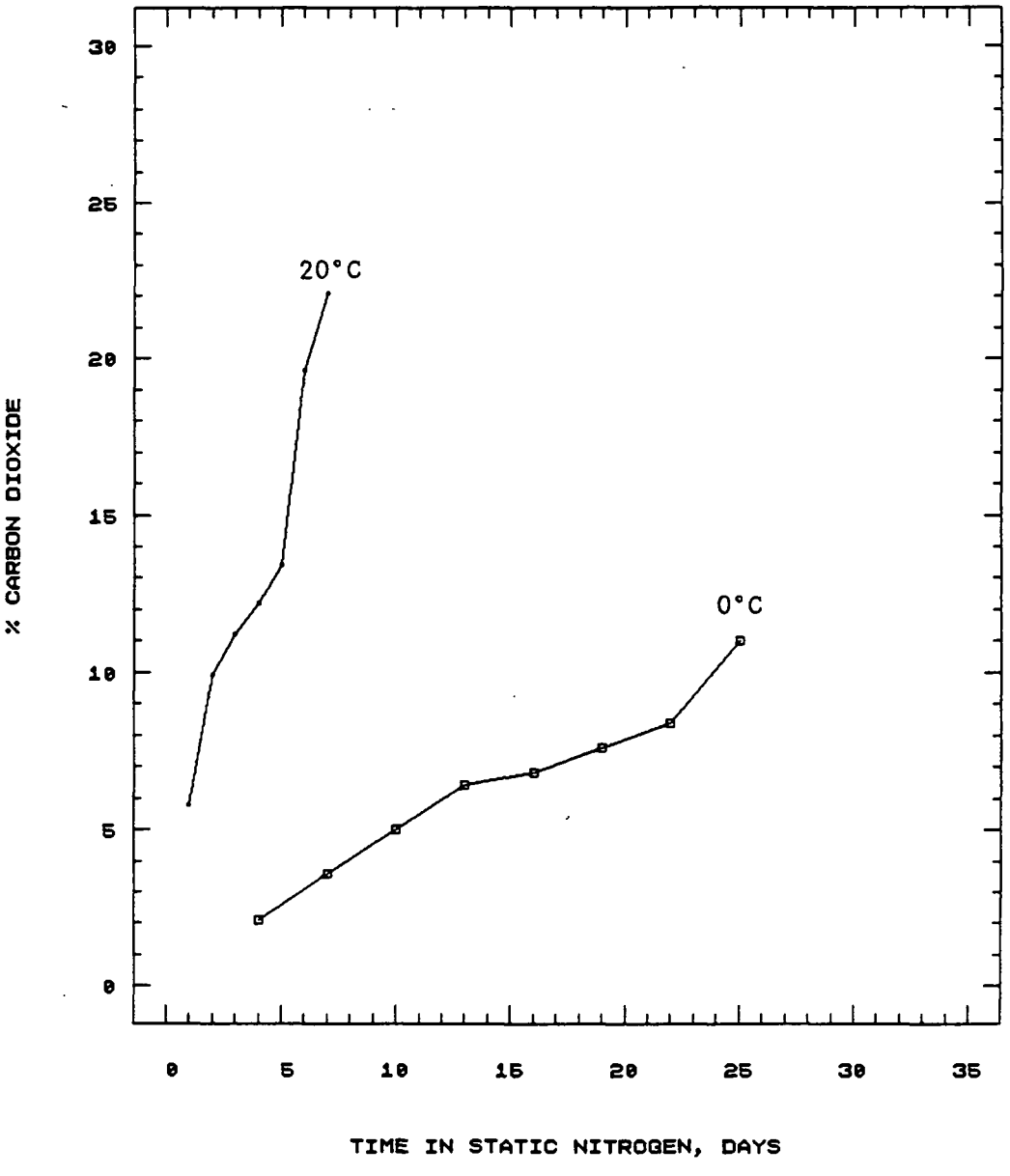

Fig. III.3. CO_2 accumulation of 'Anjou' pears in static N_2 at 0° and 20°C. Each point represents the mean of 3 observations.

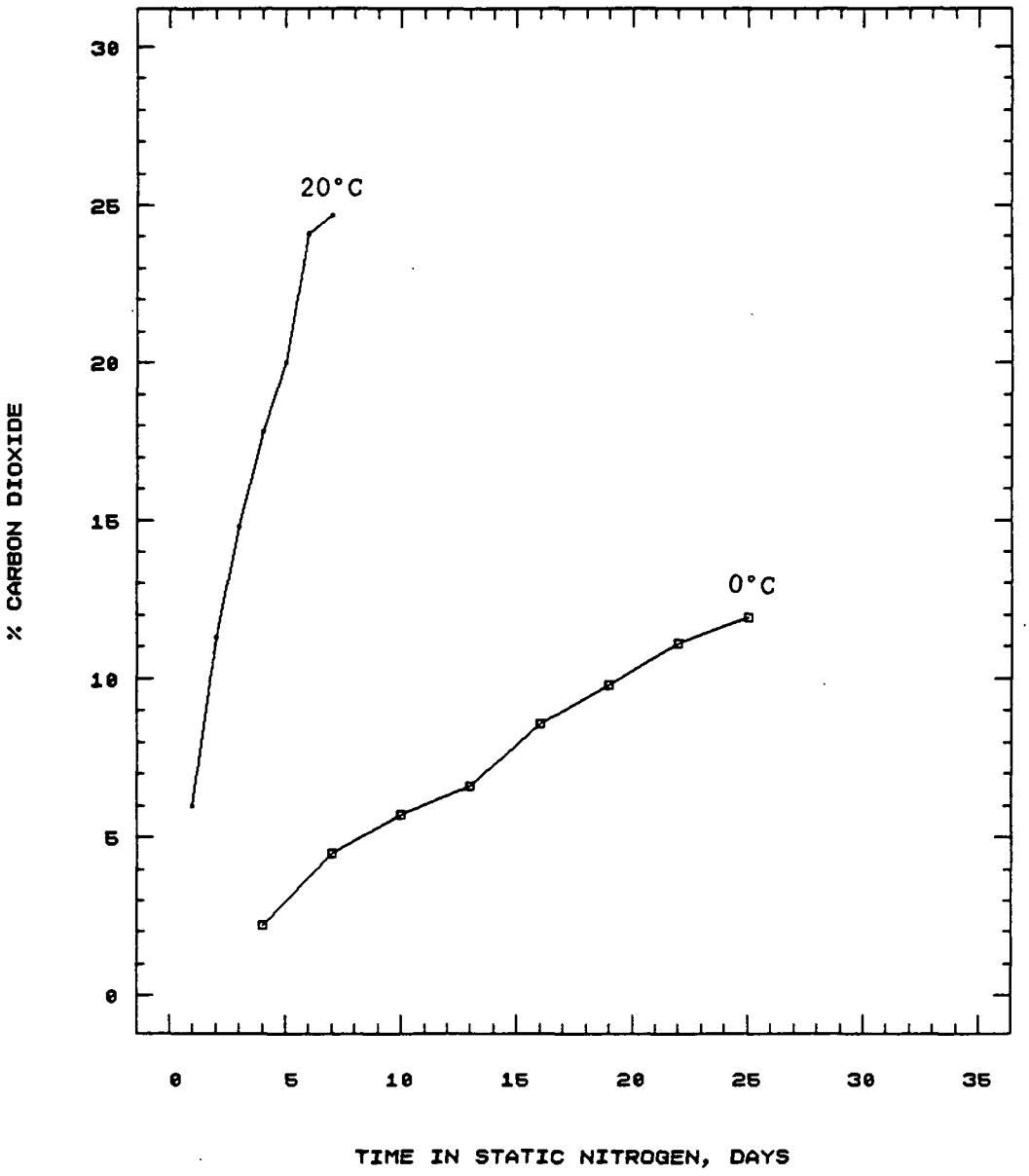

Fig. III.4. CO_2 accumulation of 'Bartlett' pears in static N_2 at 0° and 20°C. Each point represents the mean of 3 observations.

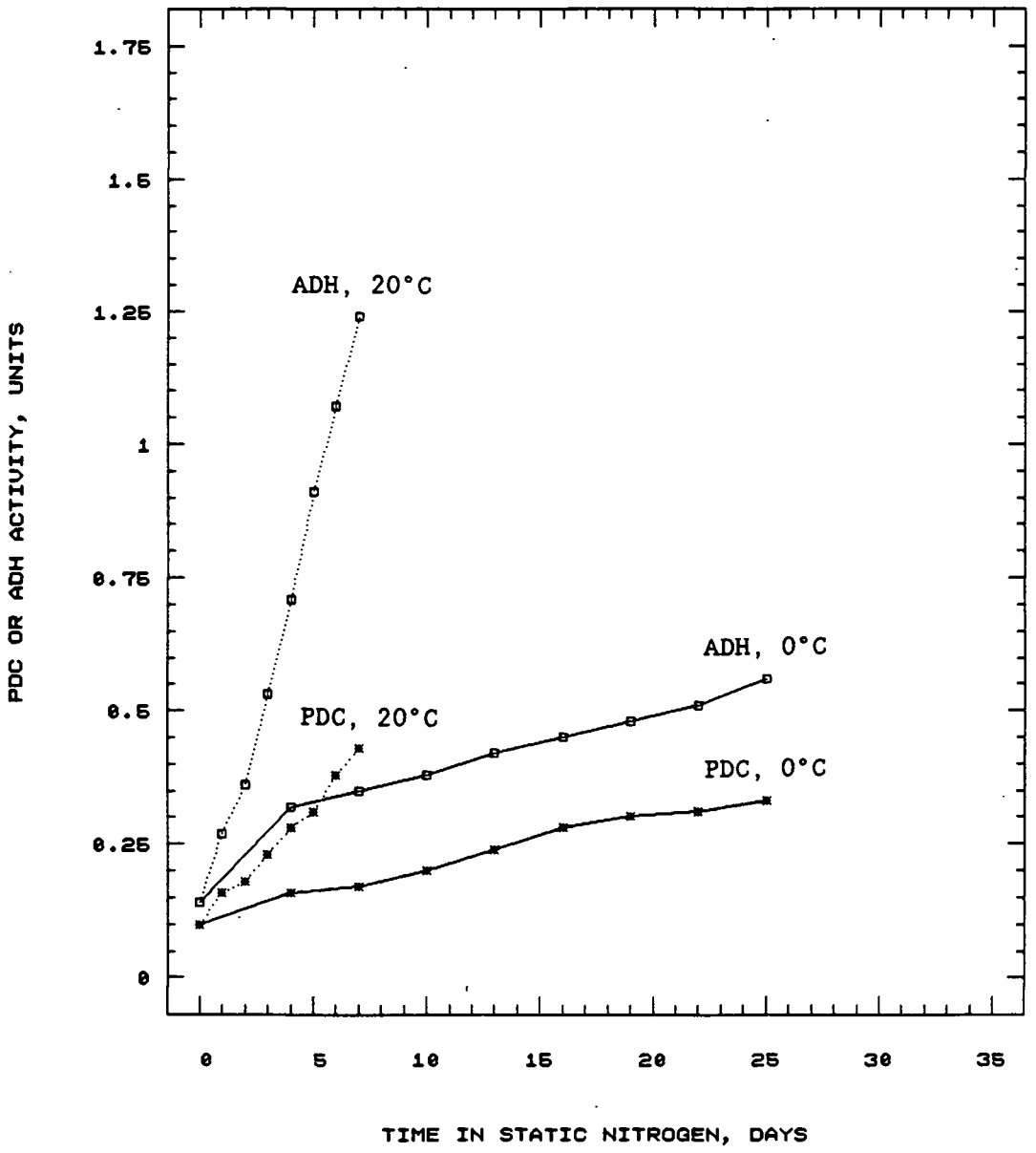

Fig. III.5. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Golden Delicious' apples under static N_2 at 0° and 20°C. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

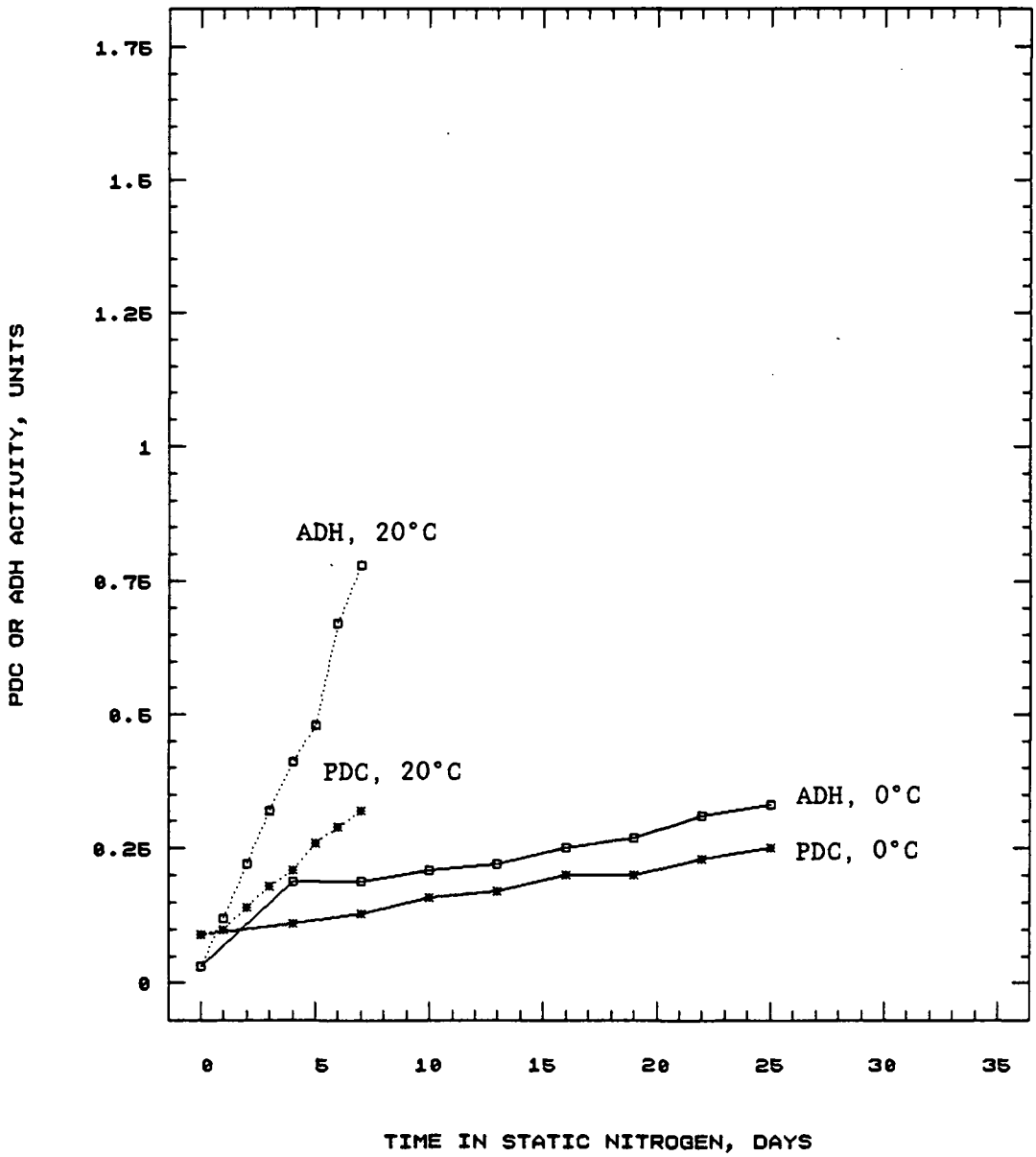

Fig. III.6. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Granny Smith' apples under static N_2 at 0° and 20°C. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

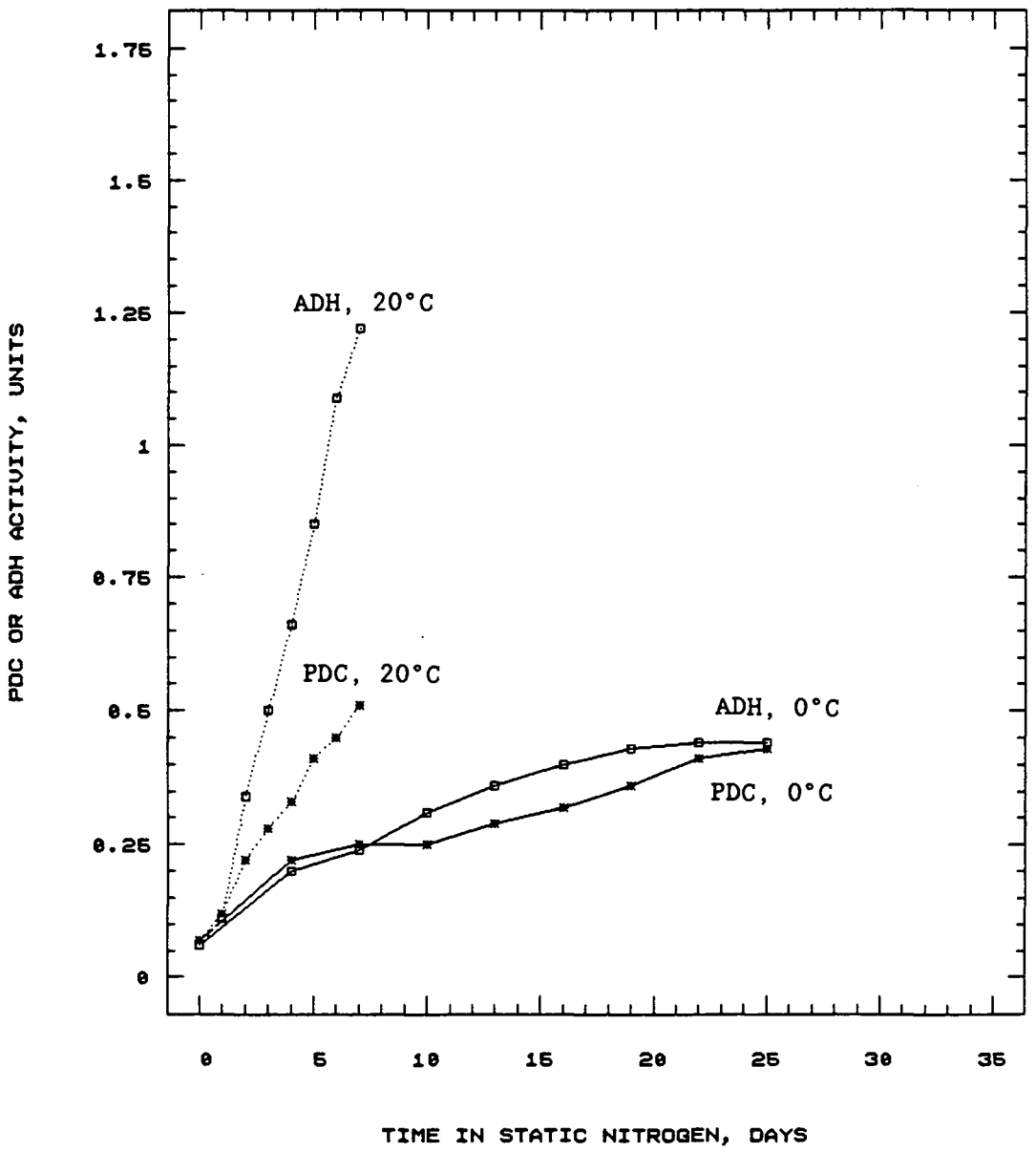

Fig. III.7. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Anjou' pears under static N_2 at 0° and 20°C. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

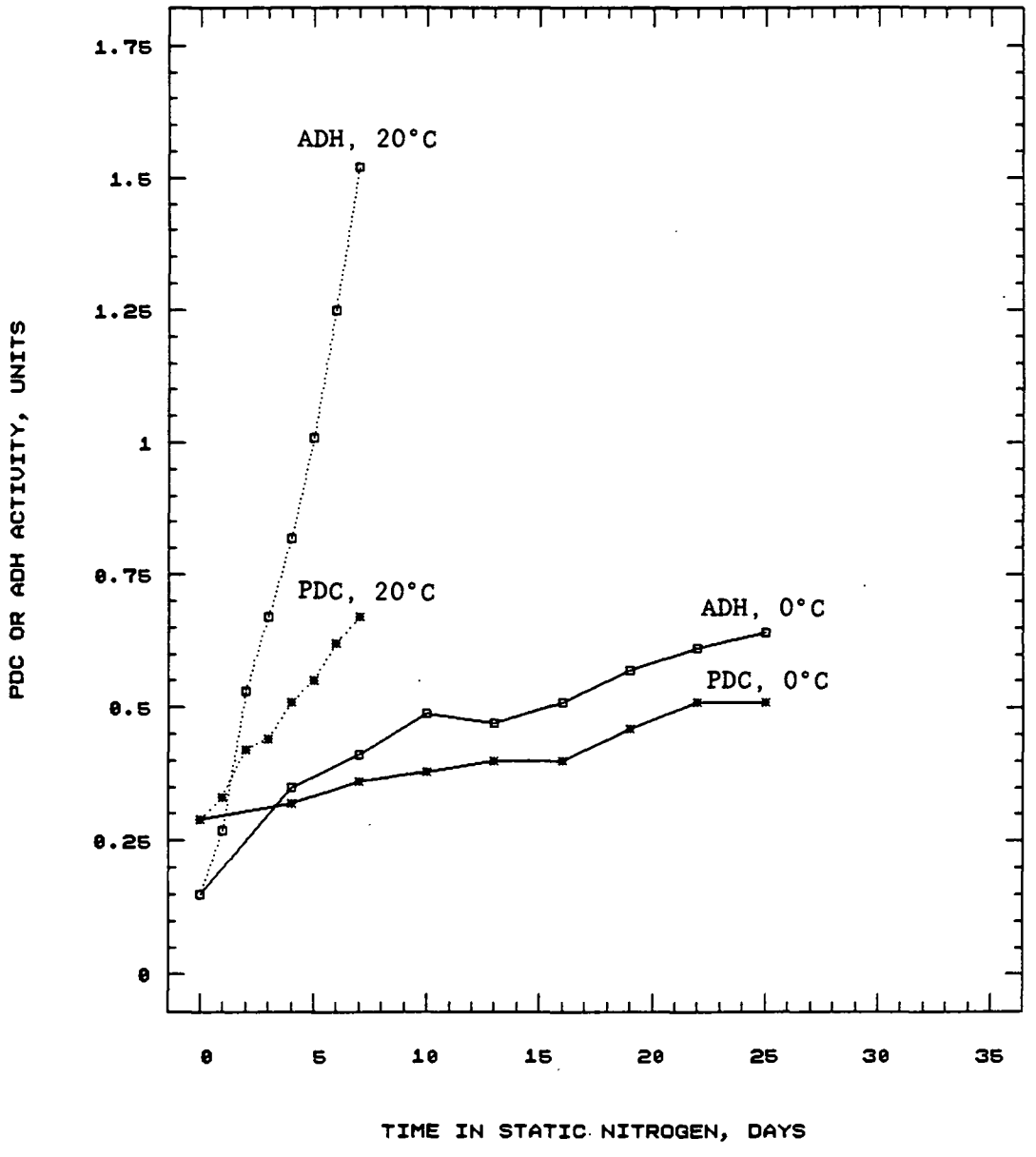

Fig. III.8. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Bartlett' pears under static N_2 at 0° and 20°C. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

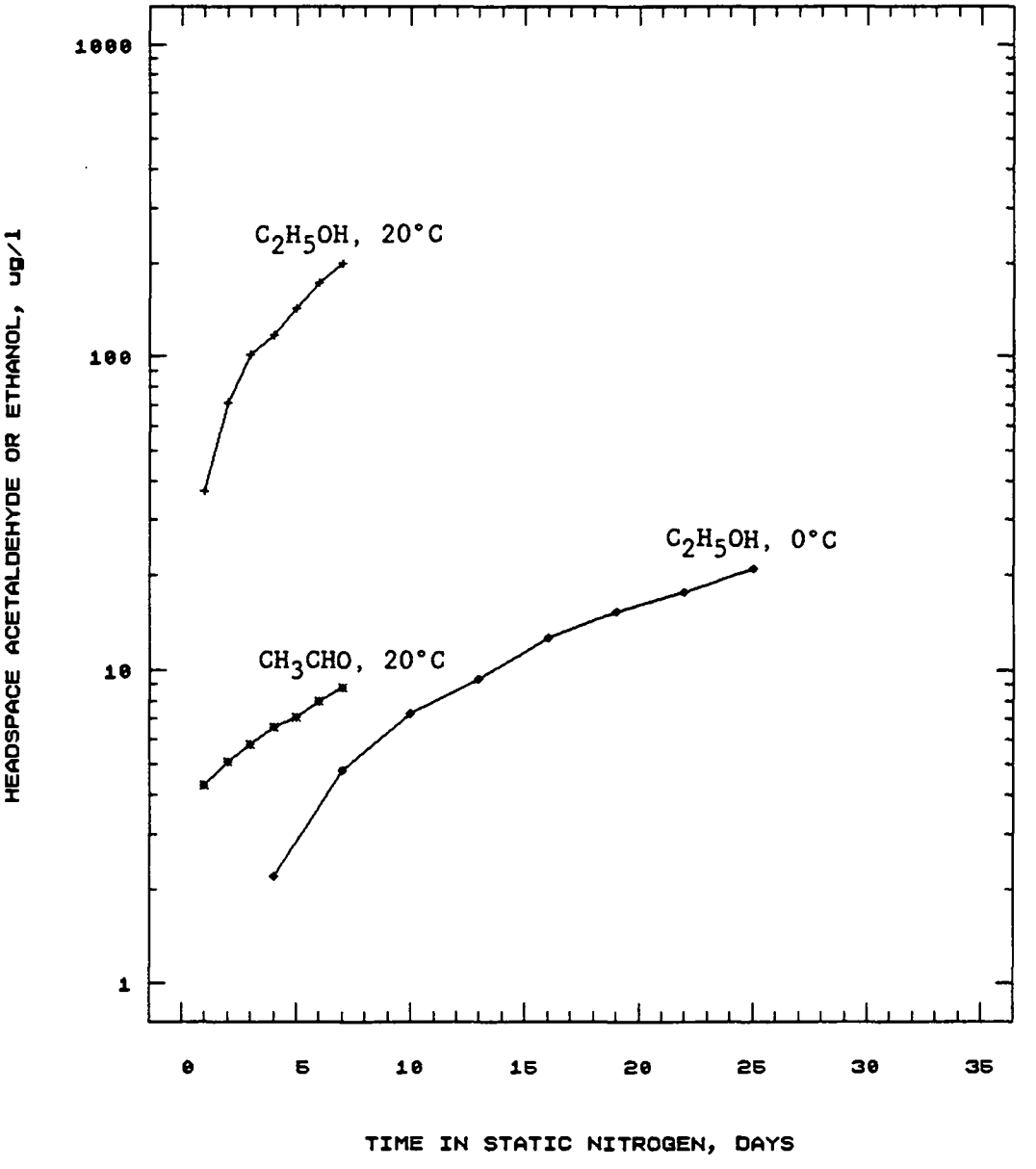

Fig. III.9. Headspace acetaldehyde and ethanol of 'Golden Delicious' apples under static N_2 at 0° and 20°C. Each point represents the mean of 3 observations. At 0°C, acetaldehyde was below detection limits (0.5 ug/l) at all sampling times.

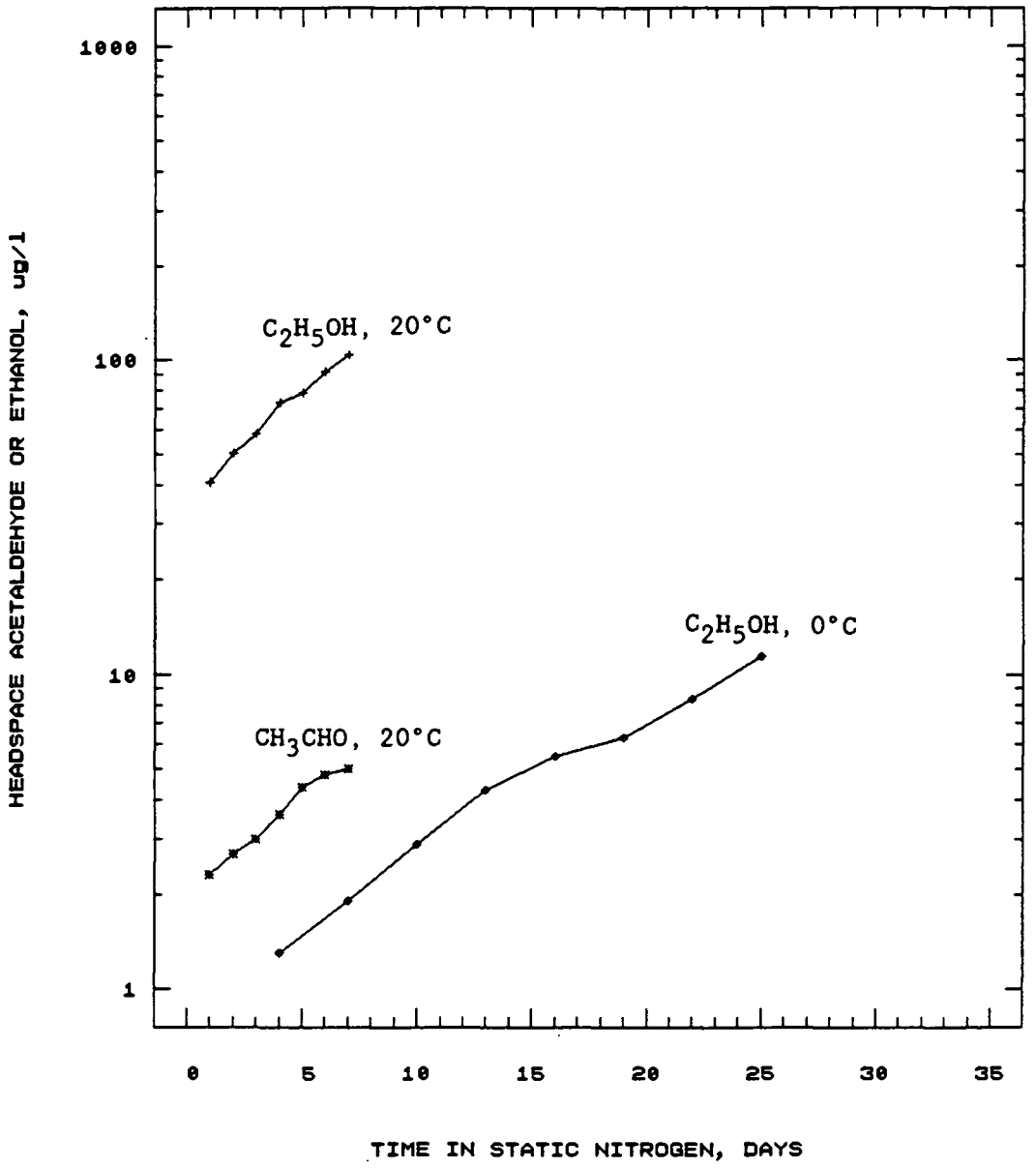

Fig. III.10. Headspace acetaldehyde and ethanol of 'Granny Smith' apples under static N_2 at 0° and 20°C. Each point represents the mean of 3 observations. At 0°C, acetaldehyde was below detection limits (0.5 ug/l) at all sampling times.

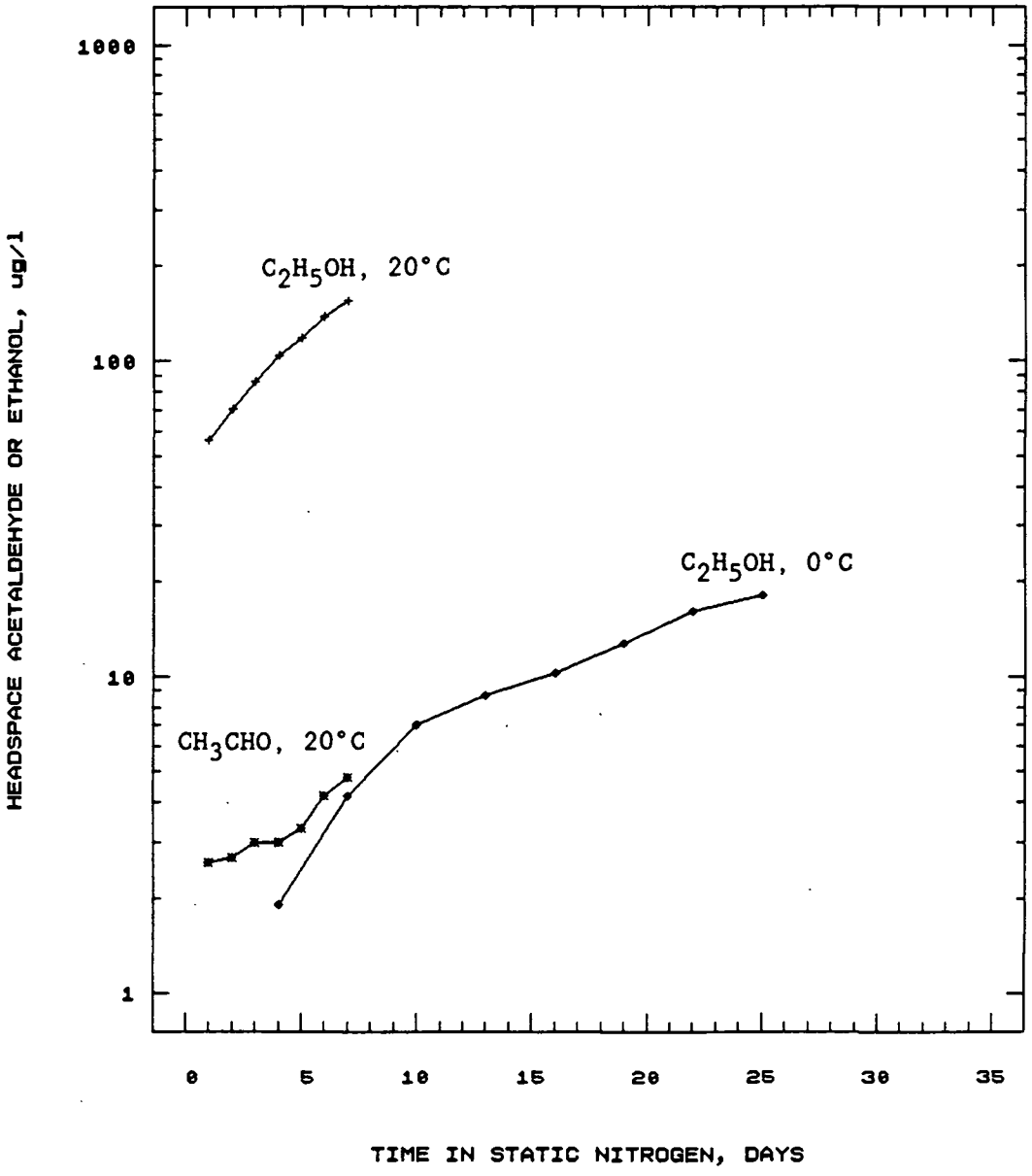

Fig. III.11. Headspace acetaldehyde and ethanol of 'Anjou' pears under static N_2 at 0° and 20°C. Each point represents the mean of 3 observations. At 0°C, acetaldehyde was below detection limits (0.5 ug/l) at all sampling times.

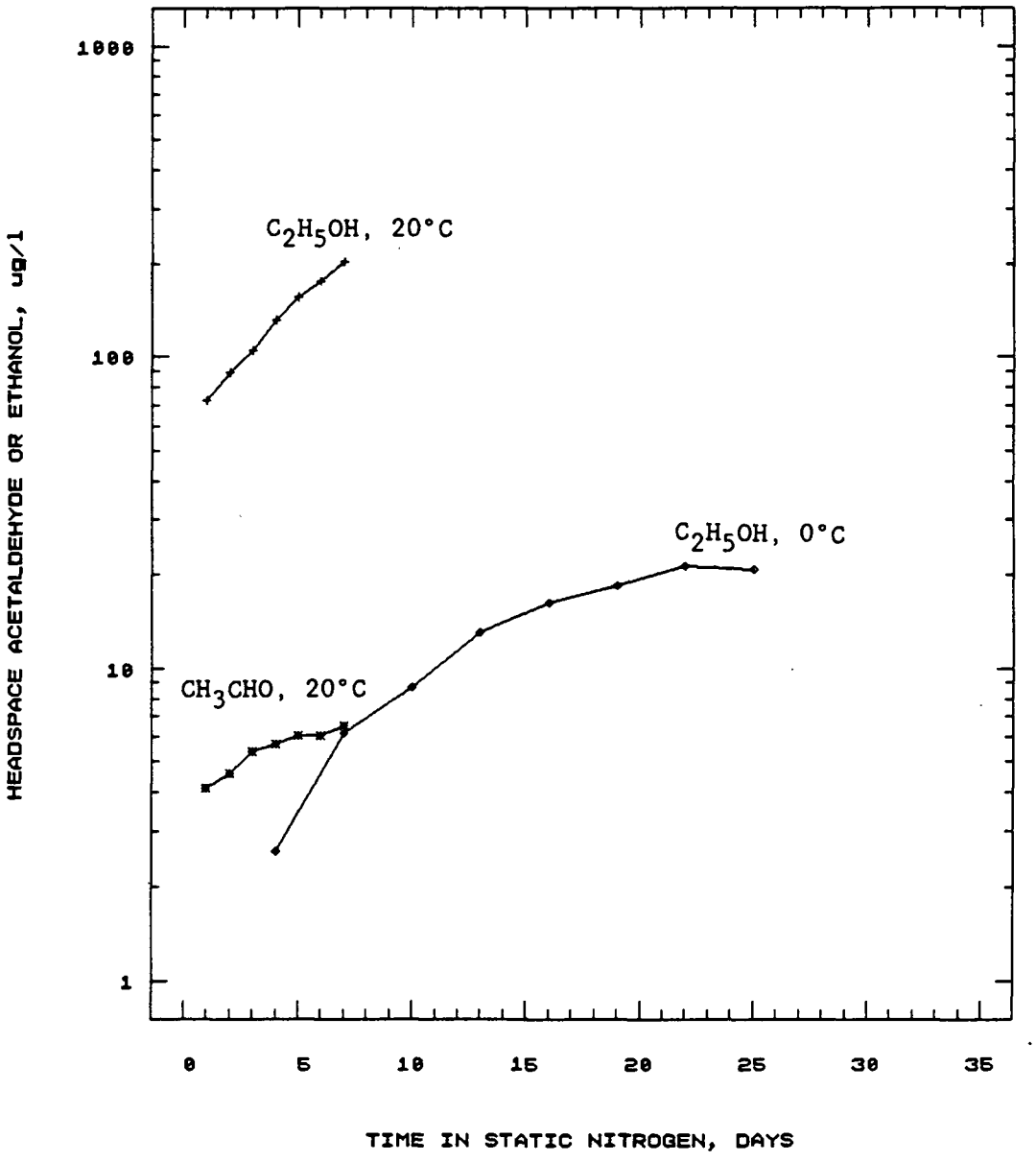

Fig. III.12. Headspace acetaldehyde and ethanol of 'Bartlett' pears under static N_2 at 0° and 20°C. Each point represents the mean of 3 observations. At 0°C, acetaldehyde was below detection limits (0.5 ug/l).

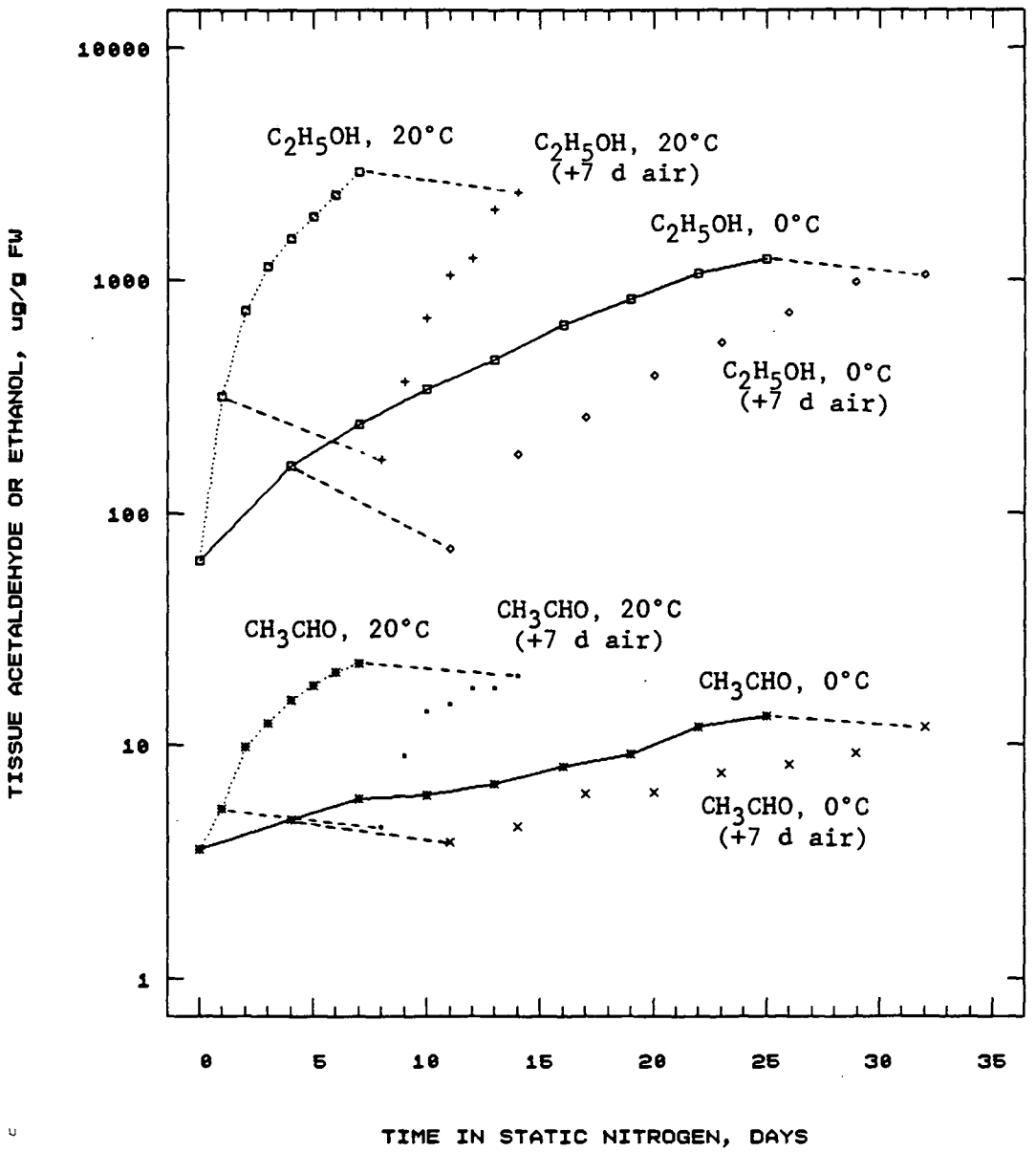

Fig. III.13. Tissue acetaldehyde and ethanol in 'Golden Delicious' apples under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

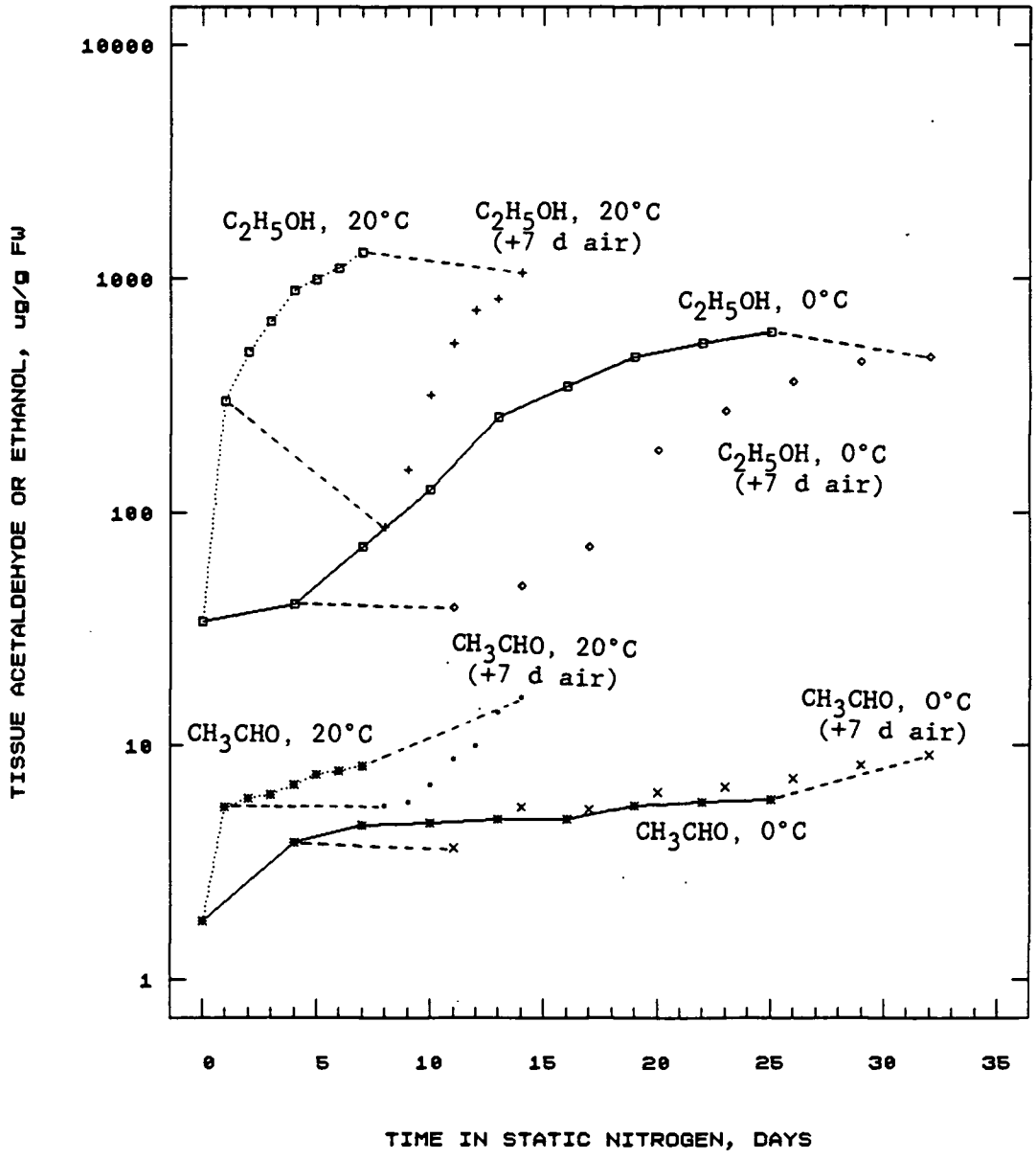

Fig. III.14. Tissue acetaldehyde and ethanol in 'Granny Smith' apples under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

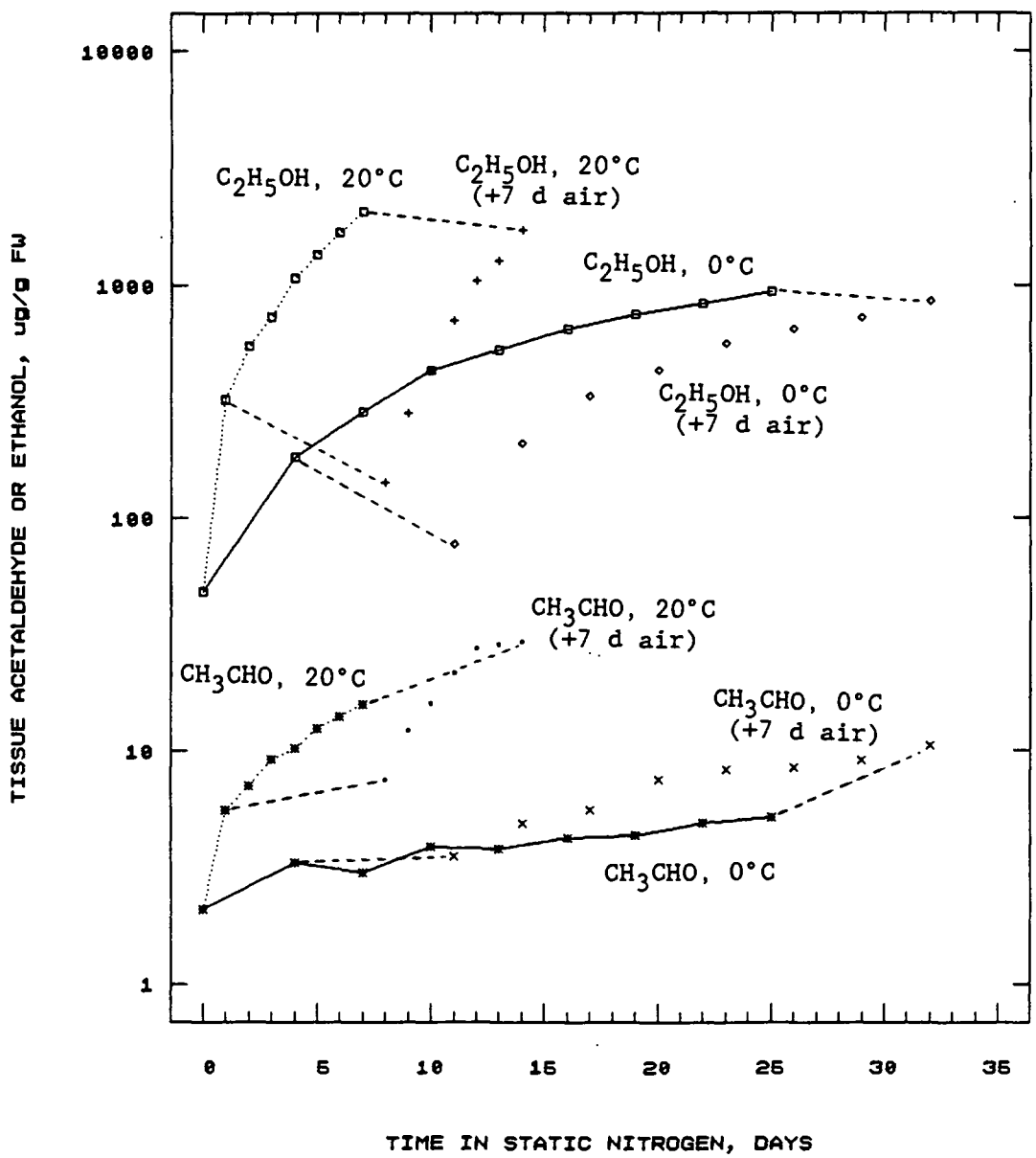

Fig. III.15. Tissue acetaldehyde and ethanol in 'Anjou' pears under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

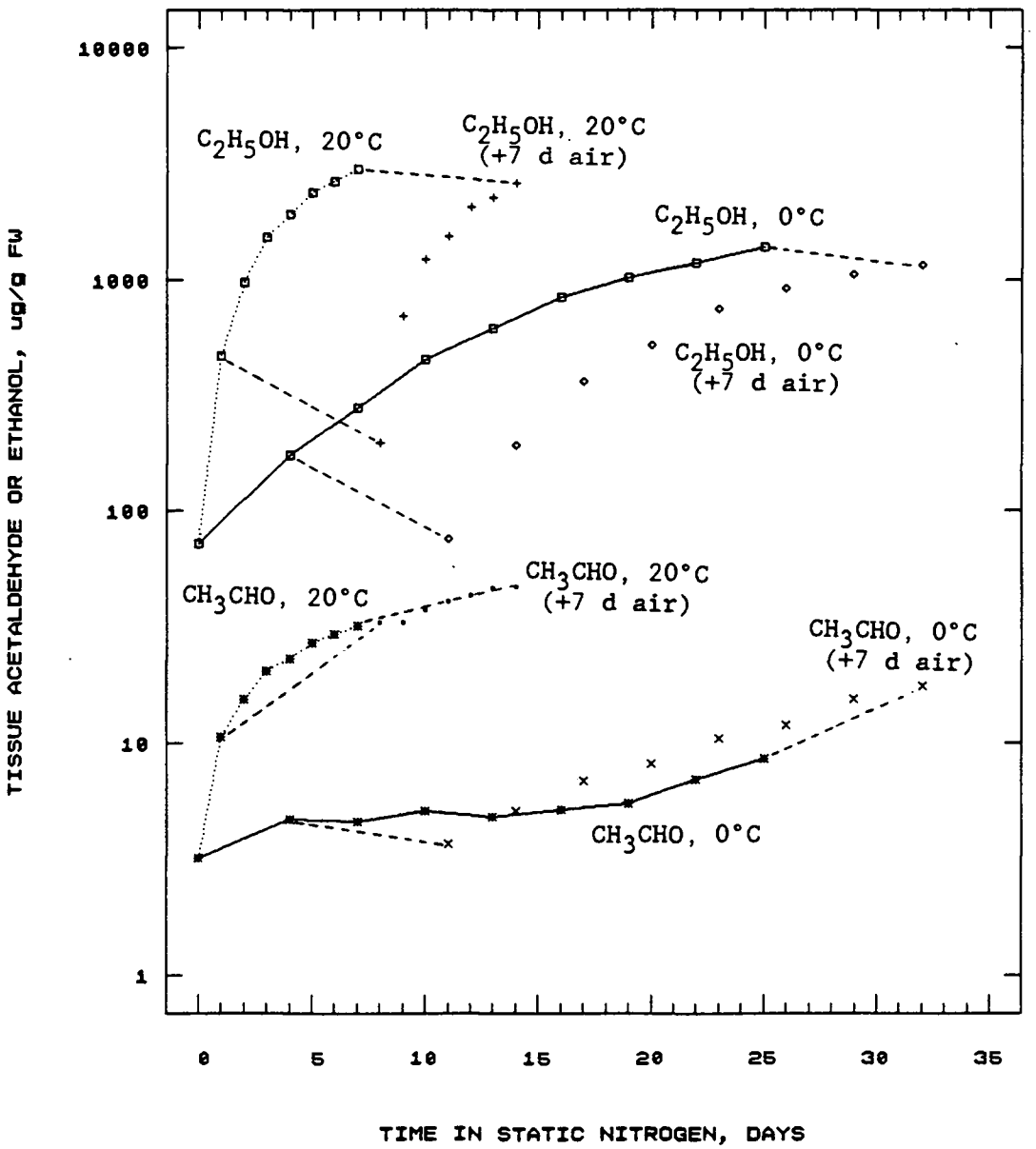

Fig. III.16. Tissue acetaldehyde and ethanol in 'Bartlett' pears under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

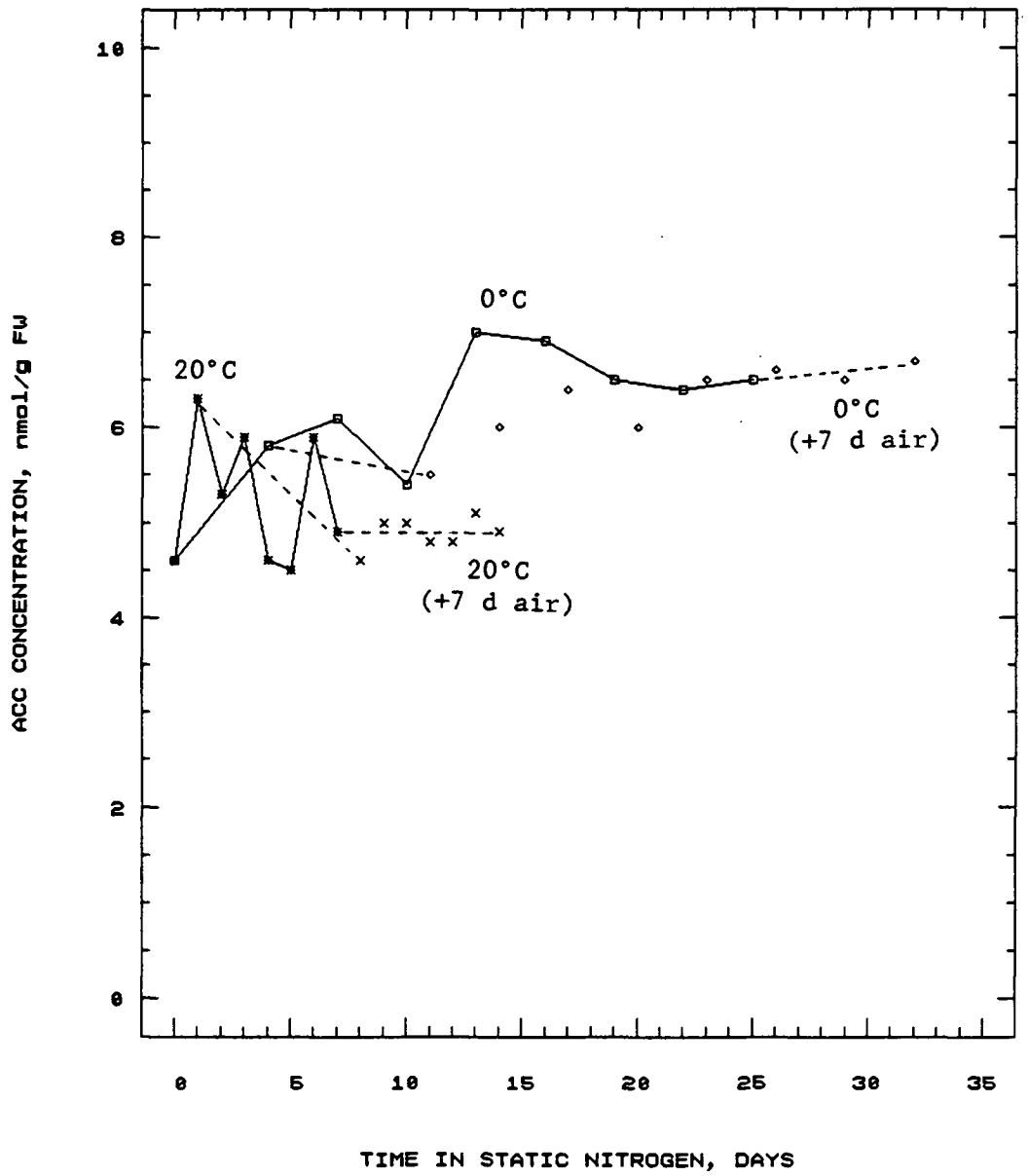

Fig. III.17. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration in 'Golden Delicious' apples under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

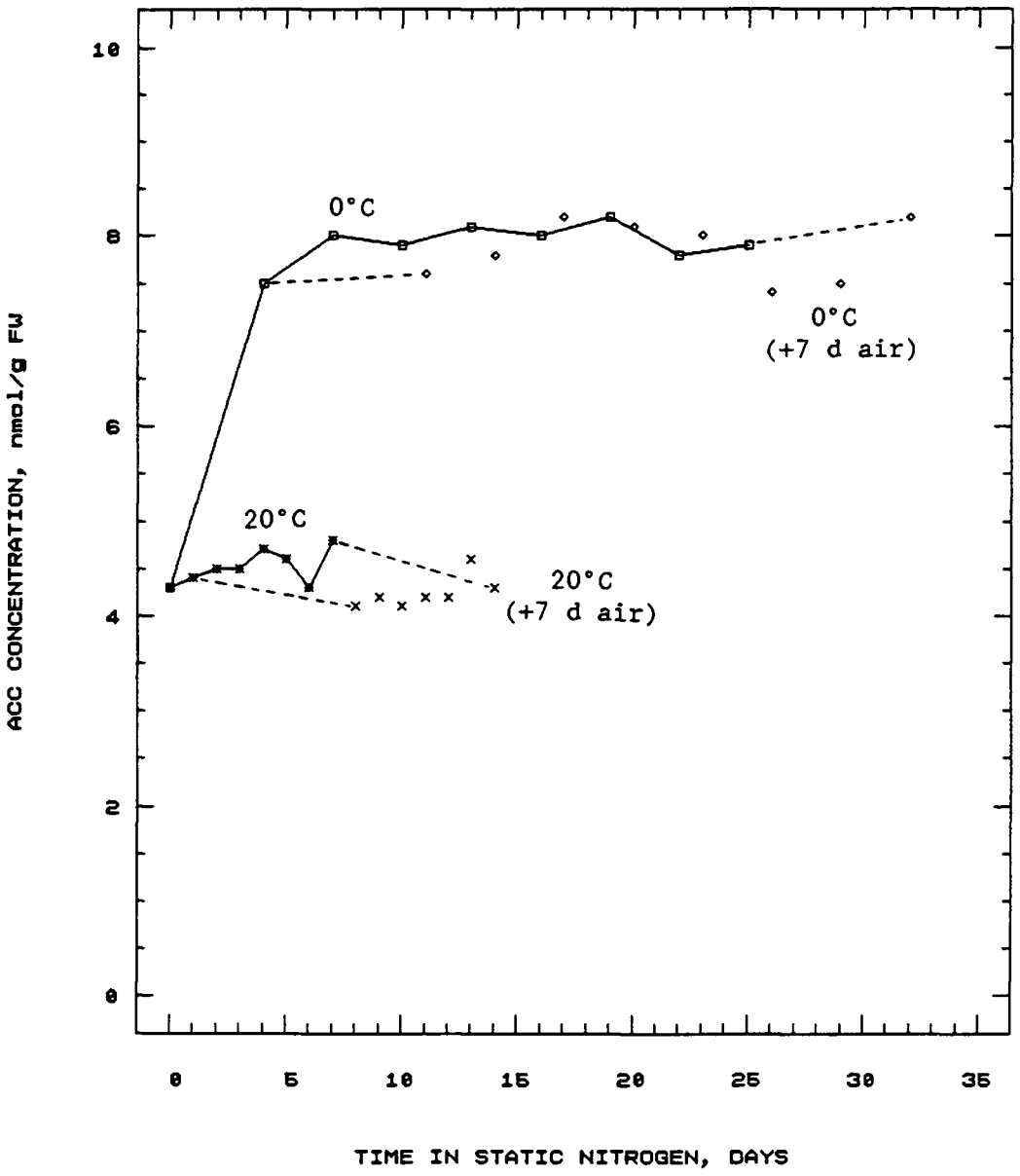

Fig. III.18. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration in 'Granny Smith' apples under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

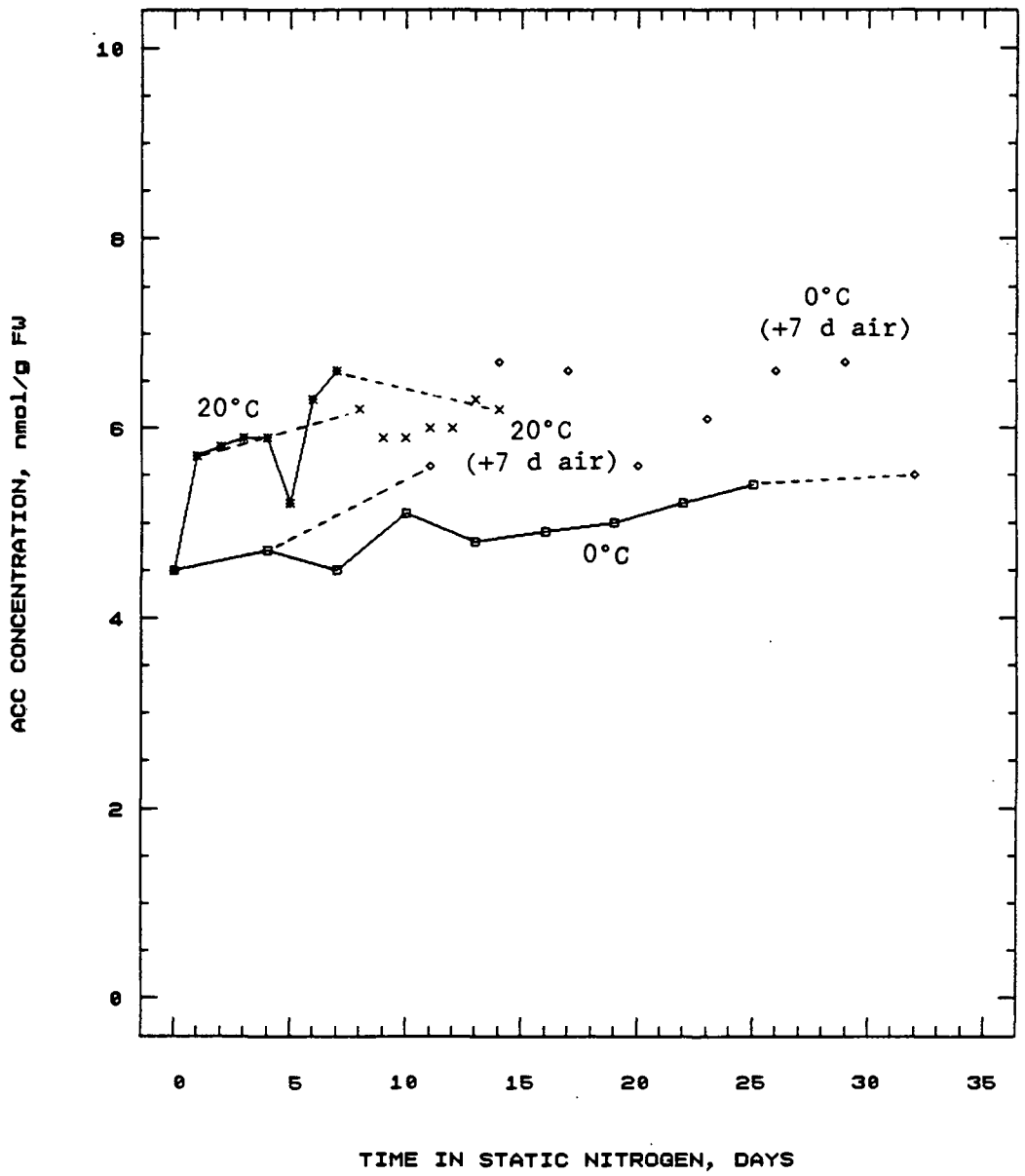

Fig. III.19. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration in 'Anjou' pears under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

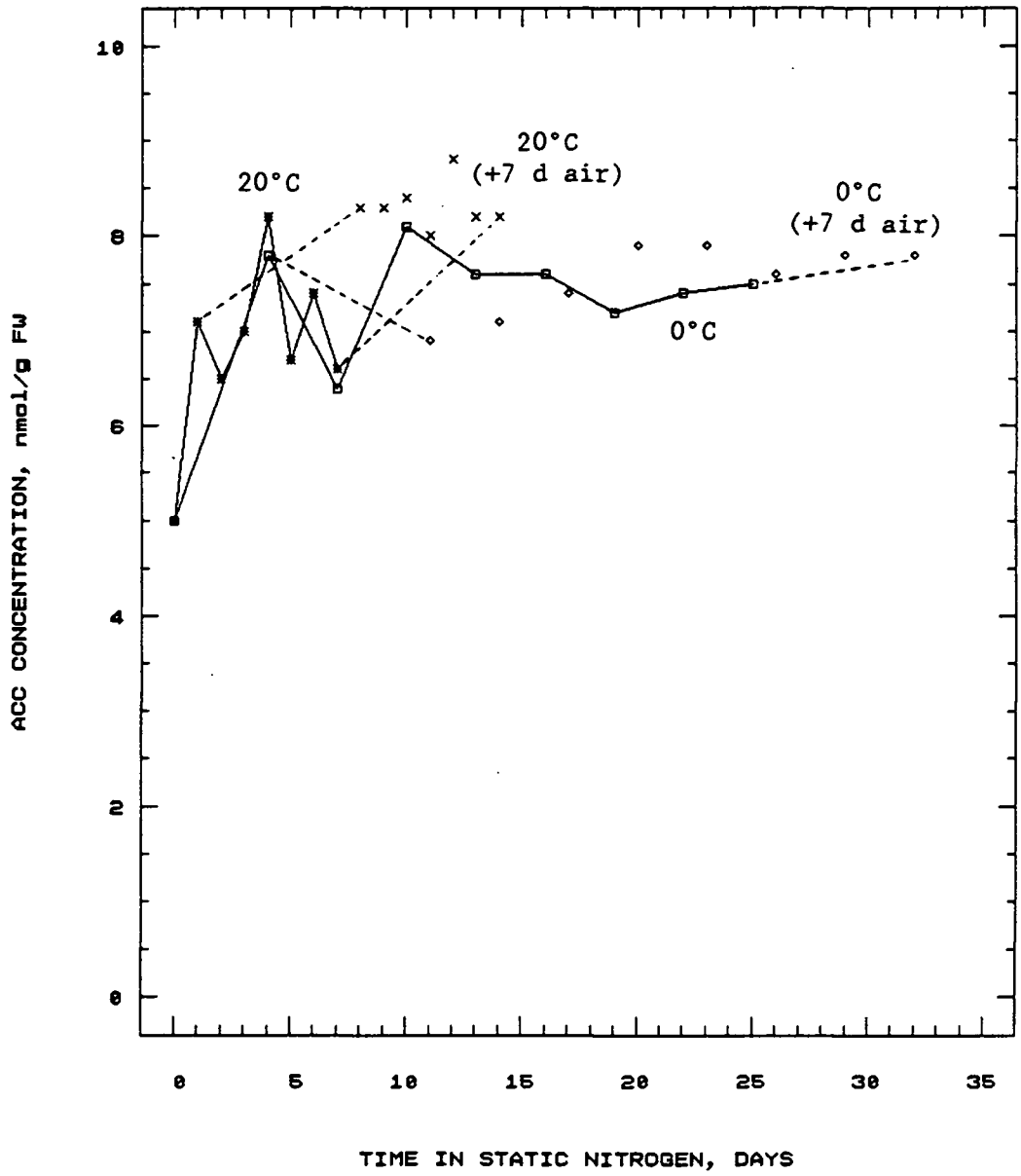

Fig. III.20. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration in 'Bartlett' pears under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

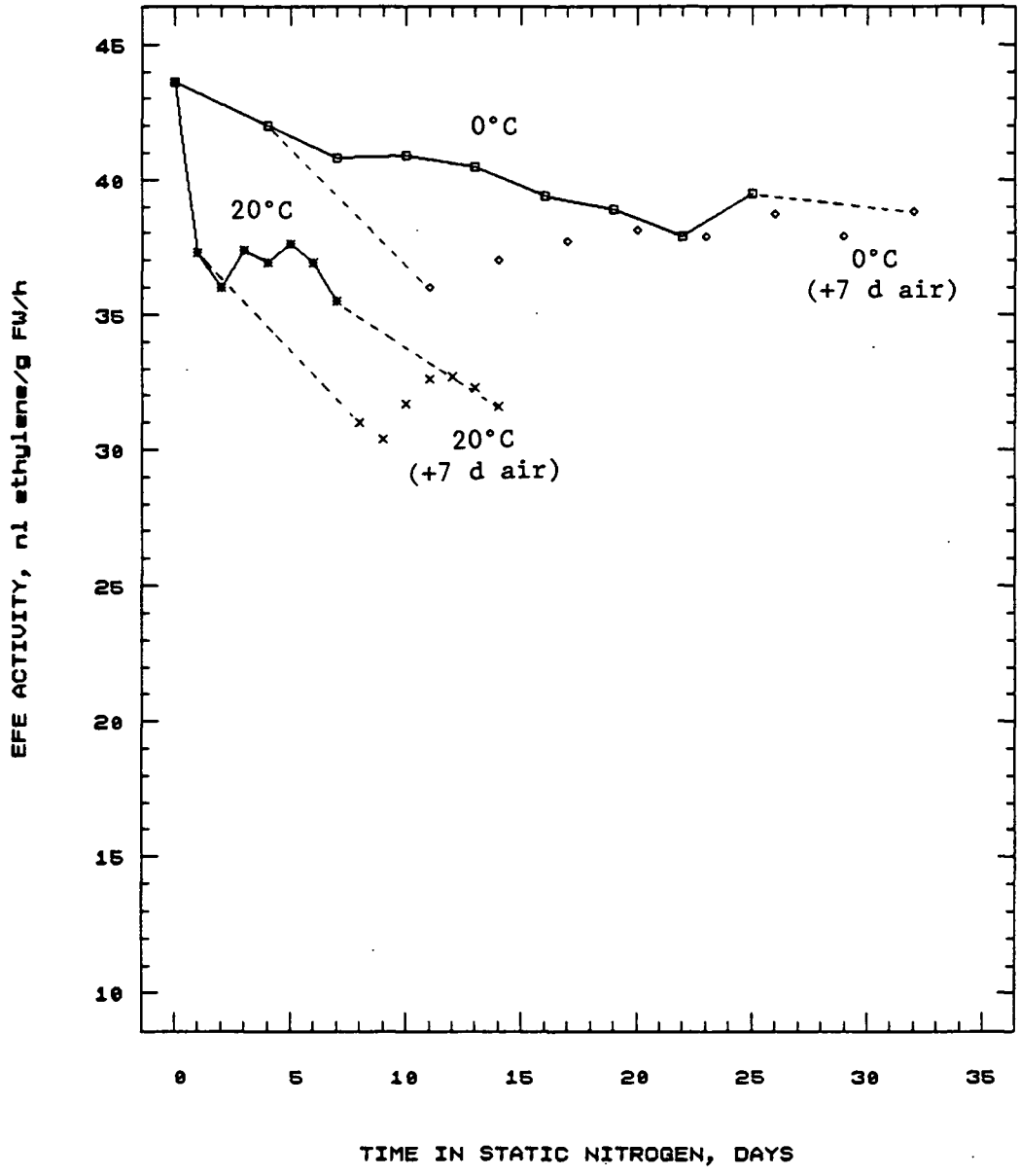

Fig. III.21. Ethylene forming enzyme (EFE) activity in 'Golden Delicious' apples under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

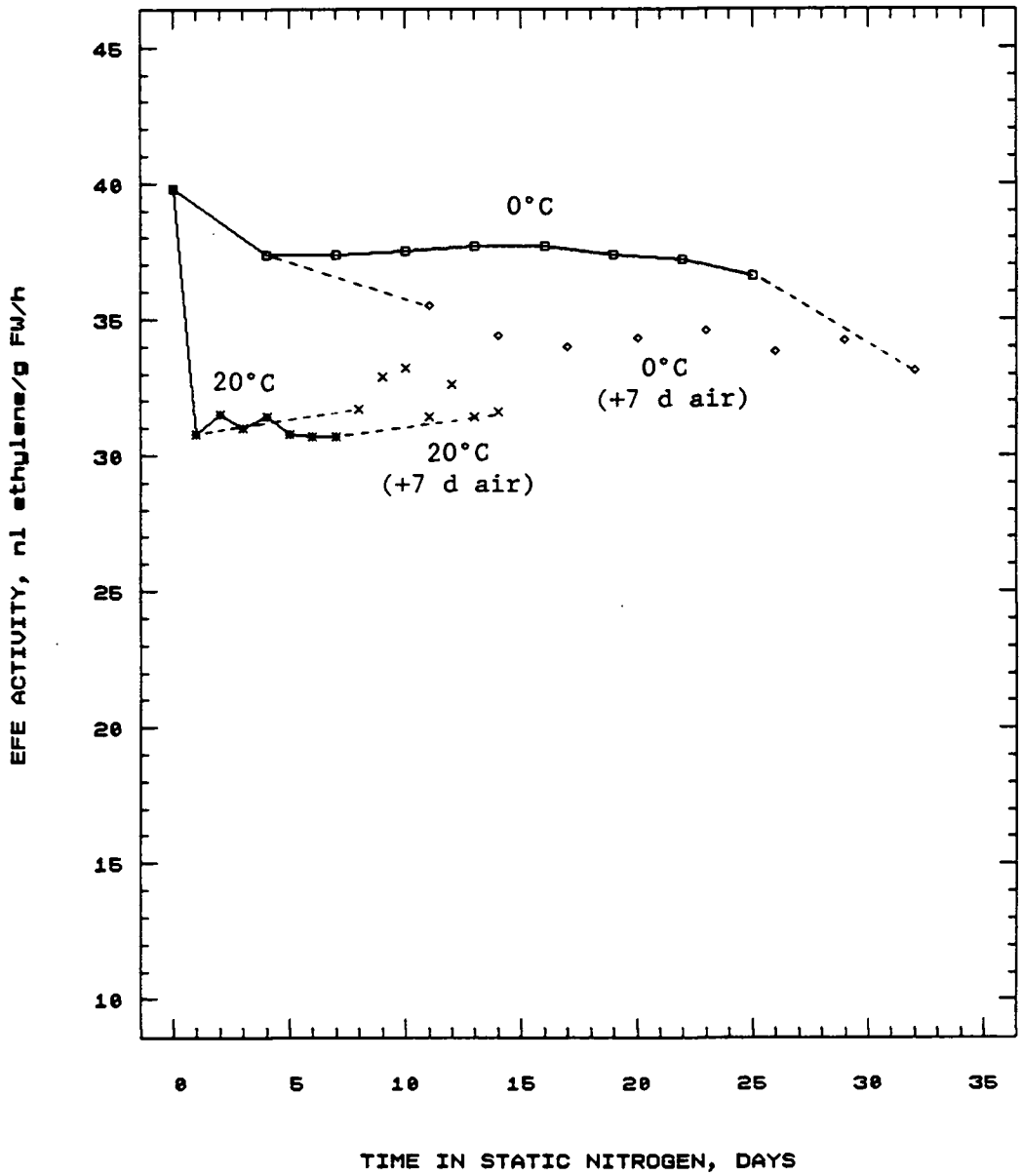

Fig. III.22. Ethylene forming enzyme (EFE) activity in 'Granny Smith' apples under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

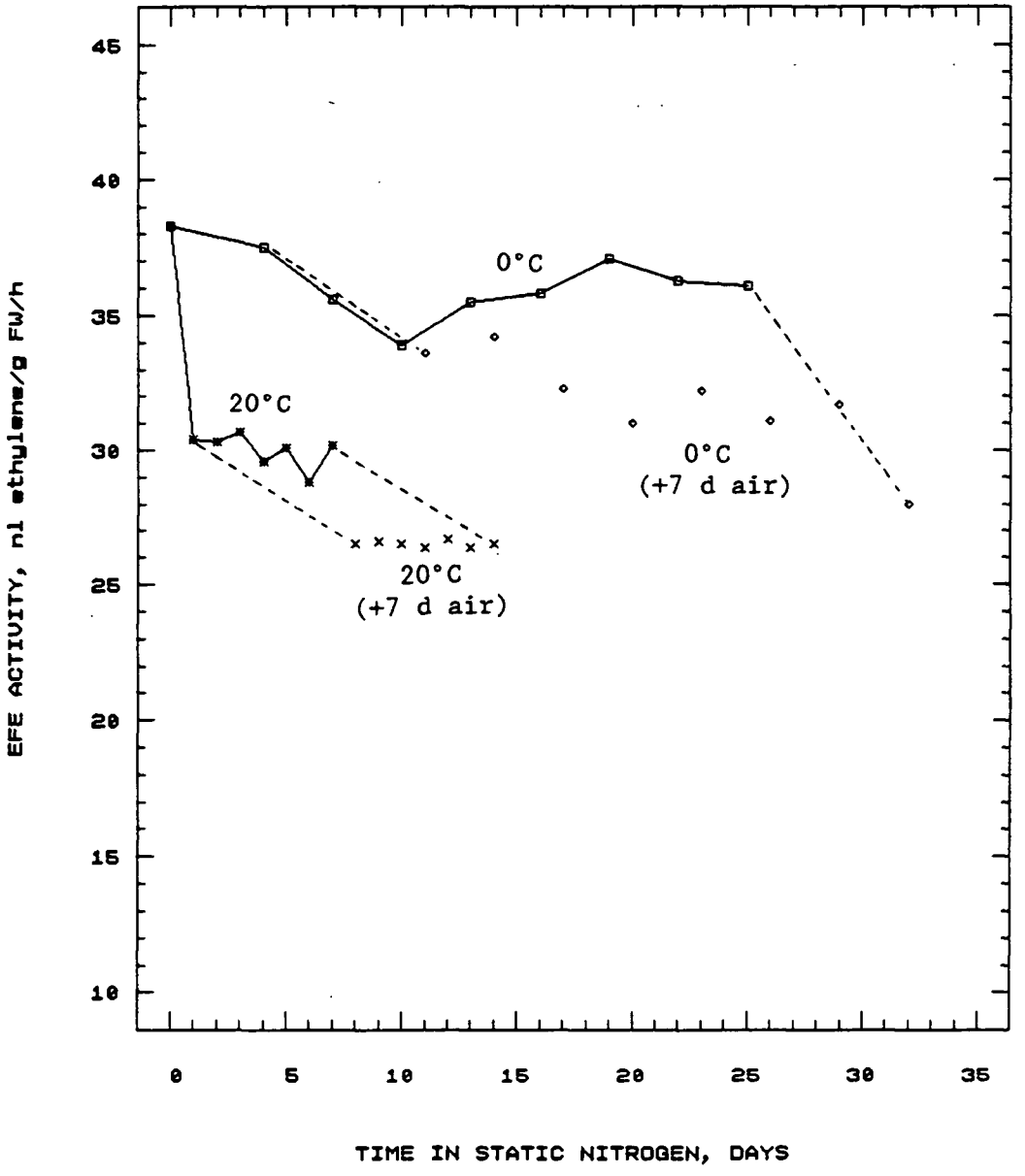

Fig. III.23. Ethylene forming enzyme (EFE) activity in 'Anjou' pears under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

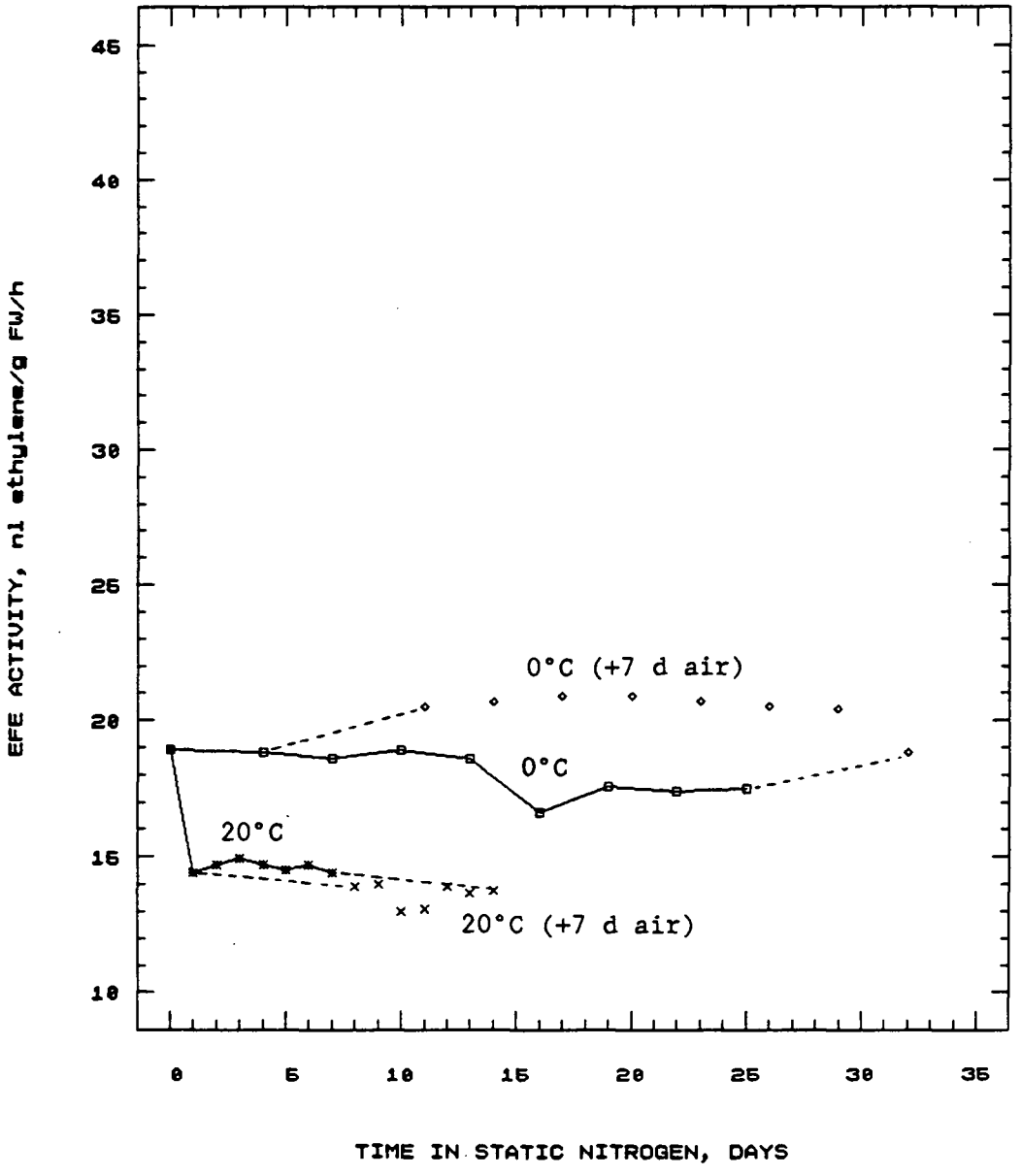

Fig. III.24. Ethylene forming enzyme (EFE) activity in 'Bartlett' pears under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 3 observations.

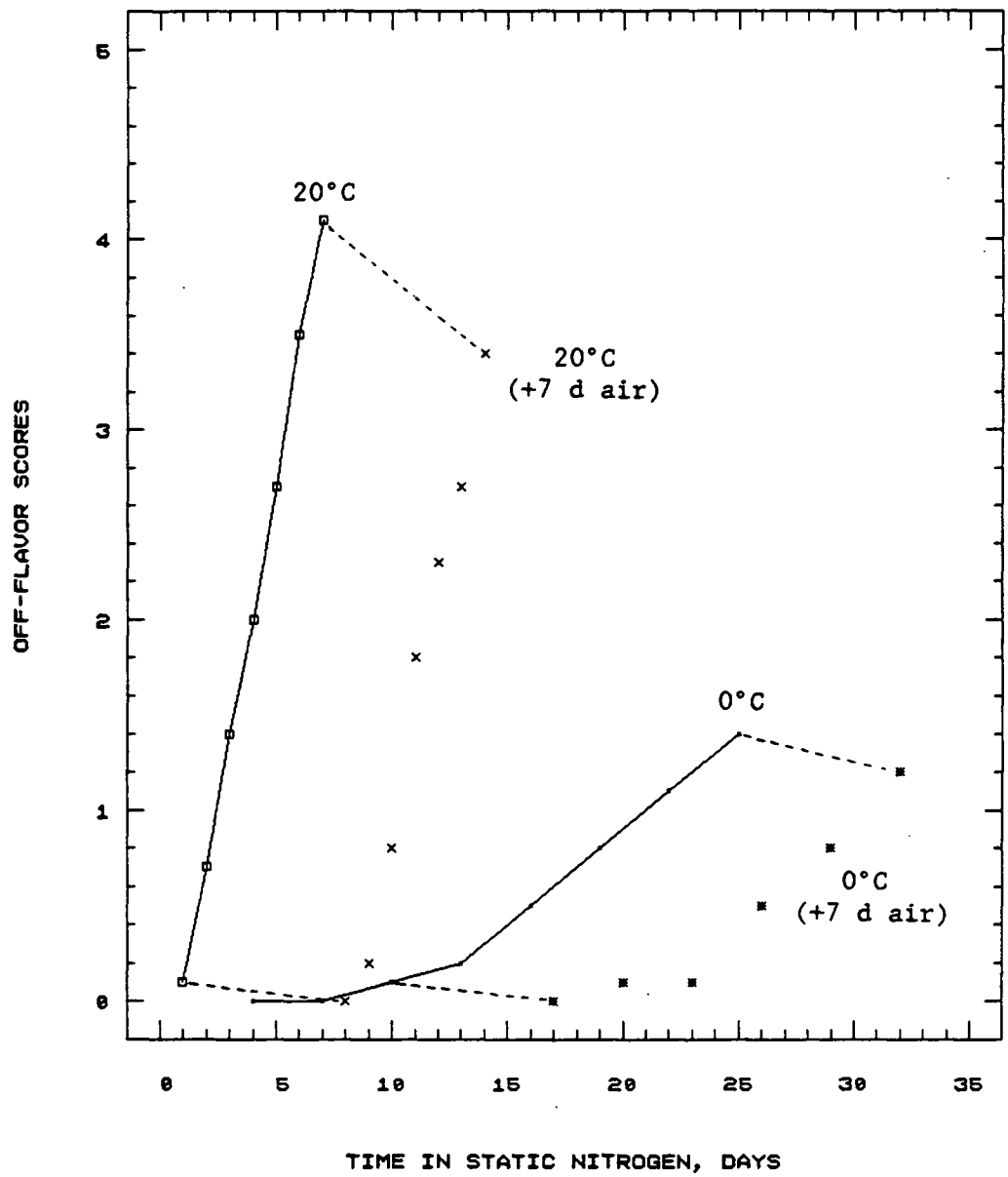

Fig. III.25. Off-flavor (scale 0 to 8, 0 = no off-flavor) development and partial reversal in 'Golden Delicious' apples under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 10 observations.

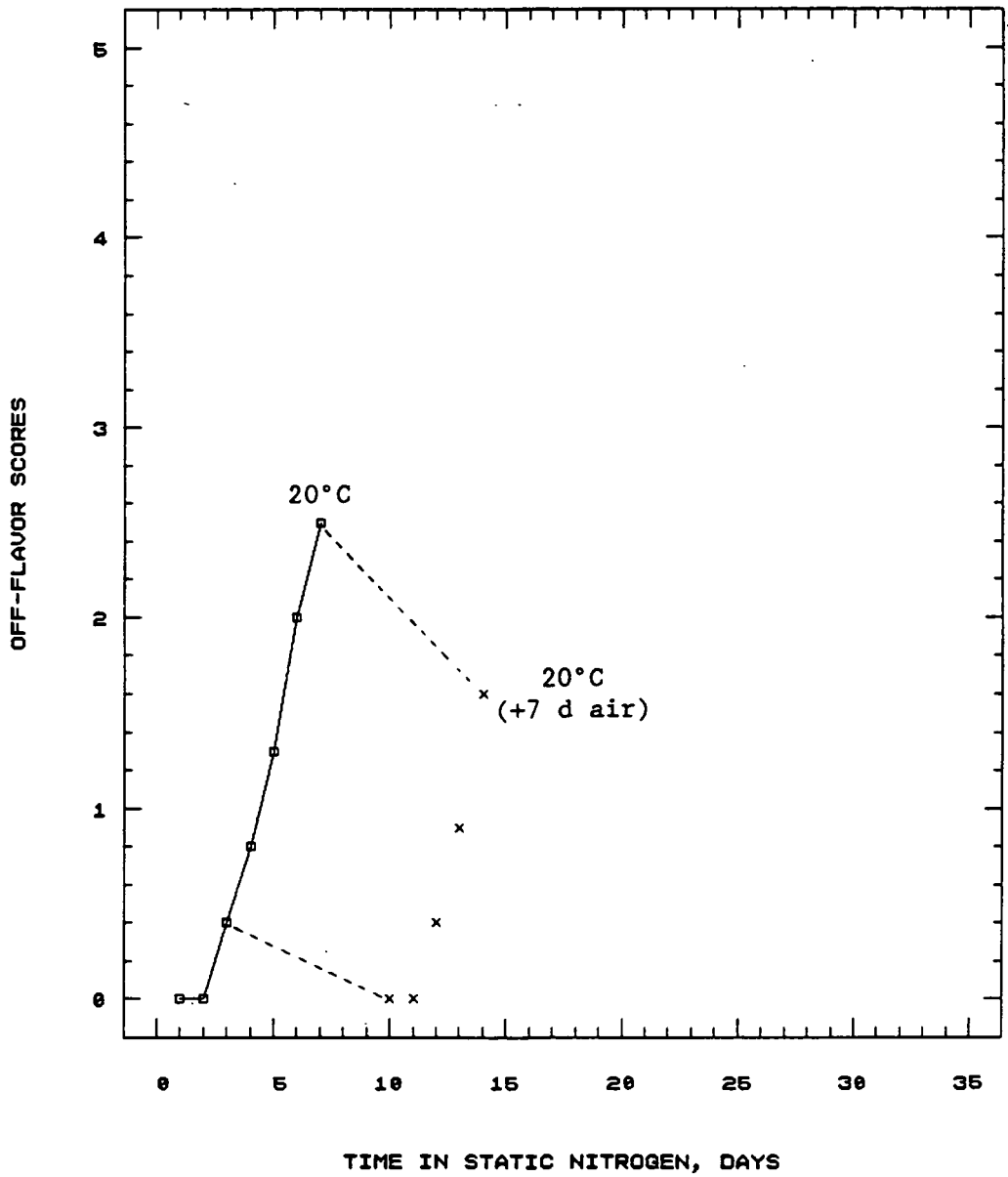

Fig. III.26. Off-flavor (scale 0 to 8, 0 = no off-flavor) development and partial reversal in 'Granny Smith' apples under static N_2 at 20°C, followed by 7 days in air. Each point represents the mean of 10 observations.

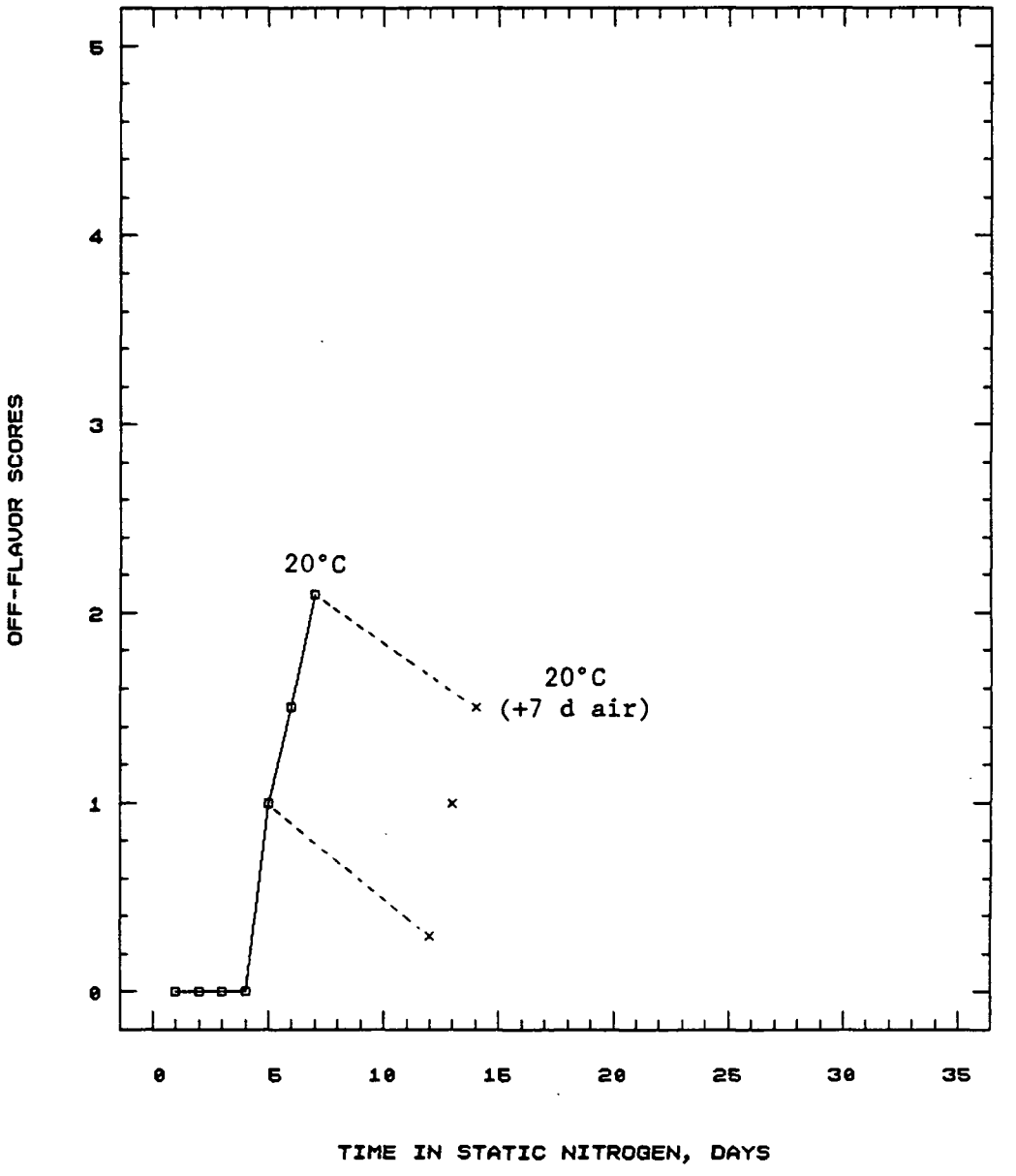

Fig. III.27. Off-flavor (scale 0 to 8, 0 = no off-flavor) development and partial reversal in 'Anjou' pears under static N_2 at 20°C, followed by 7 days in air. Each point represents the mean of 10 observations.

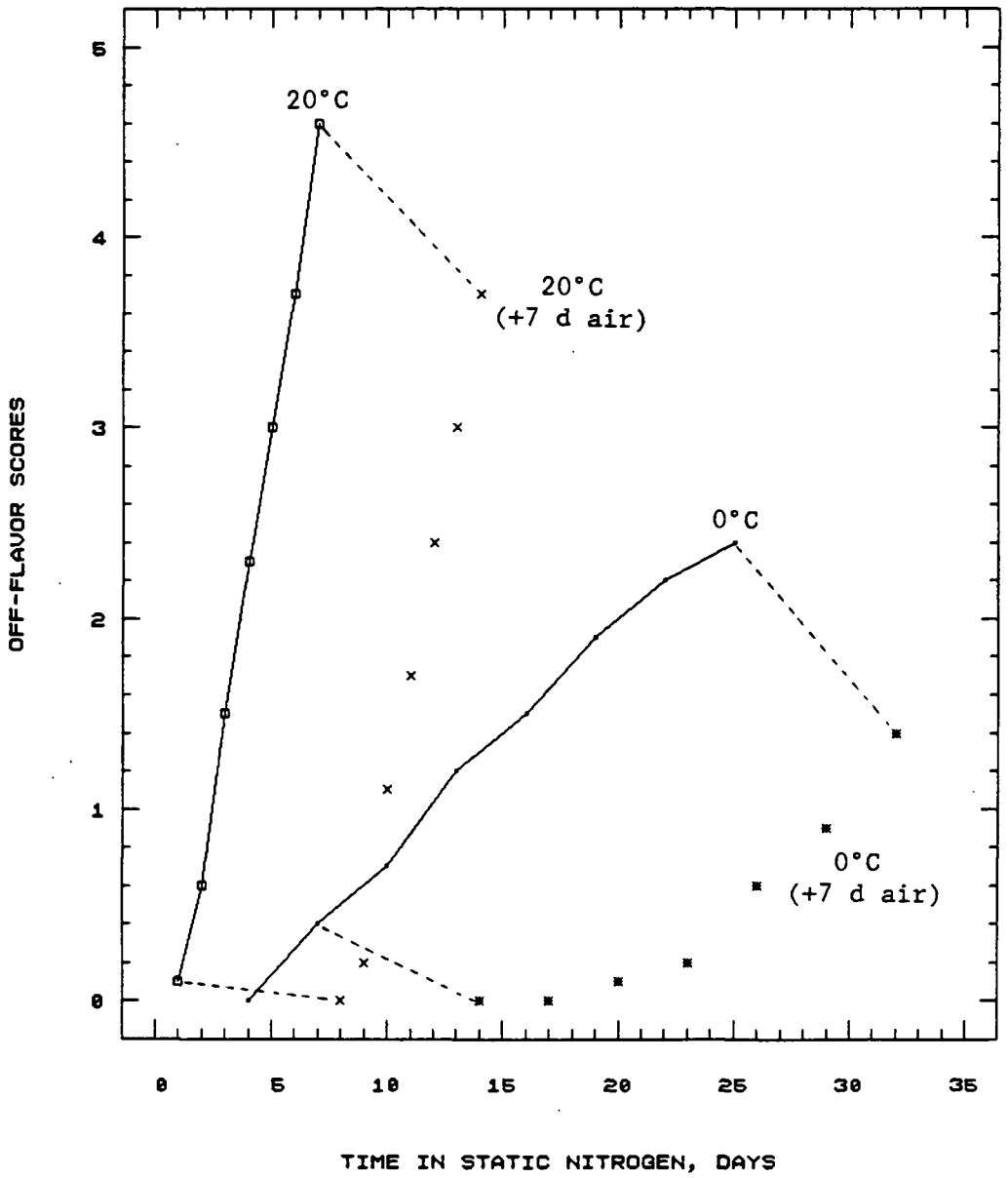

Fig. III.28. Off-flavor (scale 0 to 8, 0 = no off-flavor) development and partial reversal in 'Bartlett' pears under static N_2 at 0° and 20°C, followed by 7 days in air. Each point represents the mean of 10 observations.

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CHAPTER IV

Mortality of Eggs and Larvae of *Rhagoletis pomonella* Walsh (Diptera: Tephritidae) in 'Golden Delicious' Apples as Influenced by Storage Temperatures and Nitrogen Atmosphere

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Additional index words: apple maggot, *Malus domestica*, refrigeration, anaerobic condition, quarantine treatment.

ABSTRACT

Mature 'Golden Delicious' apples were exposed to adult apple maggots (*Rhagoletis pomonella* Walsh) in a cage at 24°C and 75% RH for 3 days. Subsequently, infested fruits were stored in air or nitrogen atmosphere at 0°C and in nitrogen atmosphere at 20°C. Mortality of the insect eggs and larvae in fruits increased with time in storage. Complete apple maggot mortality was obtained after holding infested fruits in the aforementioned conditions for 35, 24 and 7 days, respectively.

INTRODUCTION

The apple maggot (*Rhagoletis pomonella* Walsh) is native to the Northeastern United States and Canada (Joos, et al., 1984). First detected in the Pacific Northwest in the fall of 1979 near Portland, Oregon, it is now well established throughout the Willamette Valley of Oregon and in Southwestern Washington (AliNiazee, 1986). The maggot develops in fruits of apple, quince and hawthorn (Fisher and AliNiazee, 1984). Occasionally this insect pest infests cherry, plum, peach, blueberry and pear particularly when the insect population is high (Schotzko, 1982). At the moment, the apple maggot is considered a potential threat to the apple industry, but in the future it may also create political problems in exporting other fruits. A rigorous insecticide management program coupled with effective postharvest treatments is required to produce fruits free from injuries and contamination caused by apple maggot,codling moth and other insects of quarantine importance.

In the past ethylene dibromide (EDB) fumigation was effectively used as a quarantine treatment in a variety of fruits and vegetables destined for both domestic and foreign markets. In 1984 the United States Environmental Protection Agency (EPA) banned EDB from most agricultural uses due to safety concerns. Methyl bromide (MB) may be used instead of EDB. Unfortunately the use of MB is limited because of its phytotoxicity to many commodities (Harvey and Harris, 1982; Hatton and Cubbedge, 1979; O'Loughlin and Ireson, 1977). More recently attempts have been

made to replace EDB with acetaldehyde (Aharoni, et al., 1979a, 1979b, 1980; Stewart, et al., 1980) and ethyl formate (Stewart and Aharoni, 1983; Stewart and Mon, 1984) with varying degrees of success. However, both fumigants are hazardous to workers who handle these chemicals.

Approaches to quarantine treatments are discussed in detail by USDA (1984). Although gamma irradiation has been publicized by a few individuals as a safe, effective and economical treatment now ready for commercial use as a quarantine treatment (Burditt, 1982; Moy, 1977), it may cause loss of firmness, injuries and adverse changes in quality of fresh produce (Maxie, et al., 1971; Sommer and Mitchell, 1986). Most research on fresh produce has demonstrated the detrimental effects on quality, and the treatments are not very economic even if they did work. Furthermore, the perception of using irradiation on edible produce is quite negative in the mind of the public, despite widespread use of irradiation to sterilize medical equipment and supplies. Possibilities of using modified or controlled atmospheres as a quarantine treatment have also been mentioned (Gaunce, et al., 1982; Klag, 1985). Success of hot water treatments to disinfest fruit flies seems to be limited to certain produce, particularly papayas picked between colorbreak and one-quarter ripe (Couey, et al., 1984,1985; Couey and Hayes, 1986). Research in support of low temperatures as an alternative to EDB for the disinfestation of Tephritid flies keeps increasing (Adsule, et al., 1984; Back and Pemberton, 1916; Benschoter, 1983,1984; Burditt and McAlister,

1982; Burditt and Balock, 1985; Chapman, 1983; Mason and McBride, 1934). Anaerobic treatments are well established to control insect pests only in grains and stored products (Williams, et al., 1980).

The objectives of these experiments were to evaluate the potential for using low temperatures and short-term anaerobic condition as quarantine treatments based on mortality of apple maggot eggs in 'Golden Delicious' apples kept in nitrogen or in air at 0° and 20°C for various periods of time.

MATERIALS AND METHODS

Several batches of mature 'Golden Delicious' apples were exposed to adult apple maggots consisting roughly of equal numbers (25-30) of males and females in a cage held in the OSU Entomology Department incubation room (24°C and 75% RH). After 3 days of exposure to the insects, the apples were removed from the cage, and immediately used for the experiment. Groups of Four infested apples from the same batches were kept as an untreated control in the incubation room to estimate the infestation level initially present. Groups of 4 infested fruits from the same batch as the control were held in static nitrogen atmospheres in sealed 4 liter jars at 20° or 0°C and in air in perforated polyethylene film-lined cardboard cartons at 0°C. Anaerobic conditions in the jars were obtained by flushing with N_2 at a flow rate of 1.5 l/min for 25 min (until O_2 concentration as verified by a gas chromatographic method described earlier in Chapter III, was less than 0.05%). A sample of 4 fruits was removed from the treatments daily, or at 3- or 7-day intervals for the above respective treatments, and placed in the incubation room.

Survival was based on numbers of pupae recovered from treated fruits as compared with the controls. Percent mortality was calculated by subtracting percent survival from 100. Analysis of variance for the data was conducted only after the angular or arcsine transformation of the raw data from percentages to degrees according to the method outlined by Steel and Torrie (1960). Mean comparisons were made using the transformed data. However, in

presenting the results the means were transformed back to the original scale. Completely randomized design (CRD) and Duncan's multiple range test (DMRT) for mean comparisons were employed in the statistical analyses.

RESULTS

The experiments were done six different times, and the mean values of recovered pupae ranged from 40 to 56, and averaged 48. Each time the experiment was done, the mean value of the control for that experiment was used to determine mortality resulting from treatments.

At 0°C (Fig. IV.1a), mortality of eggs and larvae of *Rhagoletis pomonella* in infested 'Golden Delicious'apples kept in air increased with time in storage from 14.8% on day 7 to 100% on day 35. In nitrogen atmospheres, no significant differences in mortality percentages were found after 3, 6, 9 and 12 days in storage. The values were in the range of 15.4 to 29.4%. Thereafter, mortality significantly increased to 54.3% after 15 days in 0°C storage. Complete mortality of the insect was obtained after holding infested apples in nitrogen atmospheres at 0°C for 24 days. After 25 days in the same condition, only 'Granny Smith' apples and 'Anjou' pears did not develop detectable off-flavors (Fig. IV.1b).

At 20°C (Fig. IV.2a), mortality of the insect eggs and larvae in infested 'Golden Delicious' apples kept in nitrogen atmospheres increased steadily and significantly from 9.6% on day 3 to 100% on day 7. However, all apples and pears held in the same condition for 7 days developed off-flavors (Fig. IV.2b).

DISCUSSION

The findings in air at 0°C was in conformity with those of Chapman (1933), who attributed insect death to a condition of forced dormancy, or a dormancy imposed on the insect whose metabolism may not be adjusted to this extreme condition for any extended period. Insects may fail to emerge when maintained at certain constant temperatures, but these temperatures would not be limiting in the field, where diurnal fluctuations about the mean would permit the act of hatching at favorable times of the day (Bursell, 1974). The infested apples used in these experiments may contain a mixture of eggs and larvae of apple maggot. There can be differences in responses of various developmental stages of the insect to low temperatures. At least in the Mediterranean fruit fly, eggs are more susceptible to refrigeration than larvae (Back and Pemberton, 1916; Couey et al., 1984). Besides varied resistance of the host to larval establishment depending on the variety of apple, variation may be found in the mortality of the apple maggot in different samples of the same variety (Chapman, 1933). To be on the safe side, 45 days of storage in air at 0°C has been recommended as quarantine treatment for controlling the apple maggot in California.

Anaerobic conditions created by a nitrogen atmosphere were lethal to the larvae of Caribbean fruit flies maintained on an artificial diet (Benschoter, et al., 1981). Mortality increased with time of exposure (Benschoter, 1987). Complete mortality of apple maggot eggs in fruits kept in nitrogen atmospheres at 20°C

was obtained much more rapidly than at 0°C (Fig.IV.1). This agrees with the results of Soderstrom, et al. (1986), who found that the higher the temperature, the less time required to kill stored-product moths. One possibility is that acetaldehyde, which accumulated in fruit tissue under anaerobic conditions was toxic to the insect eggs since it has been reported that acetaldehyde inhibited energy production of mitochondria of rat liver (Cederbaum, et al., 1974).

In commercial quarantine procedures, the security level is often based on a survival rate of $\leq$32 in a population of 1,000,000. This represents 99.9968% mortality, which is equivalent to probit 9 (Baker, 1939). The minimum number of insects necessary to conduct this kind of experiment is 93,613 (Couey and Chew, 1986). It was not possible to test that many insects.

Feasibility of any quarantine procedure depends not only on its performance from the standpoint of pest mortality, but also upon non-detrimental effects to the physiology and quality of the commodity. Special attention must be given to fresh produce which may exhibit physical injury or physiological disorders under conditions used to disinfest insects of quarantine importance. Anaerobic conditions at 0°C would be feasible for 'Granny Smith' apples and 'Anjou' pears, but would be questionable for 'Golden Delicious' apples and 'Bartlett' pears. If time is not a matter of concern, refrigerated storage at 0°C remains an effective and

safe quarantine treatment against apple maggot eggs or larvae. Forty-five days in air at 0°C is a common practice in California.

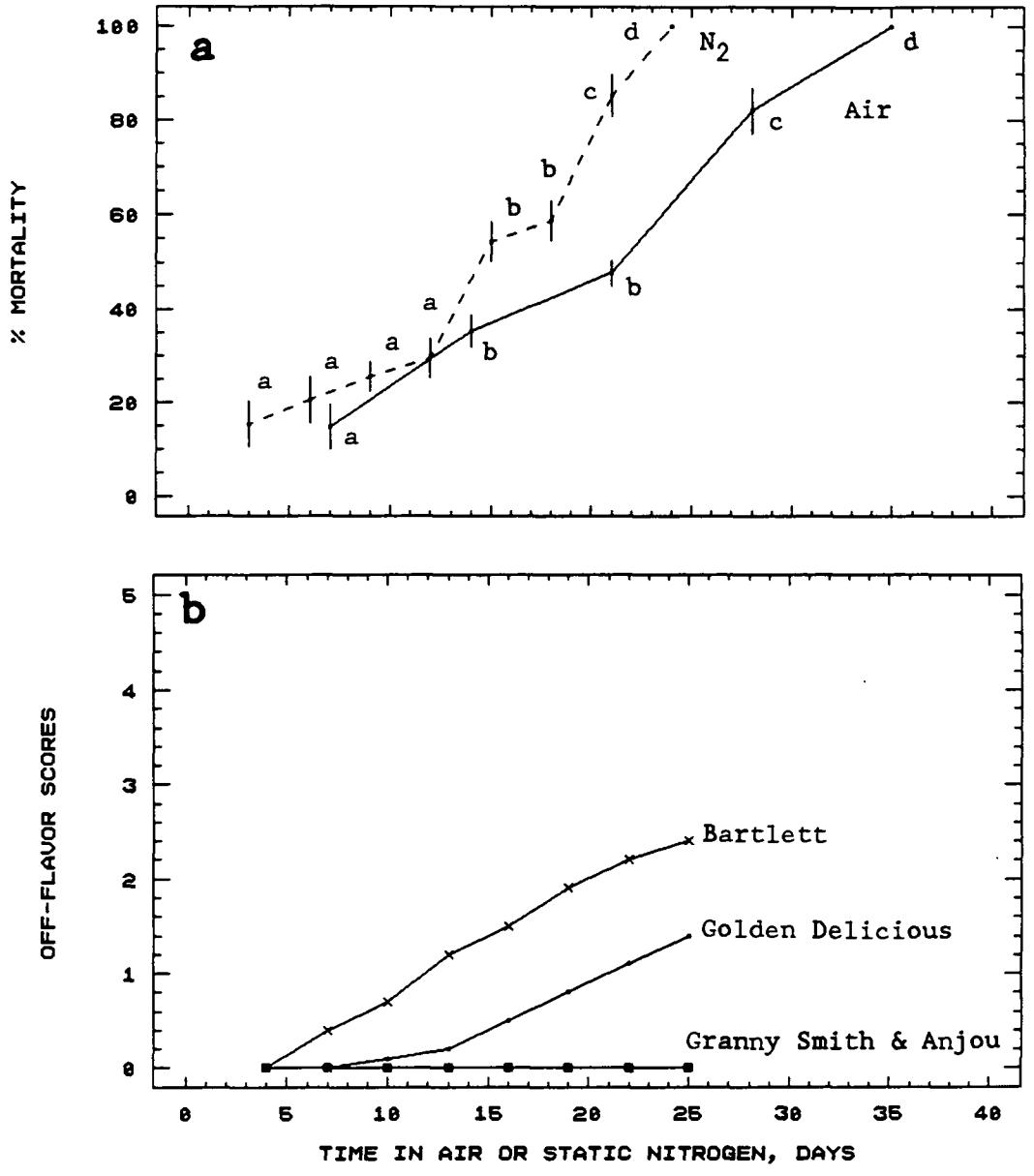

Fig. IV.1 Mortality of apple maggot (*Rhagoletis pomonella*) eggs and larvae in 'Golden Delicious' apples kept in air or static N_2 at 0°C (a), and off-flavor (scale 0 to 8, 0 = no off-flavor) development in 'Golden Delicious' and 'Granny Smith' apples, and 'Anjou' and 'Bartlett' pears held in the same condition (b). Mean separation for the insect mortality by Duncan's multiple range test, 5% level. Vertical bars represent S.E. of the mean (n = 4). Each point of off-flavor scores represents the mean of 10 observations.

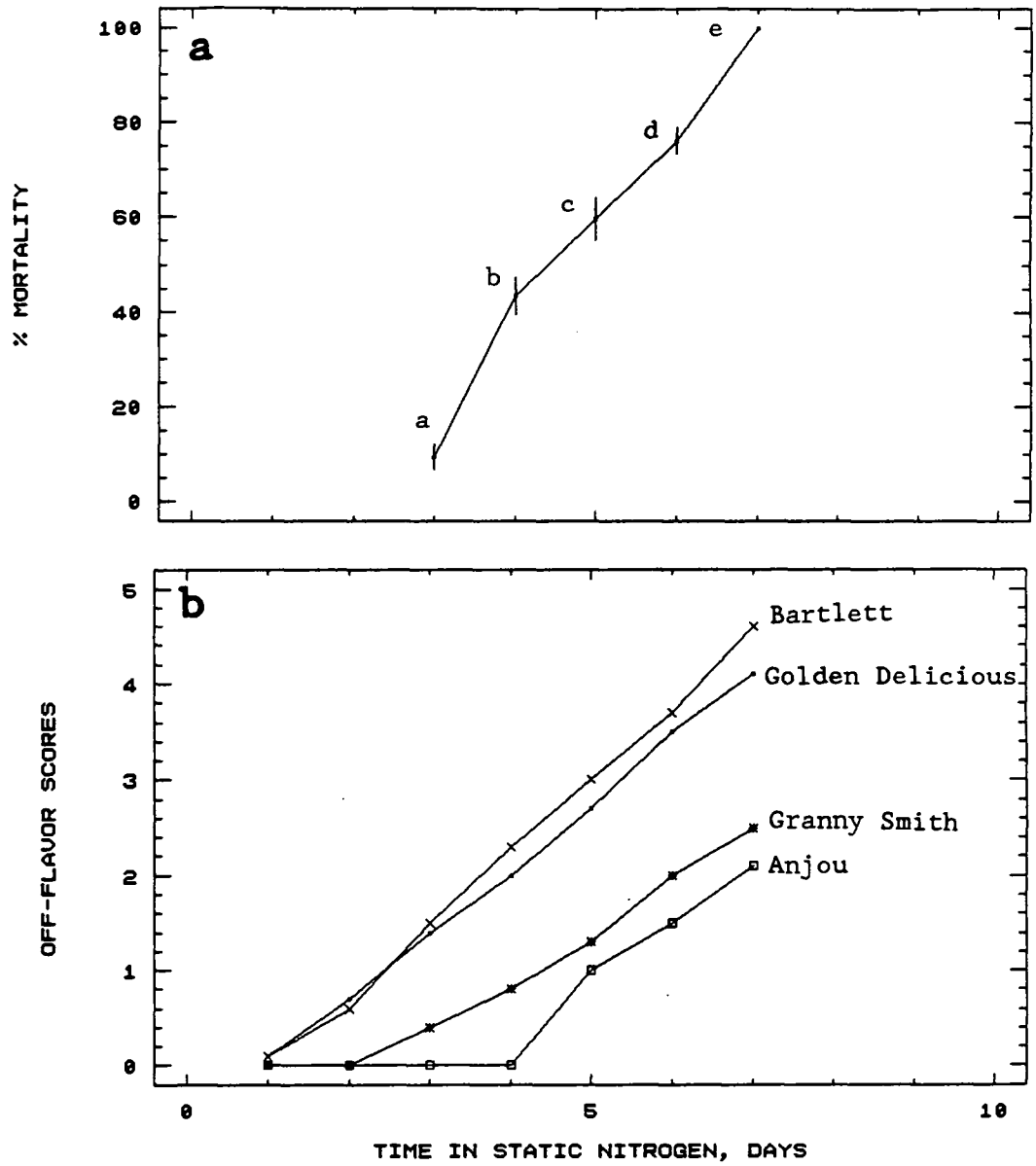

Fig. IV.2. Mortality of apple maggot (*Rhagoletis pomonella*) eggs and larvae in 'Golden Delicious' apples kept in static N_2 at 20°C (a), and off-flavor (scale 0 to 8, 0 = no off-flavor) development in 'Golden Delicious' and 'Granny Smith' apples, and 'Anjou' and 'Bartlett' pears held in the same condition (b). Mean separation for the insect mortality by Duncan's multiple range test, 5% level. Vertical bars represent S.E. of the mean (n = 4). Each point of off-flavor scores represents the mean of 10 observations.

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CHAPTER V

Effect of Short-Term Exposure to Controlled Atmospheres on Physiology and Flavor of Apples and Pears

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Additional index words: *Malus domestica*, *Pyrus communis*, acetaldehyde, ethanol, pyruvate decarboxylase, alcohol dehydrogenase, 1-aminocyclopropane-1-carboxylic acid, ethylene, ethylene forming enzyme, off-flavors

ABSTRACT

The responses of 'Granny Smith' apples and 'Anjou' pears to storage at 0°C in flowing streams (10 ml/ min) of O_2, CO_2 and N_2 mixed to generate atmospheres of 0.3, 0.5, 0.7, 1.4, and 2.8% O_2 plus 1.4% CO_2 were investigated. Low-O_2 atmospheres delayed and suppressed ethylene production of apples and pears. Headspace ethanol was detectable only in fruits held in 0.3% O_2. Fruits kept in controlled atmospheres (CA) for 24 days had higher pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities, tissue acetaldehyde and ethanol, and 1-aminocyclopropane-1-carboxylic acid (ACC) concentration. Generally, the lower the O_2 level in storage atmospheres, the higher the values of the mentioned parameters. The reverse was true for ethylene forming enzyme (EFE) activity. Exposure to air for 7 days did not result in appreciable changes in any of the parameters studied. No external or internal symptoms of physiological disorders were observed during 24 days in CA storage

and 7 days in air. Off-flavor development did not occur in CA-stored fruits, even in the lowest (0.3%) oxygen treatments for these short exposures.

INTRODUCTION

Controlled atmosphere (CA) storage has long been successfully used to extend postharvest life, and maintain quality of certain horticultural commodities, especially apples and pears. However, its potential application as an alternative to chemical fumigation to control insects of quarantine importance has not been thoroughly investigated. Storage in low-O_2 (1.0 to 1.5%) atmospheres with and without CO_2 (0 to 2.0%) resulted in better retention of flesh firmness and titratable acidity in apples (Chen, et al., 1985; Lau, 1983; Lau, 1985; Lidster, et al., 1981; Lidster, et al., 1983; Smith, 1984), and better dessert quality and suppression of superficial scald and stem-end decay in pears (Chen, et al.,1981; Hansen, 1957; Mellenthin, et al., 1980). Use of CA as a quarantine treatment against insects in grains and stored products has been also well documented (Soderstrom and Brandl, 1984; Storey, 1977; Storey and Soderstrom, 1977). Low oxygen concentration was more important in reducing the time to kill insects than CO_2 concentration (Brandl, et al., 1983). Low-O_2 ($\leq$1.5%) storage may offer a means to retain fruit quality and yet act as a biocide for insects on the fruit (Gaunce, et al., 1982). Exposure of fresh produce to high CO_2 or low O_2 beyond tolerance limits may increase anaerobic respiration and the consequent accumulation of acetaldehyde and ethanol, causing off-flavors (Kader, et al., 1989). However, aeration following storage in such conditions diminished the undesired flavor (Weichmann, 1986).

The purposes of this experiment were to evaluate responses of 'Granny Smith' apples and 'Anjou' pears to short-term exposure to controlled atmospheres, some of which may have potential as insecticidal treatments.

MATERIALS AND METHODS

'Granny Smith' apples and 'Anjou' pears harvested at commercial maturity were purchased from Naumes Inc., Medford, Oregon. Fruits were kept in cardboard cartons lined with perforated polyethylene film, and placed at 0° (apples) and -1°C (pears), for about 3 - 4 weeks before being used in this experiment. About 1 kg (8 fruits) of apples or pears were placed in 4 liter jars, and stored at 0°C for up to 24 days in continuous 10 ml/min flow of 0.3, 0.5, 0.7, 1.4 and 2.8% O_2 combined with 1.4% CO_2. CO_2, N_2 and O_2; headspace ethylene, acetaldehyde and ethanol were measured at 3-day intervals. Each value is the mean of 2 replications (jars). On day 24, 4 individual fruits from each treatment (2 fruits per replication) were analyzed for pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities, tissue acetaldehyde and ethanol, ethylene forming enzyme (EFE) activity, 1-aminocyclopropane-1-carboxylic acid (ACC) concentration and off-flavor development immediately after removing treated fruits from CA storage. The remaining fruits were subject to the same analyses after holding in air for 7 days. All methods are described in detail in Chapter III.

RESULTS

'Granny Smith' Apples

Evolved ethylene concentration of 'Granny Smith' apples in various combinations of low O_2 and 1.4% CO_2 at 0°C decreased with decreasing O_2 levels (Fig. V.1). Ethylene became detectable in lower O_2 atmospheres later than in higher O_2 atmospheres. On day 24, ethylene concentrations in 0.3, 0.5, 0.7, 1.4 and 2.8% O_2 were 2.6, 2.7, 3.9, 5.0 and 5.7 ppm, respectively. Headspace ethanol was detected only in 0.3% O_2 (Fig. V.2). It increased steadily with time in storage from 2 ug/l on day 3 to 7 ug/l on day 24.

After 24 days in 0°C storage, apples in 0.3% O_2 showed highest PDC activity (0.23 units), which was significantly different from the values of apples in other atmospheres (Fig. V.3). PDC activity increased when O_2 levels were $\leq$1.4%. ADH activity of CA-stored apples was significantly greater than that of air-stored apples when O_2 levels were decreased from 1.4 to 0.3% (Fig. V.4). The ADH values in apples under 1.4, 0.7, 0.5 and 0.3% O_2 were 0.27, 0.37, 0.44 and 0.51 units, respectively. When fruits were removed from CA storage and subsequently held in air at 0°C for 7 days, both PDC and ADH activities remained unchanged (Appendix Fig. B.1 - B.2)

As O_2 levels decreased below 1.4%, acetaldehyde concentration in fruit tissue increased significantly (Fig. V.5). After 24 days in storage, the highest level of 6 ug acetaldehyde/g fresh wt was found in apples held in 0.3% O_2. Tissue ethanol was also

significantly highest (222 ug/ g fresh wt) in fruits kept under 0.3% O_2 (Fig. V.6). However, a drastic change in tissue ethanol was noticed when O_2 levels in storage atmospheres were $\leq$0.5%. Acetaldehyde concentration of apples in all storage atmospheres remained unchanged when fruits were removed from storage atmospheres and exposed to air for 7 days (Appendix Fig. B.3). Tissue ethanol in air-stored apples increased by 4% , but that in CA-stored fruits decreased by 1 to 10 % (Appendix Fig. B.4).

ACC concentration of CA-stored apples was significantly greater than that of air-stored apples (Fig. V.7). Storage in 0.3% O_2 at 0°C for 24 days resulted in the highest ACC concentration of 9 nmol/g fresh wt. EFE activity in fruits decreased as O_2 levels in storage atmospheres decreased from 1.4 to 0.3% O_2 (Fig. V.8). There were no significant differences in EFE activity between apples in 0.5% O_2 and those in 0.3% O_2. ACC concentration in CA-fruits decreased by 7 to 15% (Appendix Fig. B.5), whereas EFE activity increased by 6 to 66% after apples were transferred to air for 7 days (Appendix Fig. B.6).

'Anjou' Pears

Ethylene concentration of 'Anjou' pears in low-O_2 atmospheres decreased with a decrease in the O_2 concentration (Fig. V.9). Ethylene was first detected on day 12 in 2.8% O_2 and 1.4% O_2 , but not until day 24 in 0.5 or 0.3% O_2. Headspace ethanol of fruits in 0.3% O_2 increased progressively from 1 ug/l on day 3 to 5 ug/l on day 24 (Fig. V.10).

CA-stored 'Anjou' pears had significantly greater PDC and ADH activities than air-stored pears (Fig. V.11 - V.12). As O_2 levels decreased, PDC and ADH activities increased. However, PDC activity of pears stored in 1.4% O_2 at 0°C for 24 days did not significantly differ from that of pears stored in 0.7% O_2. The highest PDC activity of 0.27 units was found in pears in 0.3% O_2. There were no significant differences in ADH activity between pears in 0.5% O_2 (0.63 units ADH) or 0.3% O_2 (0.64 units). Exposure to air for 7 days after removing fruits from low O_2 storage atmospheres did not affect PDC and ADH activities at all (Appendix Fig. B.7 - B.8).

CA-stored 'Anjou' pears had significantly higher tissue acetaldehyde than air-stored fruits when kept in $\leq$1.4% O_2 (Fig. V.13). There were no significant differences in this regard between fruits in 0.7% O_2 and those in 0.5% O_2 . The greatest acetaldehyde concentration of 5 ug/g fresh wt was found in tissue of pears held in 0.3% O_2. Significantly high concentrations of tissue ethanol were detected in fruits in $\leq$0.5% O_2 (Fig. V.14). Fruits in 0.5% O_2 contained 143 ug ethanol/g fresh wt, whereas those in 0.7% O_2 contained 65 ug ethanol/g fresh wt. Fruits in 0.3% O_2 had an exceptionally high tissue ethanol of 362 ug/g fresh wt. Tissue acetaldehyde stayed relatively constant while tissue ethanol decreased by 2 to 8 % when fruits were removed from CA storage and exposed to air for 7 days (Appendix Fig. B.9 - B.10).

ACC concentration in fruits increased significantly when O_2 levels in storage atmospheres were $\leq$1.4%. ACC concentrations in 'Anjou' pears in 0.7% O_2 were not statistically different from that in pears in 0.5% O_2 (Fig. V.15). There were significant differences in EFE activity among fruits in air, 1.4% O_2 and 0.5% O_2 (Fig. V.16). After fruits were removed from CA storage and subsequently held in air for 7 days, ACC concentration decreased by 8 to 23 %, whereas EFE activity increased by 3 to 8 % (Appendix Fig. B.11 - B.12).

No external or internal symptoms of physiological disorders were observed in 'Granny Smith' apples or 'Anjou' pears during 24 days of exposure to low O2 plus 1.4% CO_2 at 0°C and following 7 days in air. At the end of the experiment, off-flavor development did not occur in CA-stored fruits nor in air-stored fruits, based on scores (all zeroes) of the sensory panel.

DISCUSSION

PDC and ADH activities reported in this paper were based on oxidation of added NADH measured spectrophotometrically at 340 nm (Chang, et al., 1982). Endogenous NADH in the crude extract might also account for differences in the activities. Also NADPH may be present in the extract and, like NADH, has been reported to be a cofactor of this oxidation (Molina, et al., 1986; Rhodes, 1973). PDC and ADH activities increased with decreasing O_2 levels. This agrees with Bufler and Bangerth (1982), who found the highest activity of PDC in 'Golden Delicious' apples in hypobaric (equivalent to 1.4% O_2) storage at 4°C. Exposure to air for 7 days after removing fruits from CA storage did not change the enzyme activities. This may imply that once the enzyme activities had been increased under low-O_2 conditions, returning fruits to air caused no enzyme inhibition.

Recommended CA storage conditions for 'Granny Smith' apples are 0°C, 2% O_2 and 1% CO_2 (Meheriuk, 1985) and for 'Anjou' pears -0.5°C, 1 to 2% O_2 and 0 to 0.5% CO_2 (Richardson, 1985). The minimum O_2 level required by the fruit before injury or quality loss occurs depends on the duration of exposure, storage temperature and the physical properties of the variety which differ in respiration rate and gas diffusion characteristics (Kader, 1985). Extremely low O_2 or very high CO_2 levels result in accumulation of acetaldehyde and ethanol. However, normal aerobic respiration was maintained in apple fruits of certain cultivars in 0.5% O_2 (Bohling and Hansen, 1985). For both apples

and pears, a pronounced increase in tissue acetaldehyde occurred in $\leq$1.4% O_2 + 1.4% CO_2, whereas a marked increase in tissue ethanol took place in $\leq$0.5% O_2 + 1.4% CO_2. No alcoholic taints of flavor were reported in 'Granny Smith' apples stored in 1.0% CO_2 + 1.5% O_2 at -0.5°C (Little, et al., 1982). 'Anjou' pears had best flavor and texture without showing scald in low O_2 (1.0%) storage treatment (Hansen, 1957). When apples and pears were removed from CA storage and subsequently held in air, their tissue acetaldehyde remained unchanged, whereas tissue ethanol slightly decreased. Thomas (1925) found that exposure of apples to air did not cause any decrease in acetaldehyde and ethanol accumulated during anaerobiosis. In contrast, Patterson and Nichols (1988) reported an increase in acetaldehyde. In addition, Nichols and Patterson (1987) found that upon returning apples to air for 7 days following low-O_2 storage, up to 50% of accumulated ethanol was lost, and that fruits held at 20°C lost more ethanol than fruits held at 0.5°C. When apples containing ethanol were kept in air, some of the ethanol was lost by diffusion, but most of it was metabolized (Fidler and North, 1971). Acetaldehyde could also be metabolized by plant tissues (Fidler, 1968). Thus changes in accumulated acetaldehyde and ethanol may depend on the rate of production, the initial concentration, diffusion resistance of the commodity, and metabolic rate of converting acetaldehyde and ethanol. Aeration at high temperatures could accelerate loss of acetaldehyde and ethanol (Fidler, 1973; Weichmann, 1986).

Alcohol vapor in storage atmospheres was roughly proportional to the level of alcohol in the fruit (North and Cockburn, 1975). This might also apply to acetaldehyde. Under a flow-through system, headspace acetaldehyde was beyond the detection limits (0.5 ug/l). Only apples and pears in 0.3% O_2 + 1.4% CO_2 had high enough tissue ethanol that could produce measurable headspace ethanol.

When apples were continuously ventilated during storage, the onset of rapid ethylene production was delayed and the maximum rate of production was reduced by low O_2 concentration (Knee, 1980). Ethylene production of 'Bartlett' and 'Bosc' pears was also suppressed by 1.0% O_2 during storage at -1°C (Chen, et al., 1985). Apples stored in 1.0% O_2 atmosphere accumulated a relatively high amount of ACC (Bufler and Streif, 1986; Zhen-guo et al., 1983). Endogenous ACC levels were 20 times higher in 'Anjou' pears stored in 1.0% O_2 compared to air-stored fruits (Blankenship and Richardson, 1986). In our short experiments, CA-stored fruits had significantly higher ACC level than air-stored fruits, but the concentrations were considerably less compared with ACC values reported by others in longer duration studies. Continuous exposure of apples to low O_2 atmospheres delayed ACC synthesis initially, but eventually resulted in rapid accumulation (Zhen-guo, et al., 1984). Only twenty-four days in low O_2 storage would still be in an early stage. Winter pears used in this experiment might have only partially satisfied their chilling requirement, resulting in relatively low ACC accumulation

in fruits under low-O_2 conditions. When ACC was applied to various plant organs (with the exception of preclimacteric fruits and flowers) from a number of plant species, a marked increase in ethylene production was observed (Cameron, et al., 1979). Conversion of exogenous ACC to ethylene by fruit tissue was measured as an indication of changes in ethylene forming enzyme (EFE). CA-stored fruits had significantly less EFE activity than air-stored fruits. We used 0.5 mM ACC as against 5 mM ACC used by Blankenship and Richardson (1986), who found reduced initial EFE activity in 1% O_2-stored 'Anjou' pears. A difference in exogenous ACC concentration applied to fruit tissue would not account for a difference in EFE activity.

No external or internal low-O_2 injury symptoms as described by Porritt et al.(1982) were observed throughout the 24 day experimental period. Similarly, off-flavors could not be detected by the sensory panel. Cox's Orange Pippin apples containing up to 300 ug ethanol/g fresh wt were still acceptable (Fidler and North, 1971). The maximum ethanol concentration in apples held in 0.3% O_2 + 1.4% CO_2 at 0°C for 24 days was 222 ug/g fresh wt, which is apparently within the acceptable level. Pears kept in the same condition contained 362 ug ethanol/g fresh wt. The acceptable level of ethanol in fruit tissue seemed to vary among commodities in this case.

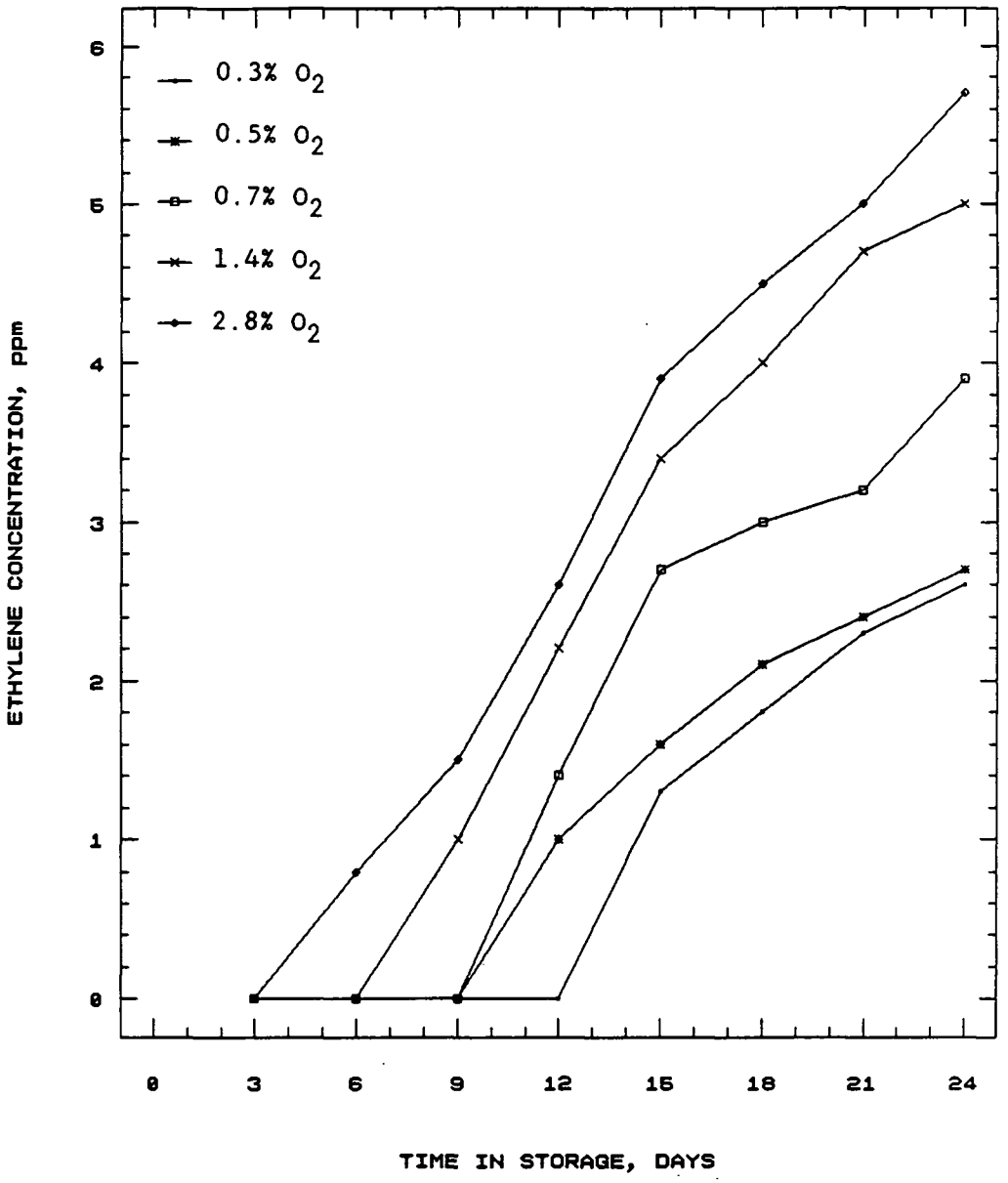

Fig. V.1. Ethylene concentration of 'Granny Smith' apples in controlled atmospheres at 0°C. Each point represents the mean of 2 observations.

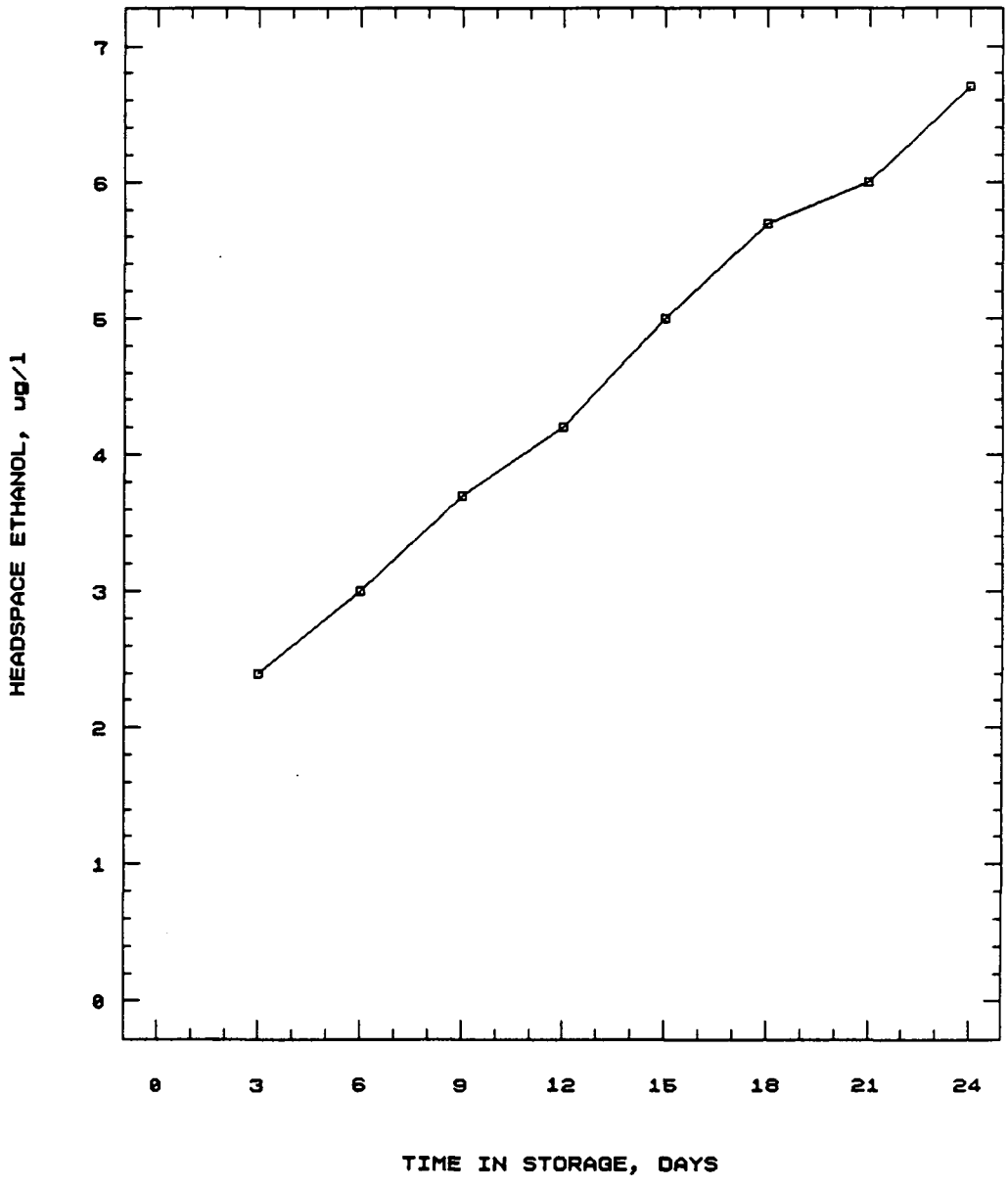

Fig. V.2. Headspace ethanol of 'Granny Smith' apples in 0.3% O_2 + 1.4% CO_2 at 0°C. Each point represents the mean of 2 observations.

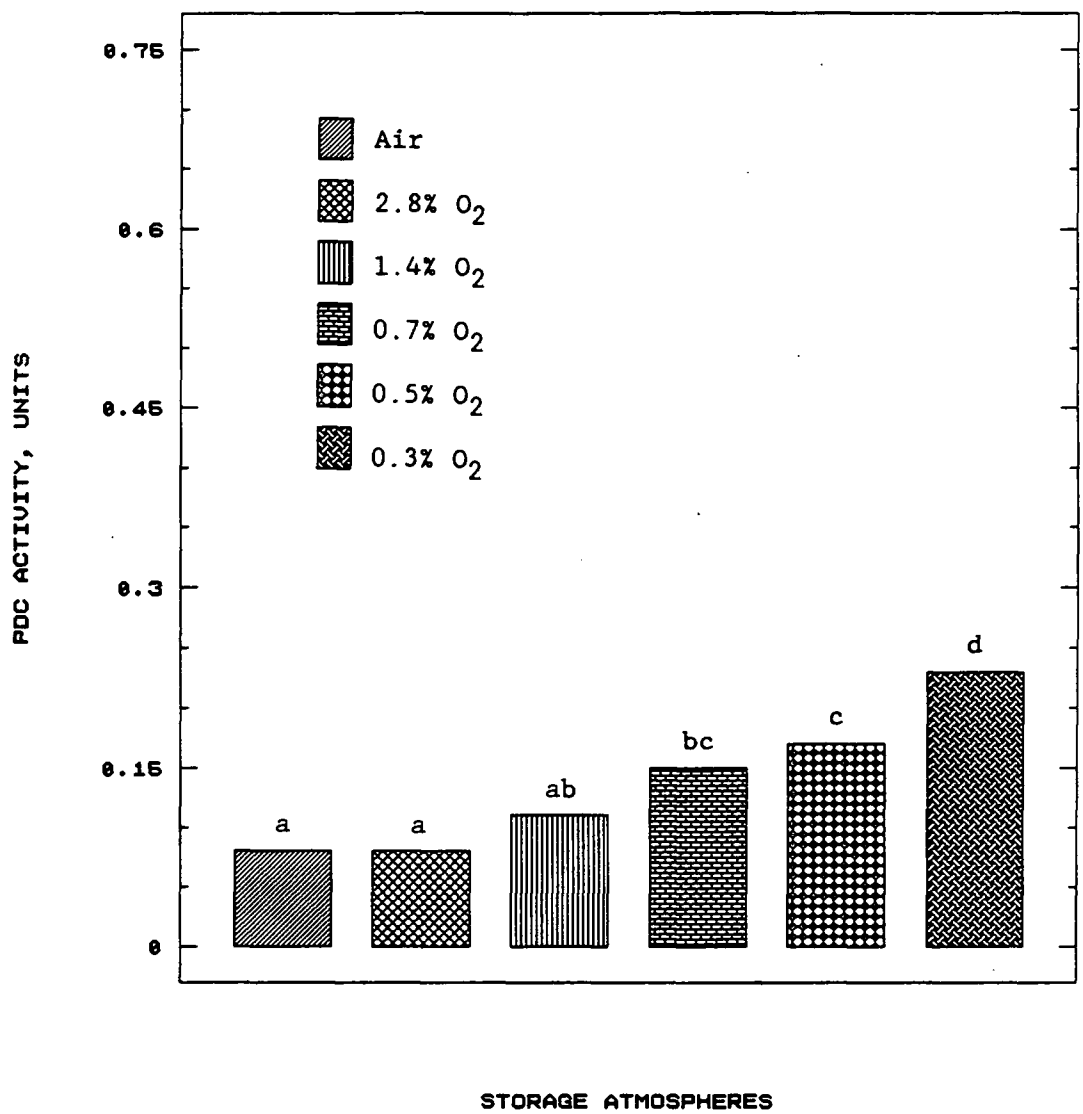

Fig. V.3. Pyruvate decarboxylase (PDC) activity of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

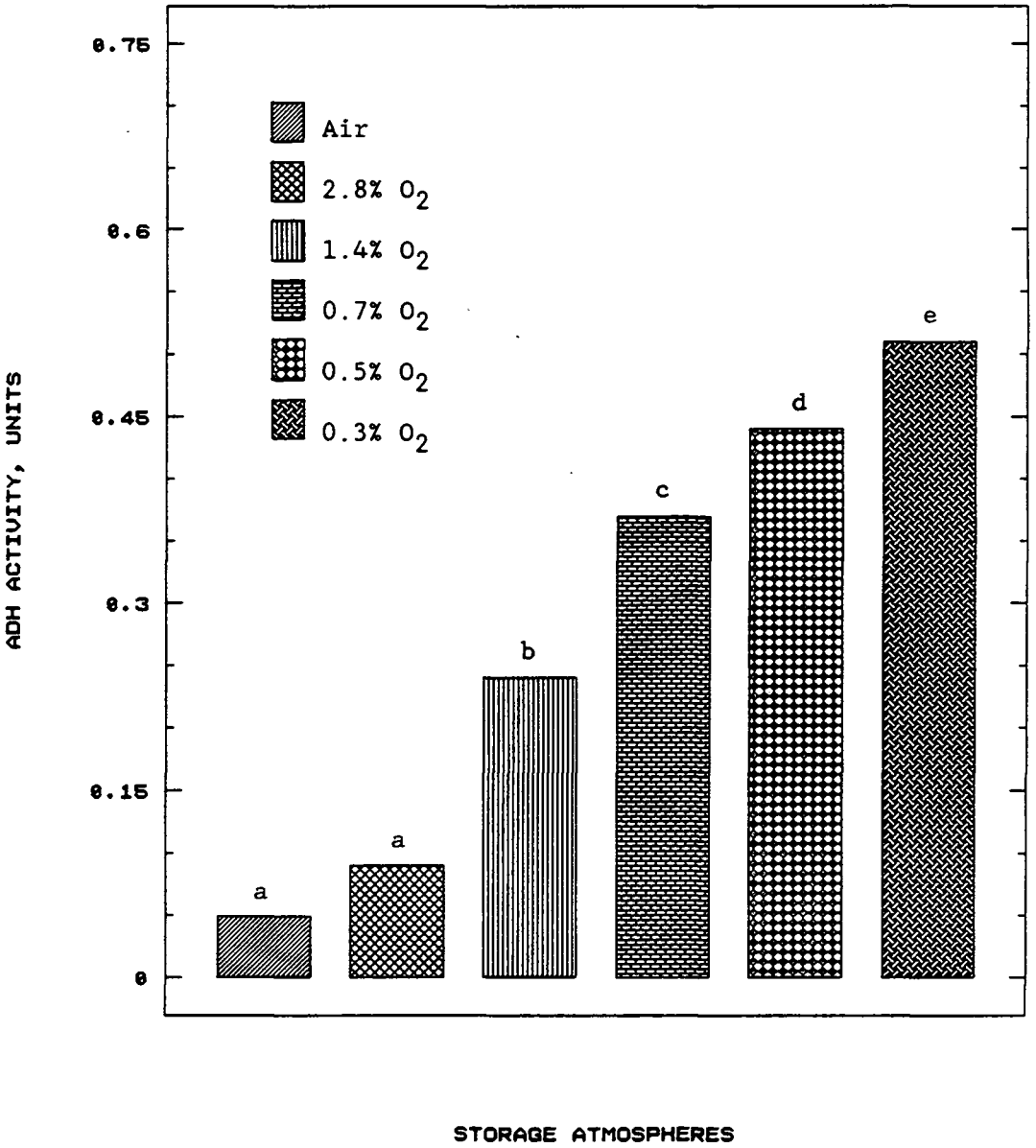

Fig. V.4. Alcohol dehydrogenase (ADH) activity of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

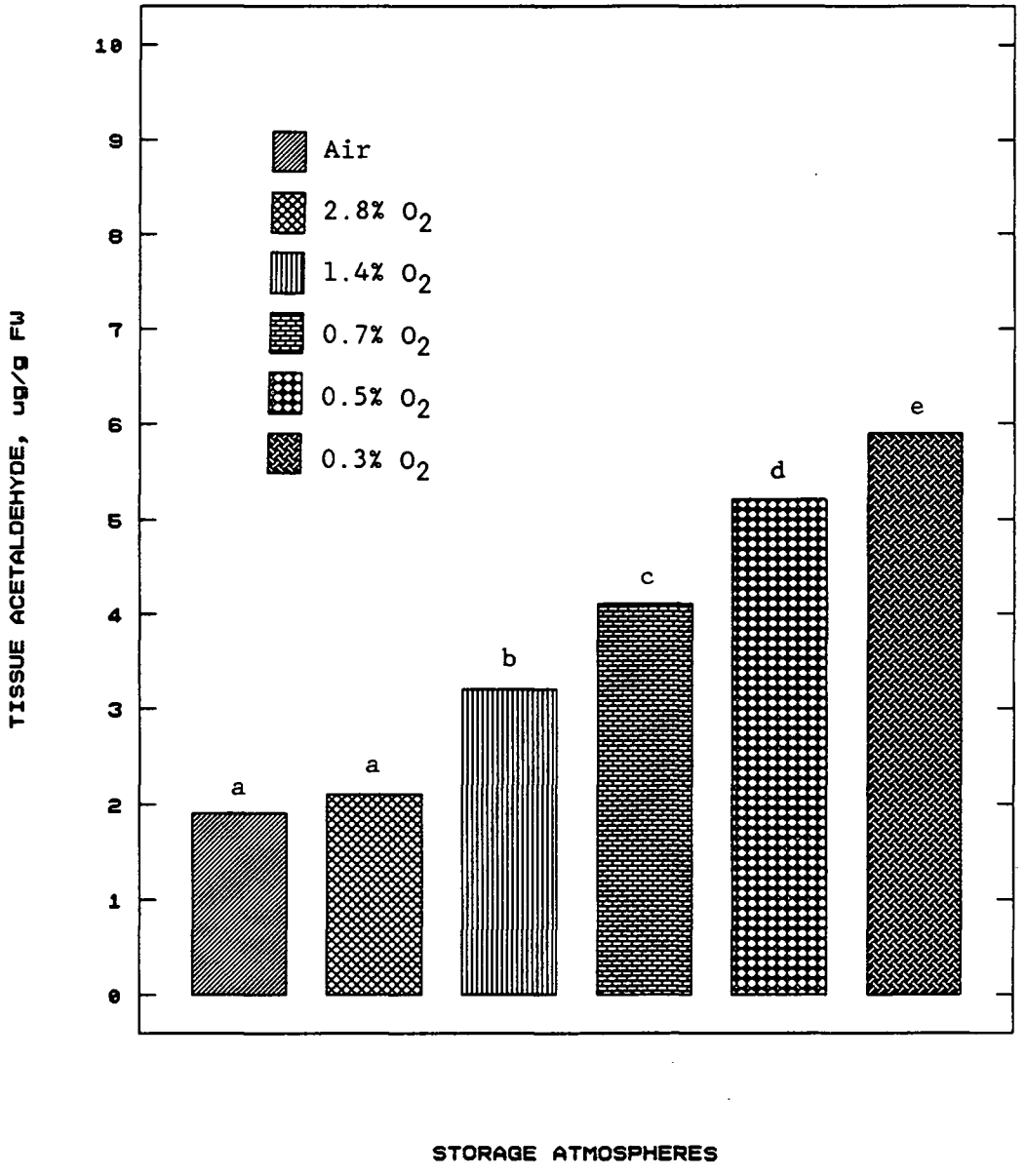

Fig. V.5. Tissue acetaldehyde of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

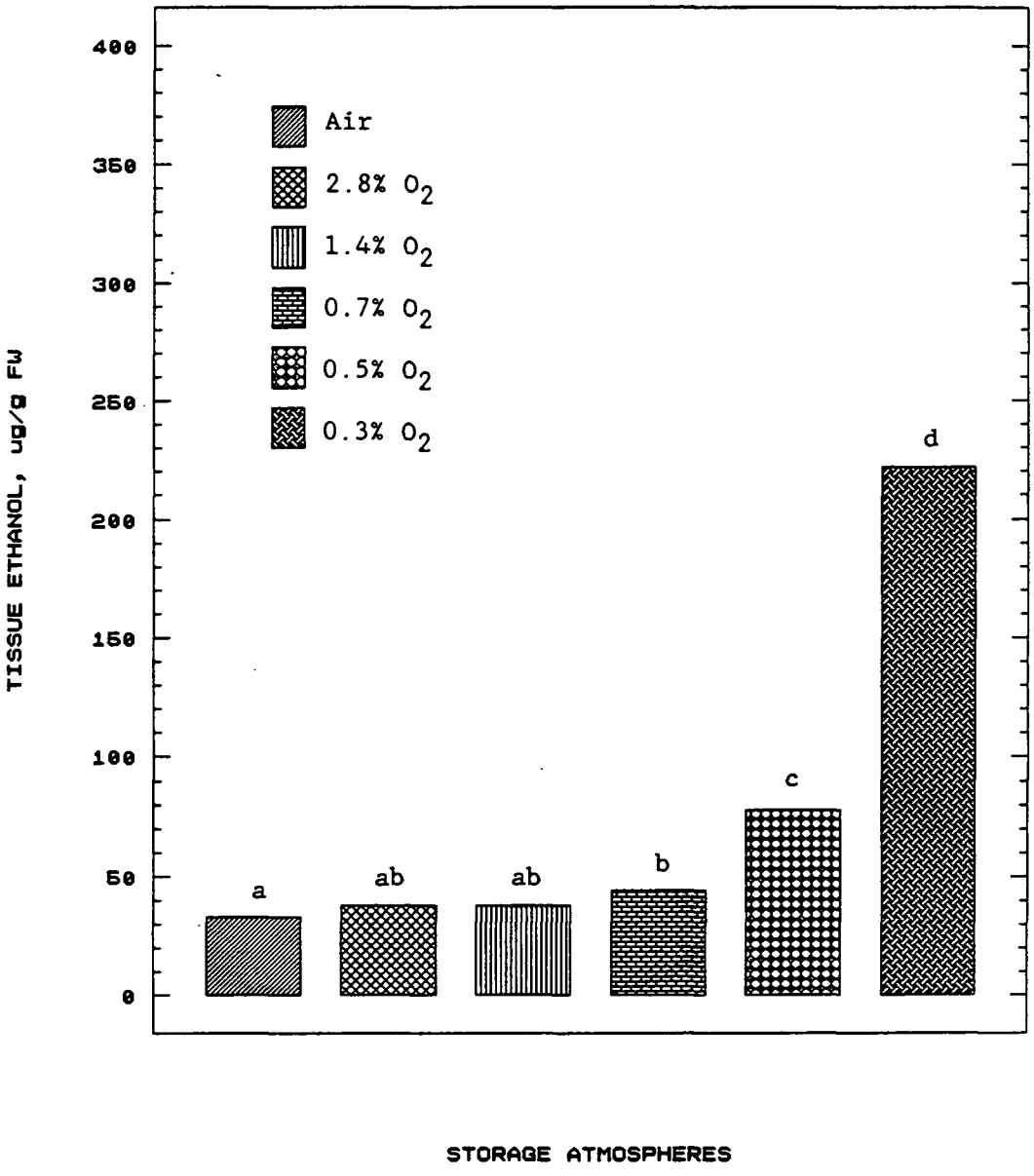

Fig. V.6. Tissue ethanol of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

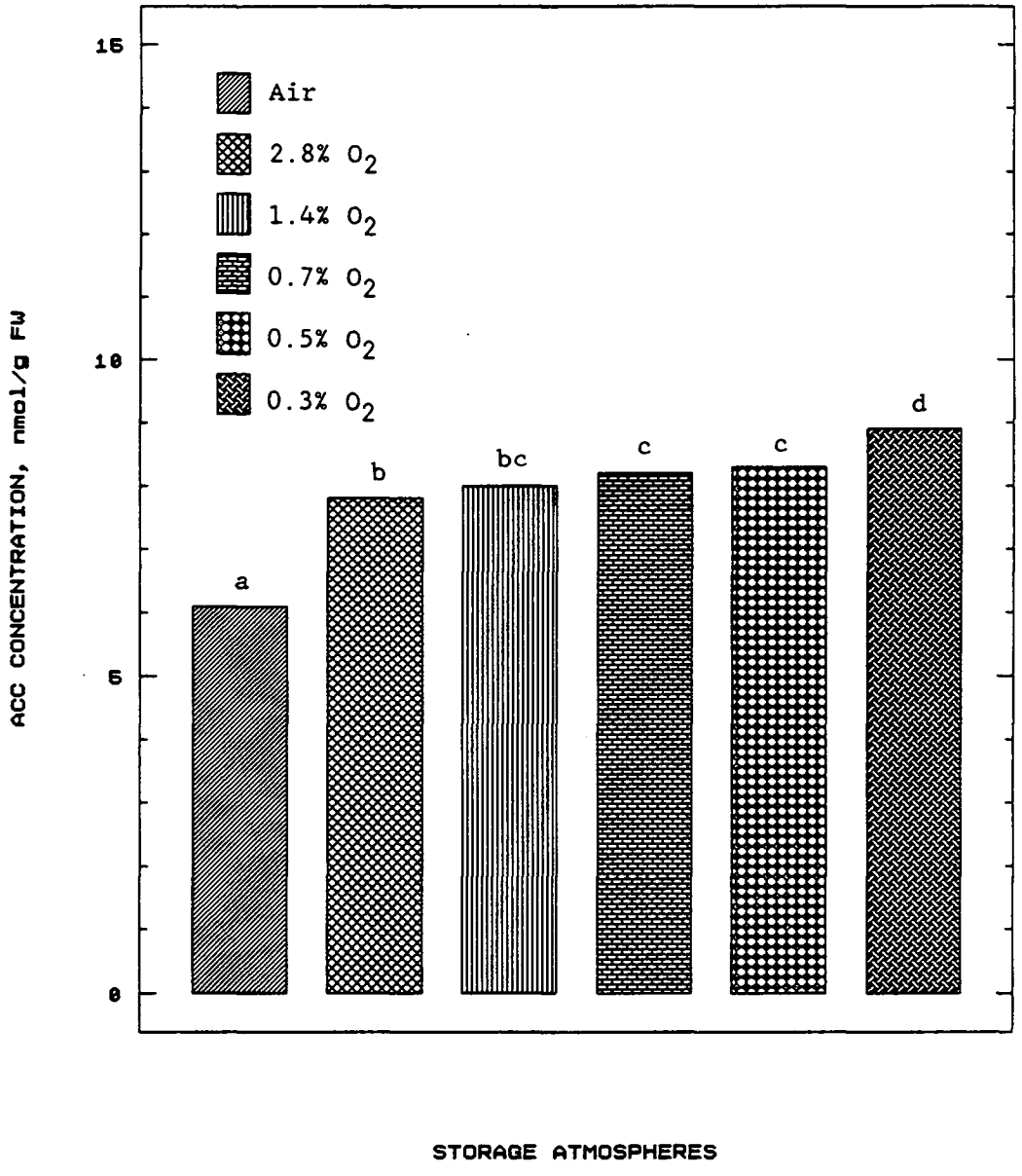

Fig. V.7. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

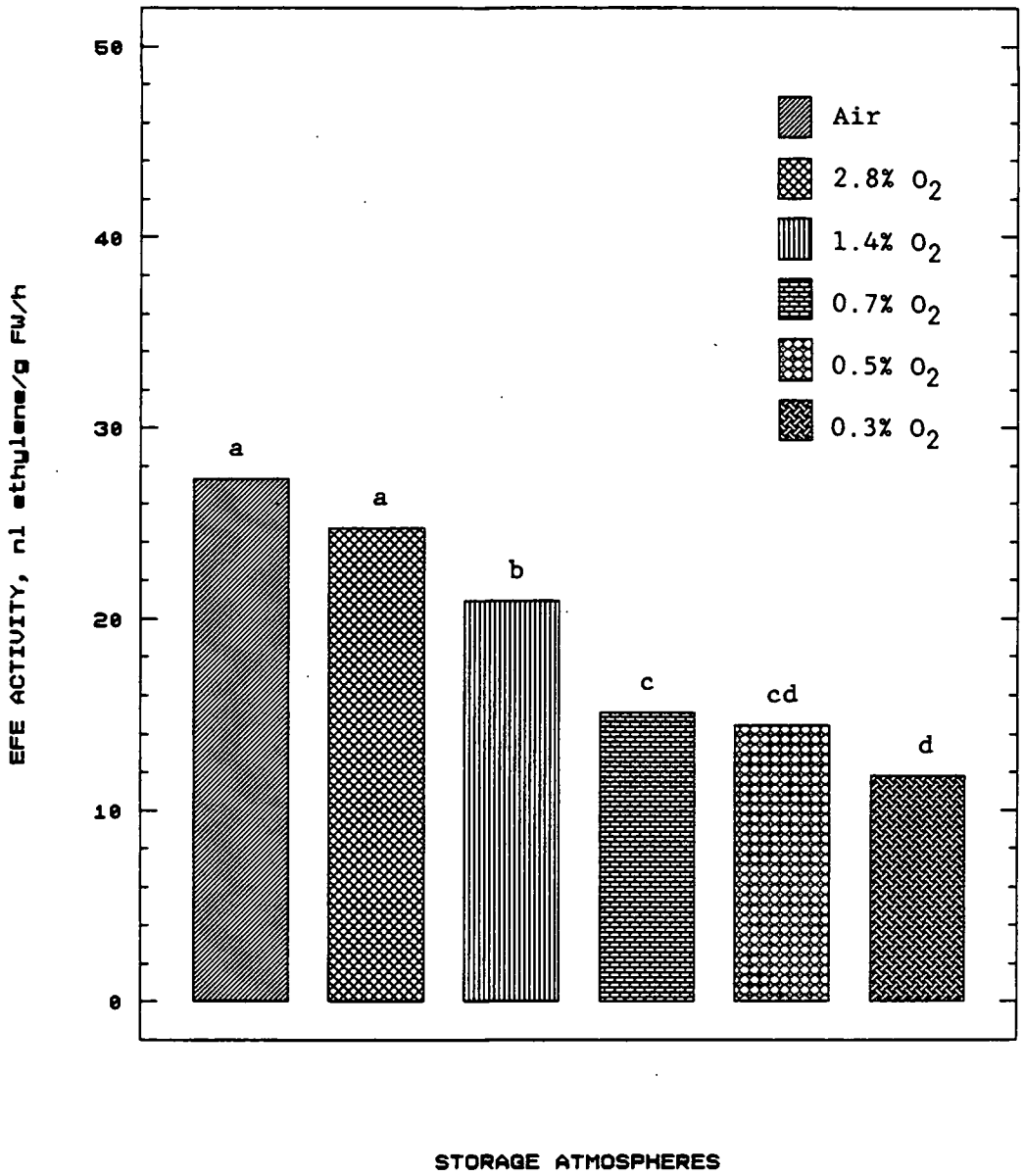

Fig. V.8. Ethylene forming enzyme (EFE) activity of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

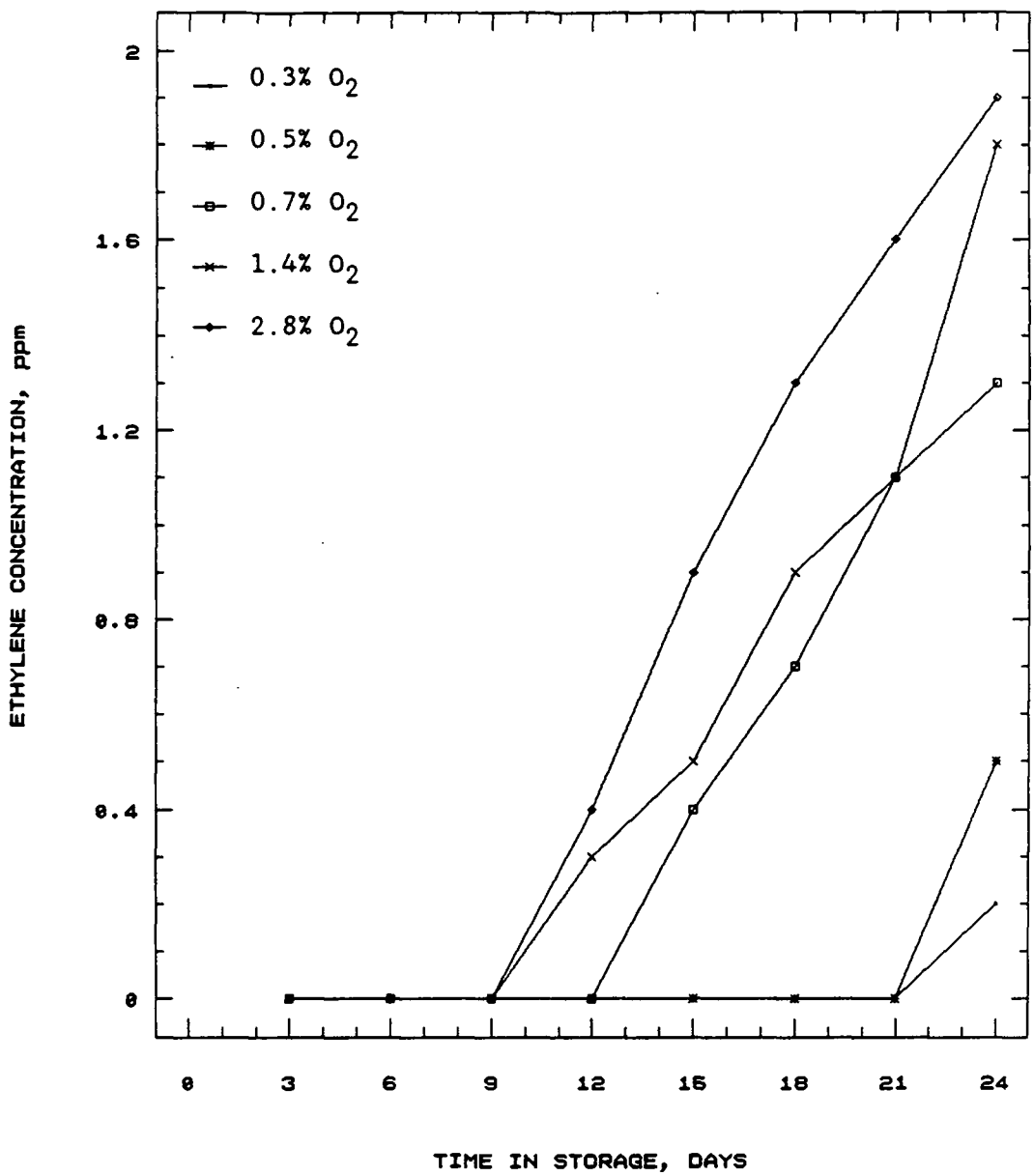

Fig. V.9. Ethylene concentration of 'Anjou' pears in controlled atmospheres at 0°C. Each point represents the mean of 2 observations.

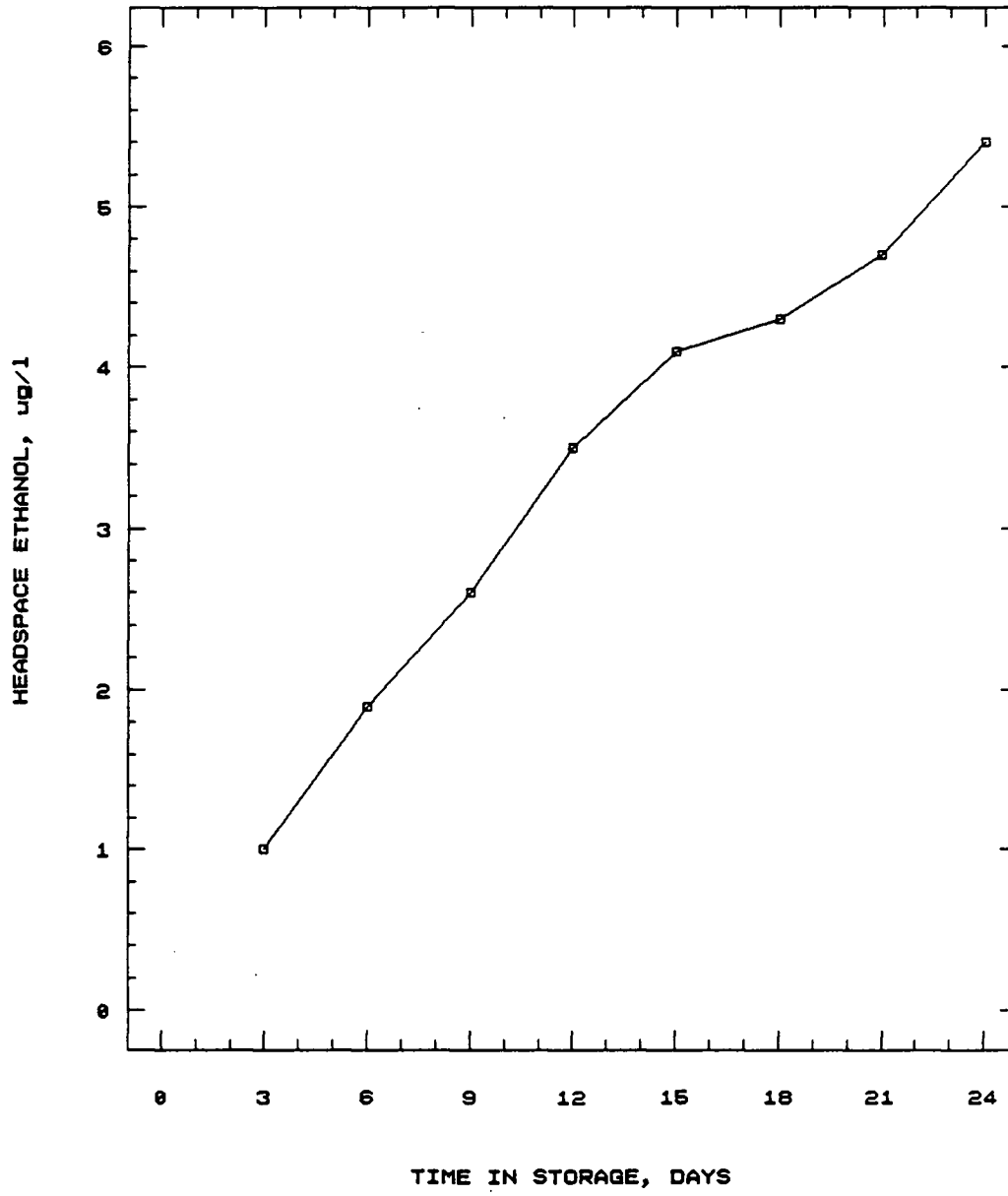

Fig. V.10. Headspace ethanol of 'Anjou' pears in 0.3% O_2 + 1.4% CO_2 at 0°C. Each point represents the mean of 2 observations.

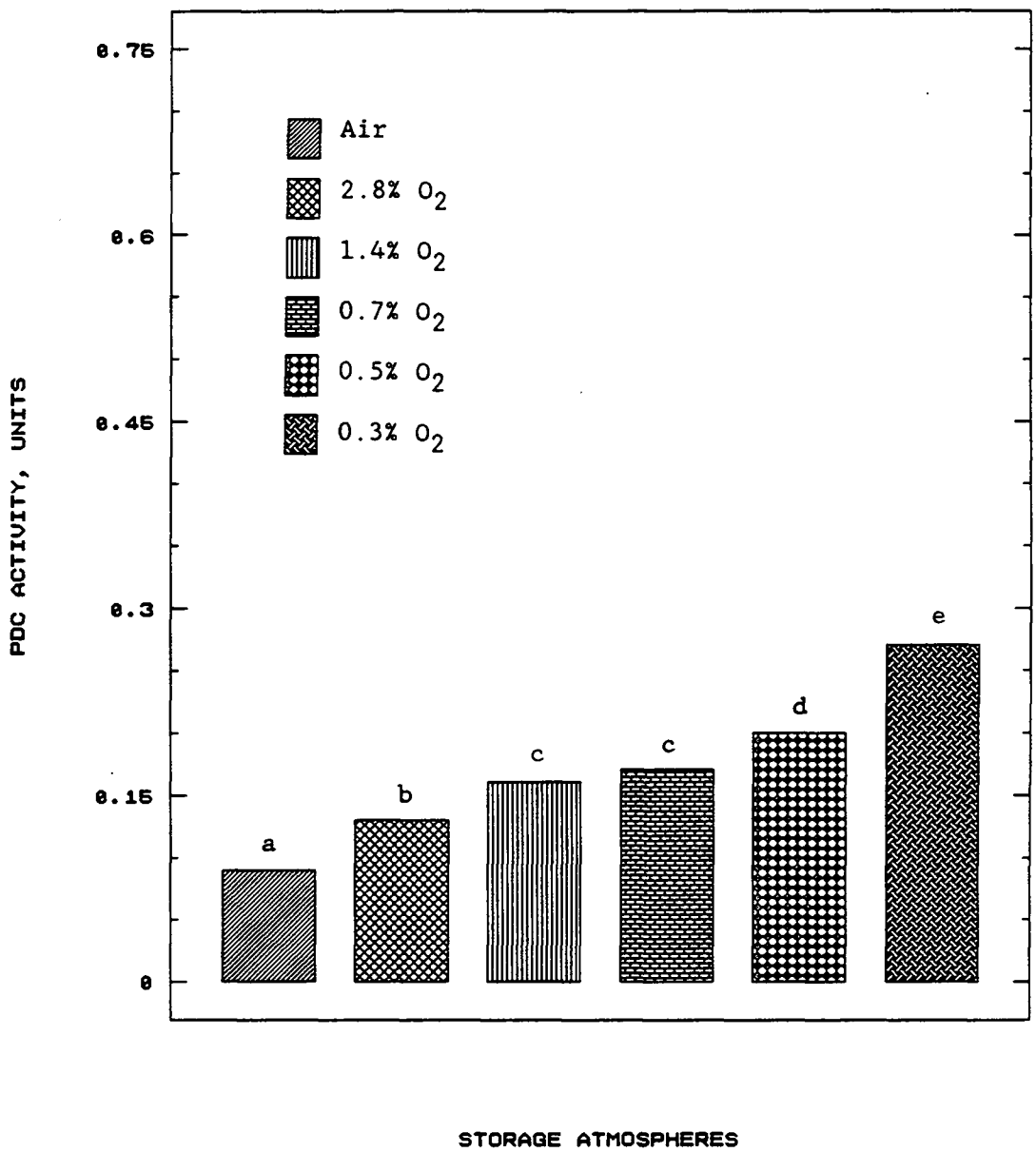

Fig. V.11. Pyruvate decarboxylase (PDC) activity of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

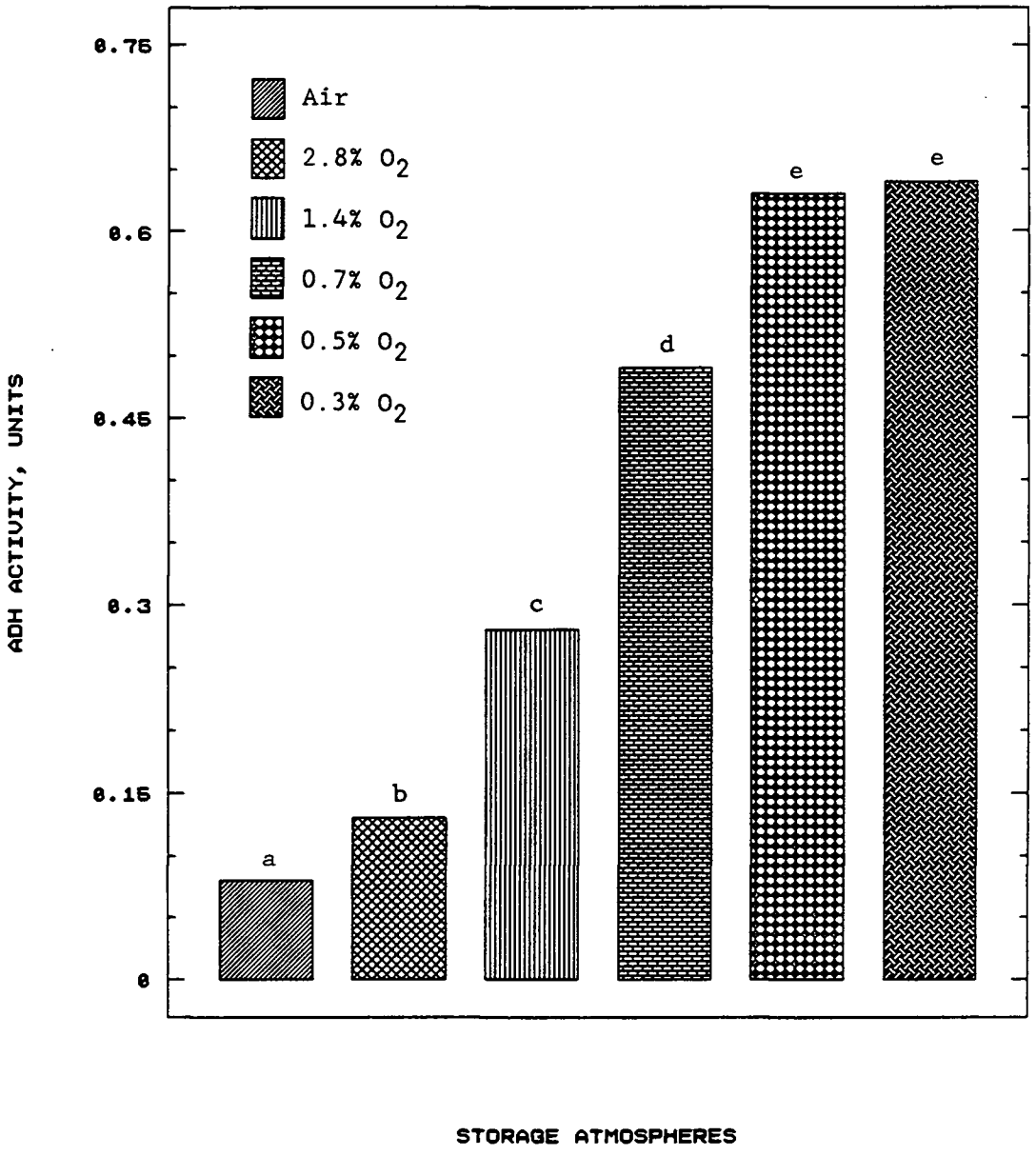

Fig. V.12. Alcohol dehydrogenase (ADH) activity of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

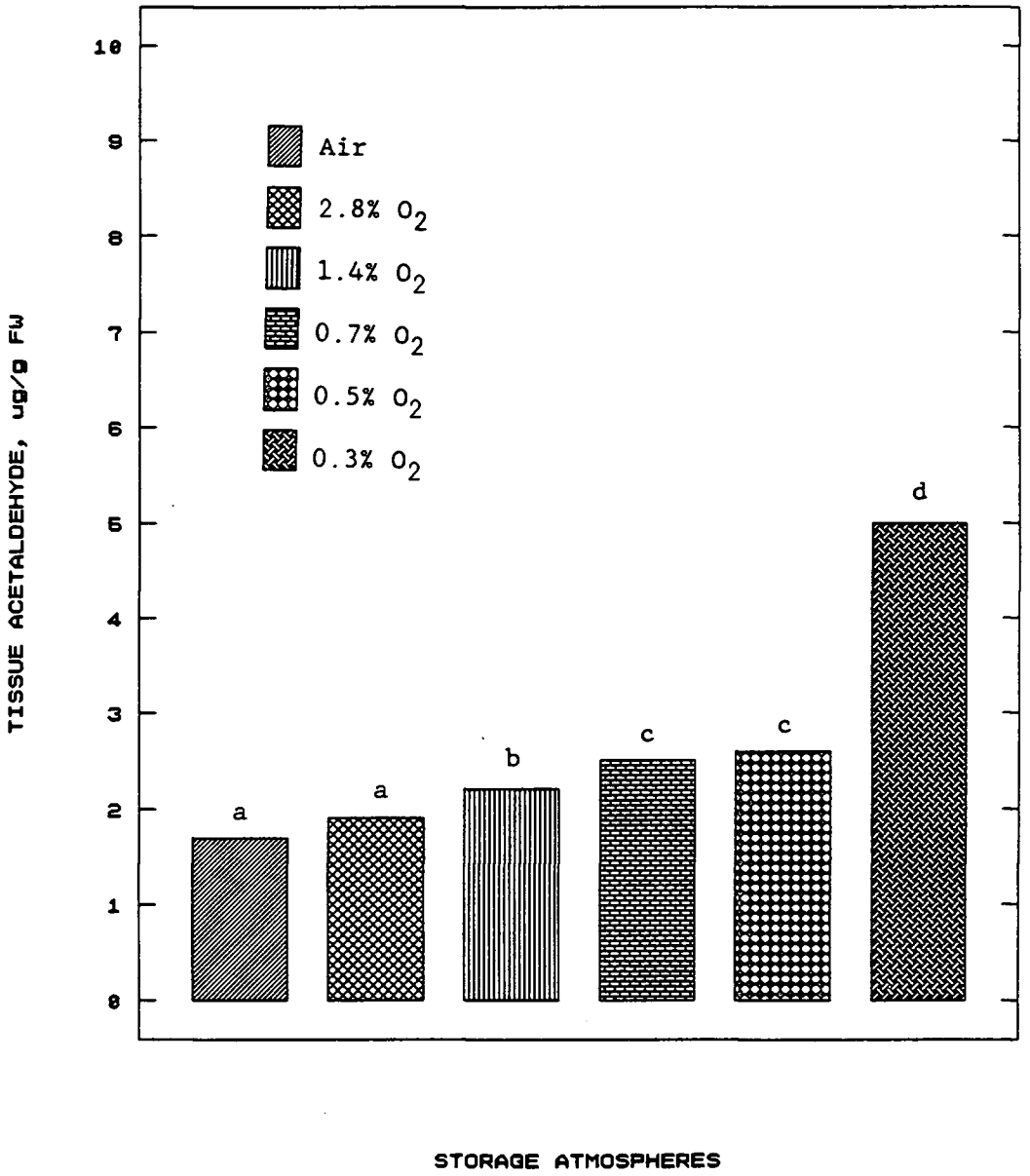

Fig. V.13. Tissue acetaldehyde of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

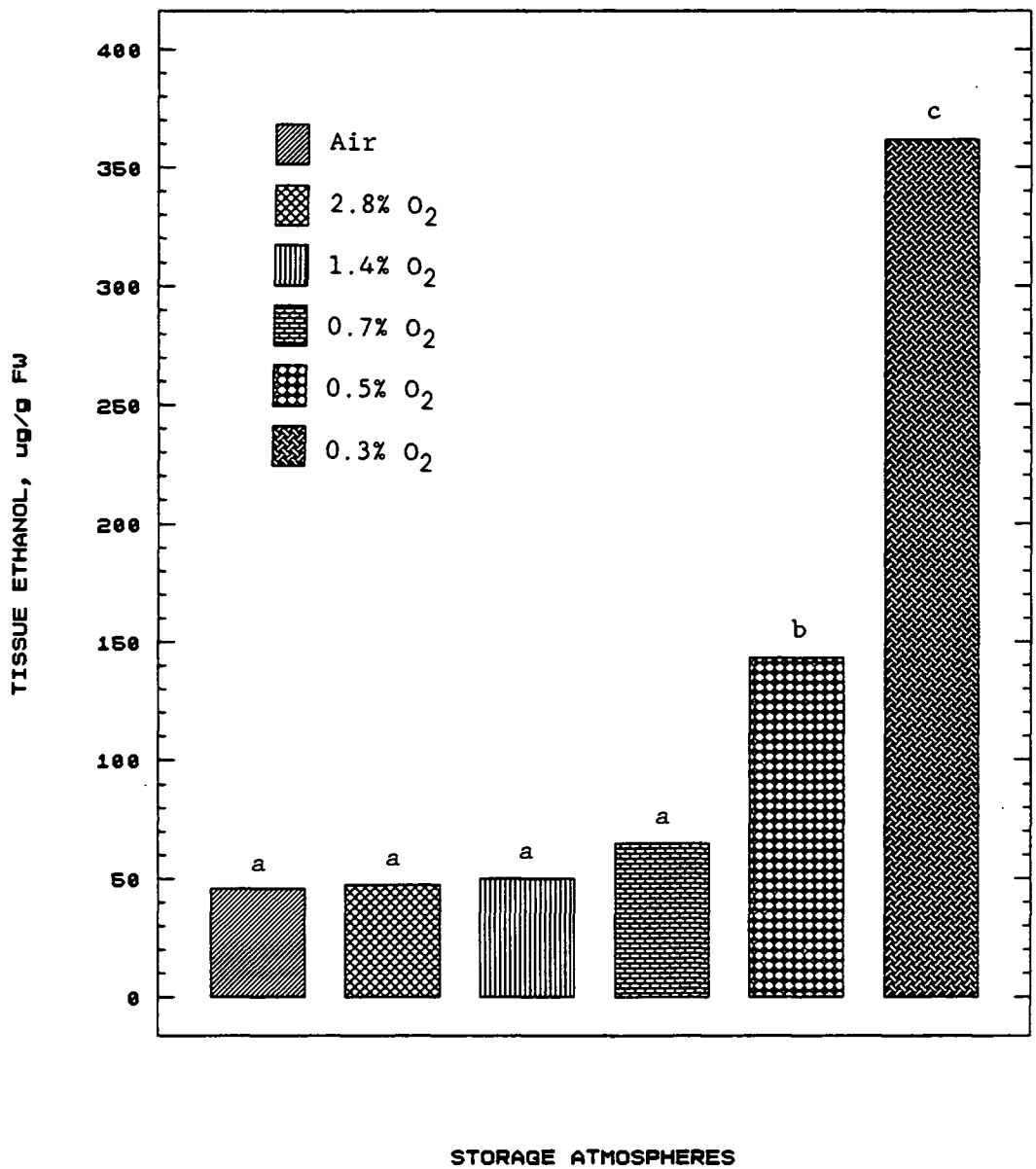

Fig. V.14. Tissue ethanol of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

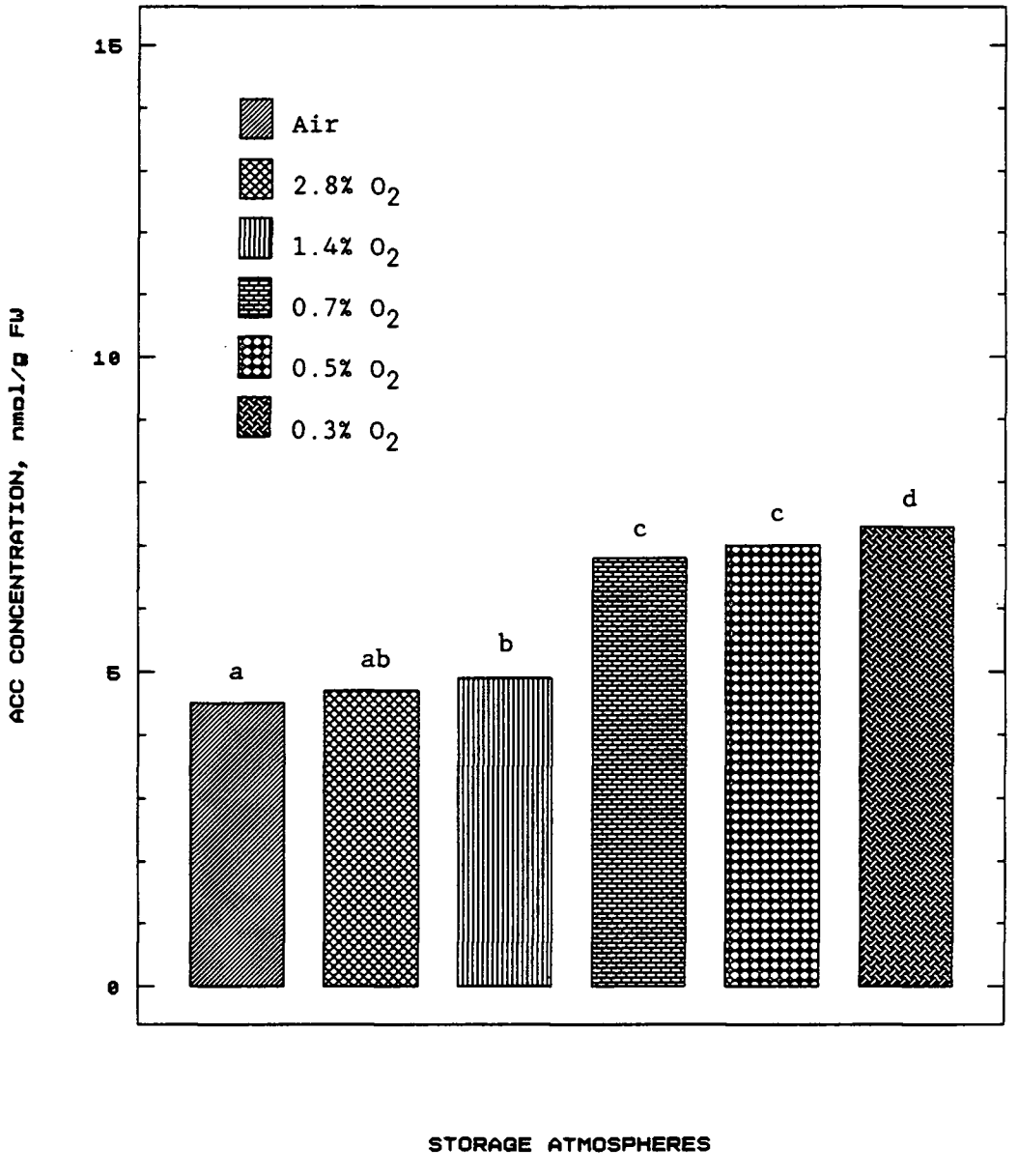

Fig. V.15. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

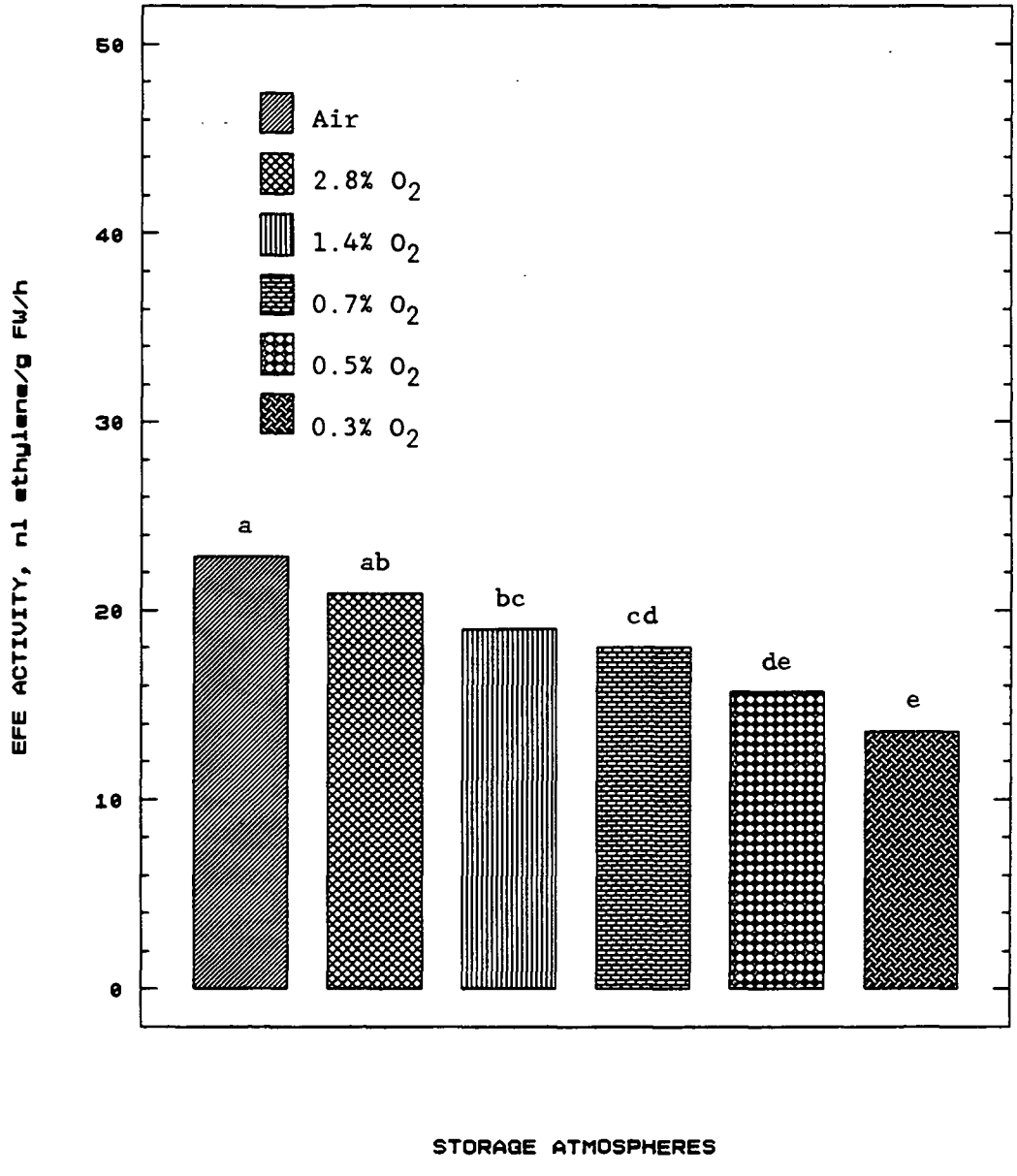

Fig. V.16. Ethylene forming enzyme (EFE) activity of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

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CHAPTER VI

Effects of Anaerobic and Low-O_2 Storage Conditions on Physiology and Flavor of Small Fruits and Plums

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Additional index words: blueberry, red raspberry, blackberry, *Rubus* spp., *Vaccinium* spp., *Prunus* spp., CO_2, acetaldehyde, ethanol, off-flavors

ABSTRACT

'Blue Jay' blueberries, 'Amity' red raspberries, 'Marion' blackberries, and 'Italian' plums were placed in static N_2 or continuous flow (10 ml/min) low O_2 (0.3 to 4.3%) in 1 pint glass jars at 0°C. For all fruits treated, accumulated CO_2 and headspace ethanol increased with time in static N_2. Blueberries, red raspberries, blackberries and plums could be kept in anaerobic conditions at 0°C for up to 9, 7, 4, and 5 days, respectively without developing off-flavors. Storage beyond these periods for a few days resulted in slight off-flavors which dissipated after transferring fruits to air for 3 days. Longer anaerobic storage resulted in off-flavors which did not completely dissipate. In low-O_2 storage conditions, headspace ethanol increased with time in storage and with decreasing O_2 concentration. Headspace ethanol was not detected in plums held in 3.4 and 1.7% O_2. Small fruits held in $\leq$1.3% O_2 and plum kepts in $\leq$0.5% O_2 for 10 days developed slight off-flavors. However, fruits recovered from this

undesired flavor within 3 days after transferring to air. No external anaerobic and low-O_2 injury symptoms were observed throughout the short experimental periods.

INTRODUCTION

Due to extremely fragile structure and high rate of respiration, the postharvest life of most small fruits is relatively short. In addition, berry shelf life is limited by fungal rots. Storage of machine-harvested blackberries in 20% and 40% CO_2 at 20°C for up to 2 days maintained raw and processed quality (Morris, et al., 1981). CO_2-enriched atmospheres could reduce postharvest decay development in blueberries during and after cold storage (Ceponis and Cappellini, 1985). Rapidly cooling fresh blueberries to a temperature just above freezing and maintaining that temperature appeared best for suppressing decay development and preserving the marketability of the fruits (Hudson and Tietjen, 1981). Temperatures near 0°C were found to be the best for storing and handling blueberries and raspberries (Hruschka and Kushman, 1963; Sjulin and Robbins, 1987). Generally, small fruits are held at 0° to 5°C in 5 to 10% O_2 plus 15 to 20% CO_2 during transport (Kader, 1985). Machine- and hand-harvested raspberries and blackberries kept in 5% CO_2 + 2% O_2 or 10% CO_2 + 2% O_2 at 0° or 5°C for 14 days had better titratable acid and soluble solid retention, resulting in higher sensory panel rating when compared with those stored in air (Varseveld and Richardson, 1980). Fruit quality of hand-harvested 'Climax' rabbiteye blueberries held in 20% CO_2 + 5% O_2 at 5°C for 42 days was as good as that of freshly harvested berries, whereas 'Woodward' blueberries stored under the same condition for 21 days exhibited a slightly fermented off-flavor (Smith and Miller,

1988). Interestingly, flavor of machine-harvested 'Climax' blueberries stored in N_2 did not change significantly, whereas that of 'Woodward' fruits became tart and fermented when berries were stored in N_2 for 21 days.

'Victoria' plums could be stored for 4 weeks or longer in 1% O_2 and less than 0.2% CO_2 at -0.5°C (Smith, 1967). Interrupting storage by 2 days at 18°C in air after day 16 resulted in further extension of storage life and reduction of internal browning and jellying. Exposure of 'Italian' plums to 20 to 60% CO_2 for 2 days at 7°C prior to storage at 0°C decreased softening and decay without off-flavors being detected after 2 weeks storage despite accumulation of aldehyde and alcohols (Ryall, 1935). Modified atmospheres created in sealed polyethylene bags, polyethylene box liners and in individually-wrapped fruits extended storage life of plums (Couey, 1960,1965).

Studies of fruit responses to short-term anaerobic and low-O_2 storage conditions are important to modified atmosphere packaging. Thus, these experiments evaluated anaerobic and low-O_2 effects on physiology and flavor of certain small fruits and plums.

MATERIALS AND METHODS

'Blue Jay' blueberries, 'Amity' red raspberries, 'Marion' blackberries and 'Italian' plums (150 g per treatment replication) were harvested at commercial maturity and placed in static N_2 or continuous flow (10 ml/min) low O_2 ranging from 0.3 to 4.3% in 1 pint glass jars at 0°C. Under anaerobic conditions, CO_2, O_2 and N_2, and headspace acetaldehyde and ethanol, and off-flavor development were determined daily according to the methods described earlier in Chapter III. Anaerobic off-flavor development was determined immediately after removing treated fruits from anaerobic conditions and again after 3 days in air. A score of 1 was arbitrarily chosen as a level of off-flavors that might be regarded as undesirable by the taste panel. Each value is the mean of 10 replications for off-flavor determination, and 4 observations for headspace analyses. In low-O_2 storage conditions, treated fruits were analyzed similarly at 2-day intervals for 10 days. Values of headspace acetaldehyde and ethanol are the means of 2 observations, whereas the off-flavor score is the mean of 10 obervations. Off-flavor development of fruits in low O_2 was determined on day 10 and after 3 additional days in air.

RESULTS

'Blue Jay' Blueberries

In static N_2 at 0°C, CO_2 accumulation increased steadily from 0.7% on day 1 to 17.3% on day 17, whereas accumulated headspace ethanol increased from 5 to 49 ug/l (Fig. VI.1). Headspace acetaldehyde could be detected on day 9 and slightly increased to 4 ug/l on day 17. The berries could be held anaerobically for 9 days without showing any off-flavors (Fig. VI.2). Ten to 14 days in static N_2 resulted in slight off-flavors with scores ranging from 1.3 to 3.3, but complete reversal was accomplished 3 days after transferring the berries to air. The berries held in N_2 longer than 16 days only slightly recovered from off-flavors when returned to air. Under low-O_2 environments, headspace ethanol increased with decreasing O_2 concentration (Fig. VI.3), but there were no differences in ethanol levels in 0.7 and 0.4% O_2. After 10 days in low-O_2 storage, slight off-flavors (scores 1.6 to 1.7) were detected in the berries in 0.7 and 0.4% O_2. Berry flavor became normal after subsequent holding in air for 3 days.

'Amity' Red Raspberries

CO_2 accumulation in static N_2 at 0°C increased from 3.4% on day 1 to 9.2% on day 10 in parallel to an increase of accumulated headspace ethanol from 11 to 61 ug/l (Fig. VI.4). The berries did not exhibit off-flavors when held in anaerobic conditions for up to 7 days (Fig. VI.5), but started to develop off-flavors on day 8. However, the berries completely recovered from the undesired

flavor after transfer to air for 3 days. Partial reversal (30 to 79%) was accomplished when the berries were in static N_2 longer than 8 days, and the off-flavor scores ranged from 0.3 to 2.3 following 3 days in air. In low-O_2 storage conditions at 0°C, headspace ethanol increased in proportion to decreased O_2 from 4.3 to 0.4% (Fig. VI.6). After 10 days in storage, the berries held in $\leq$1.3% O_2 had slight off-flavors with scores ranging from 1.3 to 2.3. However, off-flavors disappeared after transferring the berries to air (data not shown, but similar to Fig. VI.5).

'Marion' Blackberries

CO_2 accumulation increased with time in static N_2 at 0°C from 4.4% on day 1 to 18.4% on day 9, and accumulated headspace ethanol increased in the same fashion from 9 to 52 ug/l (Fig. VI. 7). Flavor of the berries held in anaerobic conditions for up to 4 days did not change when compared with the control (Fig. VI.8). The berries showed slight off-flavors (scores 1.3 to 3.0) when kept in such conditions for 5 to 8 days, but complete reversal of off-flavors was obtained 3 days after the berries were transferred to air (Fig. VI.8). Headspace ethanol could not be detected in 4.3% O_2 atmosphere (Fig. VI.9). At lower O_2 levels, ethanol concentration increased with time in storage and decreasing O_2 concentration. However, ethanol levels in 0.7 and 0.4% O_2 appeared to be the same. Ten days in low-O_2 storage resulted in slight off-flavors (scores 0.6 to 1.9) in the berries kept in 1.3 to 0.4% O_2. Flavor of these berries became normal after transfer to air for 3 days.

'Italian' Plums

In static N_2 at 0°C, CO_2 accumulation increased steadily with time in storage from 1.4% on day 1 to 9.2% on day 10, and accumulated headspace ethanol increased from 6 to 47 ug/l (Fig. VI.10). Fruits did not show off-flavors when kept anaerobically for up to 5 days (Fig. VI.11), but had slight off-flavors (scores 1.3 to 2.7) when held in N_2 for 6 to 9 days. Complete reversal of off-flavors could be accomplished by placing the plums 3 days in air after fruits were kept anaerobically for up to 9 days. In low-O_2 storage, headspace ethanol was not detected in 3.4 and 1.7% O_2 atmospheres. Ethanol levels of fruits kept in O_2 at 1.1 to 0.3% increased with time in storage and decreasing O_2 concentration (Fig. VI.12). After 10 days in storage, fruits kept in $\leq$0.5% O_2 exhibited slight off-flavors (scores 1.3 to 2.3) which disappeared when fruits were subsequently held in air for 3 days.

DISCUSSION

In anaerobic or partially anaerobic conditions, both CO_2 accumulation and headspace ethanol increased with time in storage for all fruits studied. This indicated that there was no inhibition by CO_2 on ethanol accumulation. CO_2 in the absence of O_2 inhibited anaerobic ethanol production in apples (Patterson and Nichols, 1988). In contrast, CO_2 enhanced ethanol accumulation in sweet potato roots (Chang, et al., 1983). In addition, high CO_2 enhanced acetaldehyde production in persimmon fruit (Pesis and Ben-Arie, 1984), but did not affect ethanol production in grapes (Saltveit and Ballinger, 1983b). In all fruits but blueberries, accumulated headspace acetaldehyde was not detected at these storage temperatures of 0°C. This may be because the concentrations were less than the detection limit (0.5 ug/l). Anaerobic ethanol accumulation in small fruits and plums increased with time of exposure to static N_2 as reported for blueberries and grapes (Saltveit and Ballinger, 1983a,1983b).

Acetaldehyde and ethanol increased during apple and pear fruit ripening (Nursten, 1970; Janes and Frenkel, 1978). However, both substances were present in trace amounts under aerobic conditions, but increased markedly in anaerobic conditions (Smagula and Bramlage, 1977; Laszlo and Lawrence, 1983). Headspace ethanol in all fruits investigated increased with decreasing O_2 concentration. However, it was not detected in plums held in O_2 greater than 1.7%. Oxygen tolerance limits recommended for blueberries, raspberries and blackberries stored

at 0 to 5°C were 5 to 10% O_2, whereas those for plums were 1 to 2% O_2. Our study suggests that the low oxygen tolerance should be about 1.5% O_2 for most berries, whereas that for plums was 1-2% (Kader, 1985).

Exposure of fresh fruits and vegetables to O_2 levels beyond tolerance limits may increase anaerobic respiration and the consequent acetaldehyde and ethanol accumulation, causing off-flavors (Kader, et al., 1989; Weichmann, 1986). 'Redhaven' peaches exposed to N_2 at 15.5°C for 6 days did not developed off-flavors (Parsons, et al., 1964). 'Wickson' plums stored in 1% O_2 at 15°C for 21 days exhibited off-flavors (Claypool and Allen, 1951). There were varietal differences in response to anaerobic conditions (Smittle and Miller, 1988). 'Woodward' blueberries developed off-flavors, but 'Climax' blueberries did not, when held in N_2 at 5°C for 21 and 42 days. 'Italian' plums could be stored anaerobically for up to 5 days without off-flavors being detected. Among small fruits used in this experiment, blueberries were the most tolerant to anoxia, followed by raspberries and blackberries. They could be kept anaerobically at 0°C for 9, 7 and 4 days, respectively without showing off-flavors. Holding fruits in N_2 beyond these periods for a few days resulted in slight off-flavors which dissipated after 3 days in air. Fruit ethanol which accumulated during anaerobiosis or low-O_2 storage decreased only slightly when fruits were transferred to air probably due to better diffusion and metabolic conversion (Fidler and North, 1971; Nichols and Patterson, 1987). In these experiments, no visible

physiological disorders in fruits as influenced by anaerobic conditions or low-O_2 atmospheres at 0°C were observed.

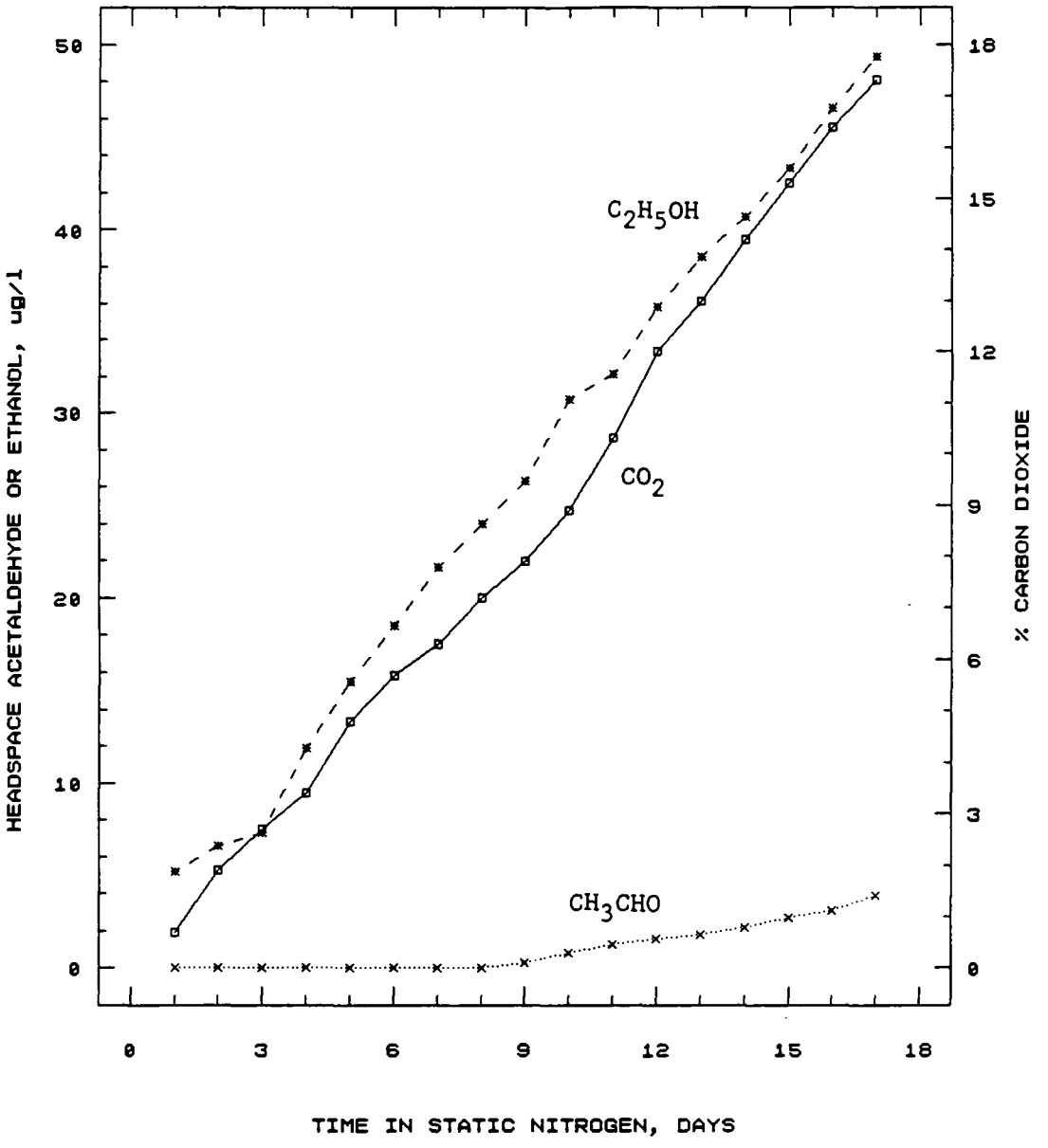

Fig. VI.1. Accumulated headspace acetaldehyde, ethanol and CO_2 concentrations of 'Blue Jay' blueberries in static N_2 at 0°C. Each point represents the mean of 4 observations.

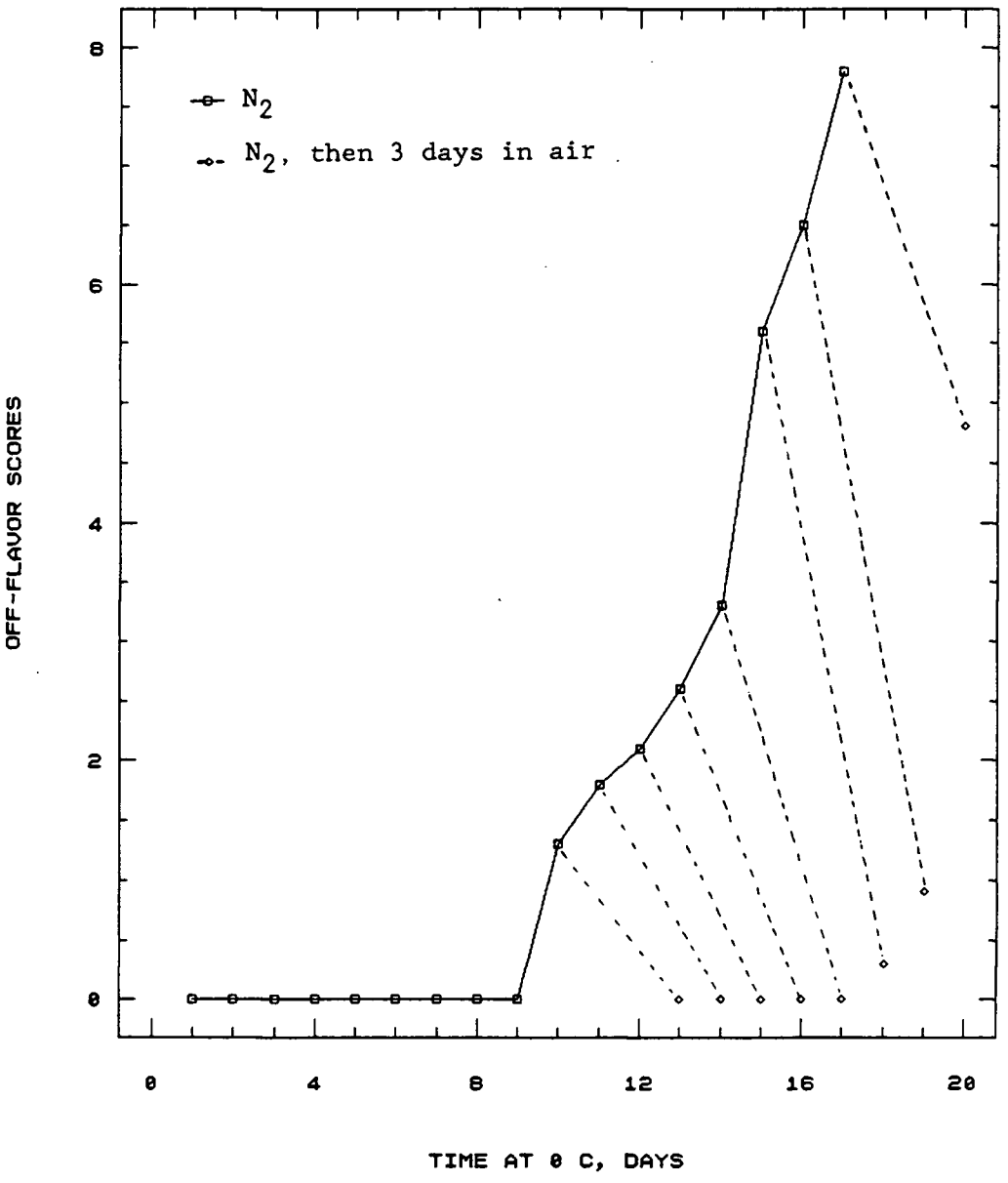

Fig. VI.2. Off-flavor (scale 0 to 8, 0 = no off flavor) development of 'Blue Jay' blueberries in static N_2 at 0°C, and partial reversal by transfer to air storage for 3 days. Each point represents the mean of 10 observations.

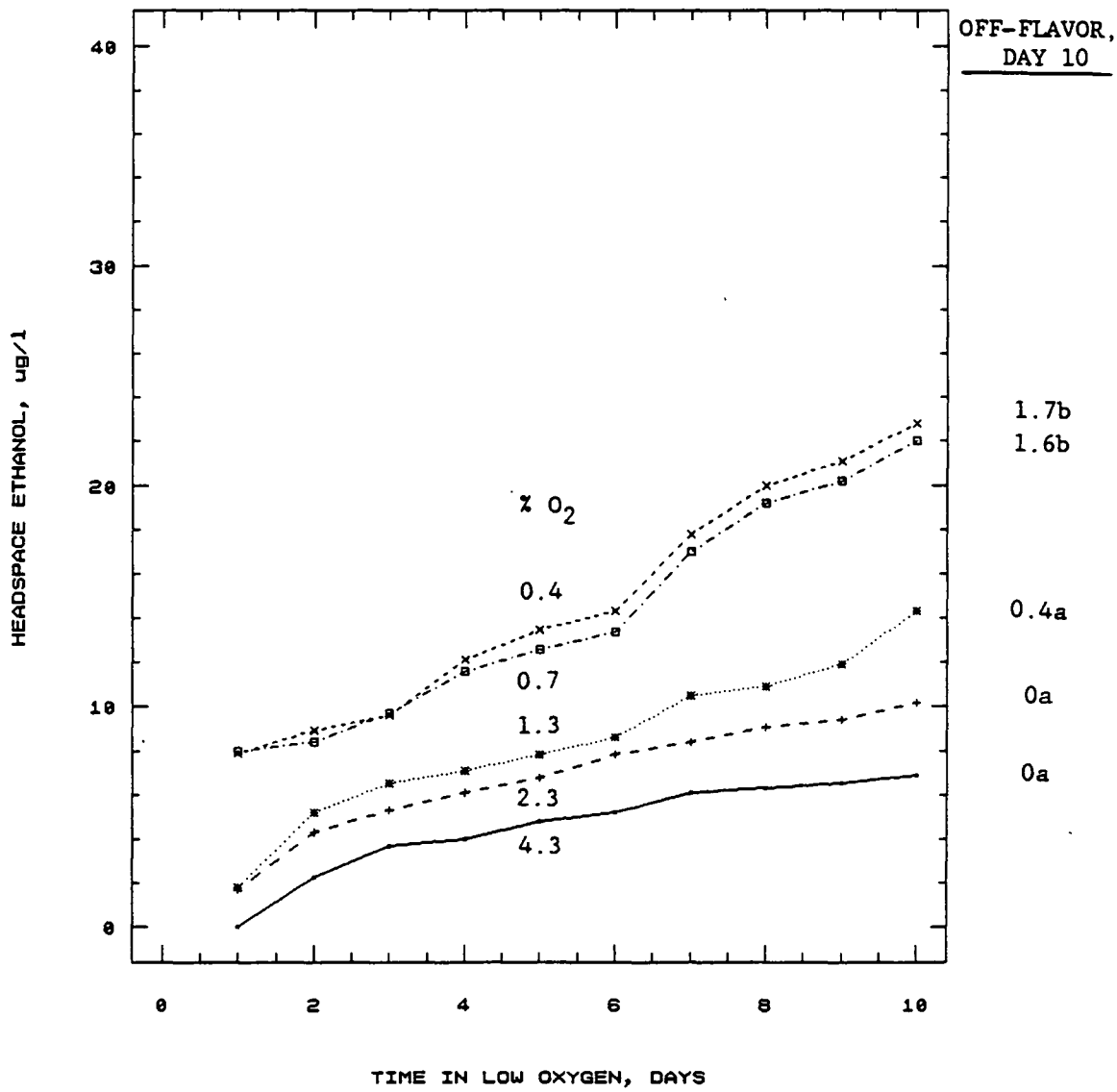

Fig. VI.3. Headspace ethanol of 'Blue Jay' blueberries in 0°C storage with continuous flow (10 ml/min) low-O_2 atmospheres; and off-flavor scores (0 to 8, 0 = no off flavor) after 10 days. Each point represents the mean of 2 observations for ethanol and 10 observations for off-flavor scores by the sensory panel. Mean separation by Duncan's multiple range test, 5% level.

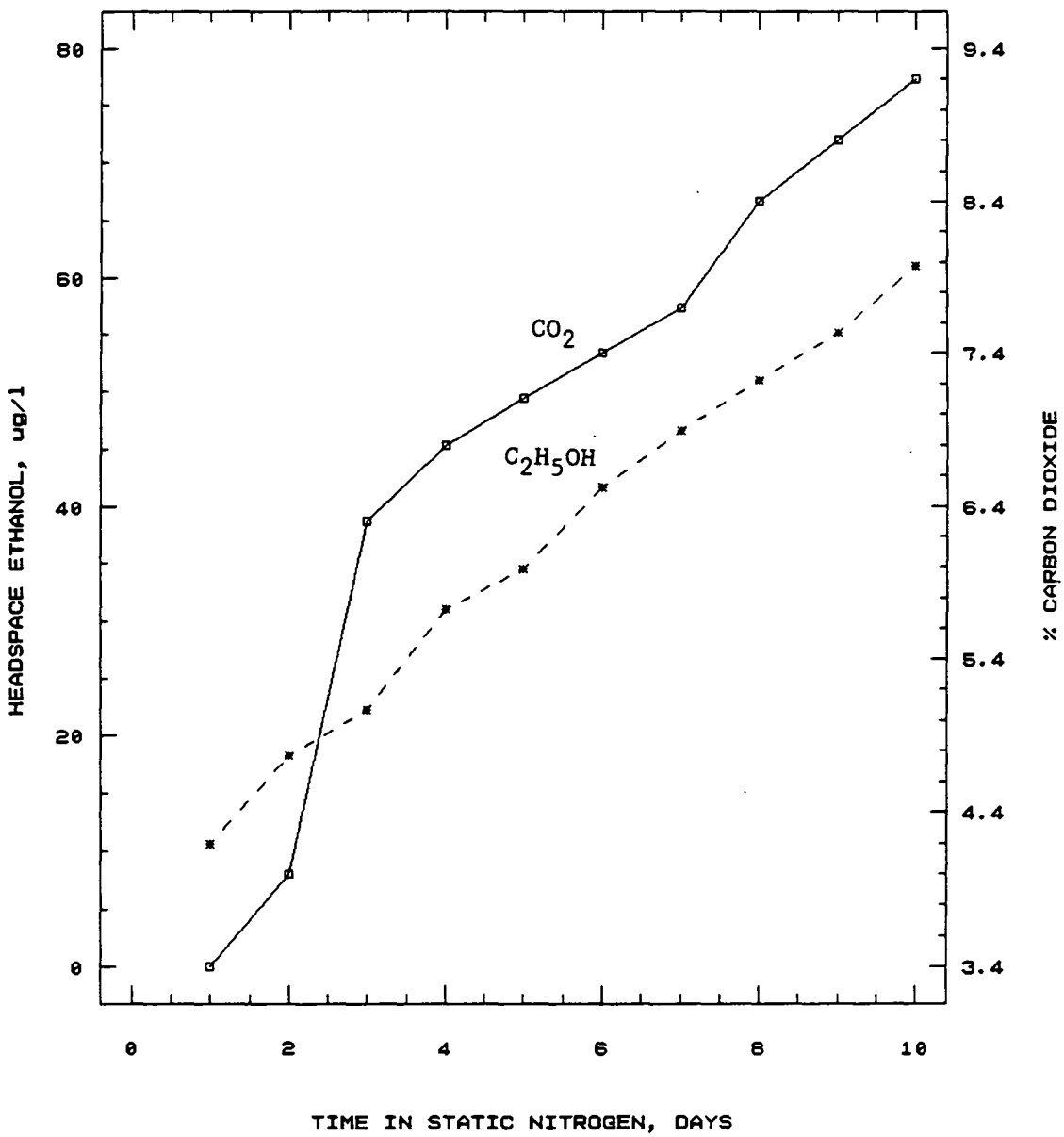

Fig. VI.4. Accumulated headspace ethanol and CO_2 concentrations of 'Amity' red raspberries in static N_2 at 0°C. Each point represents the mean of 4 observations.

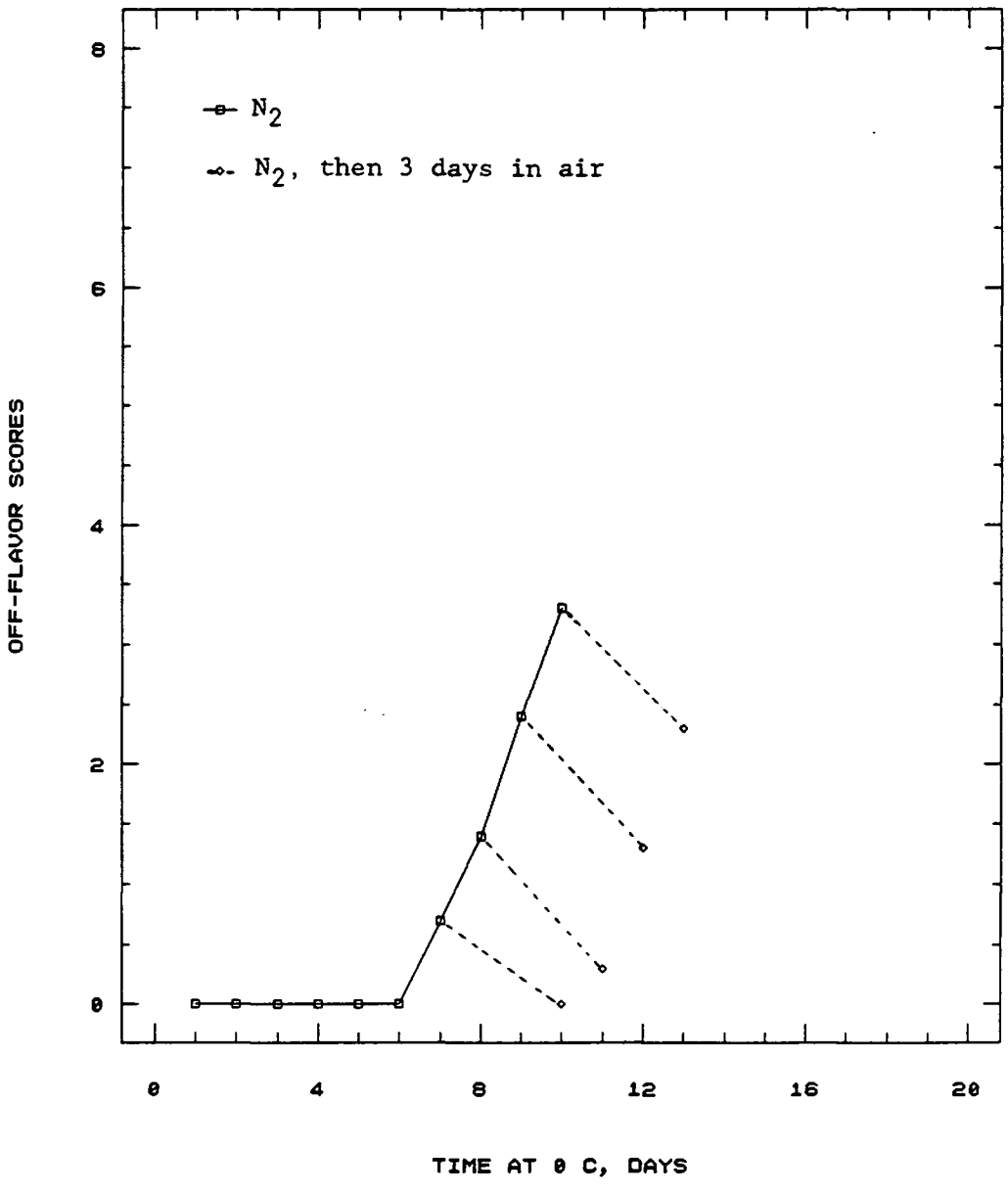

Fig. VI.5. Off-flavor (scale 0 to 8, 0 = no off flavor) development of 'Amity' red raspberries in static N_2 at 0°C, and partial reversal by transfer to air storage for 3 days. Each point represents the mean of 10 observations.

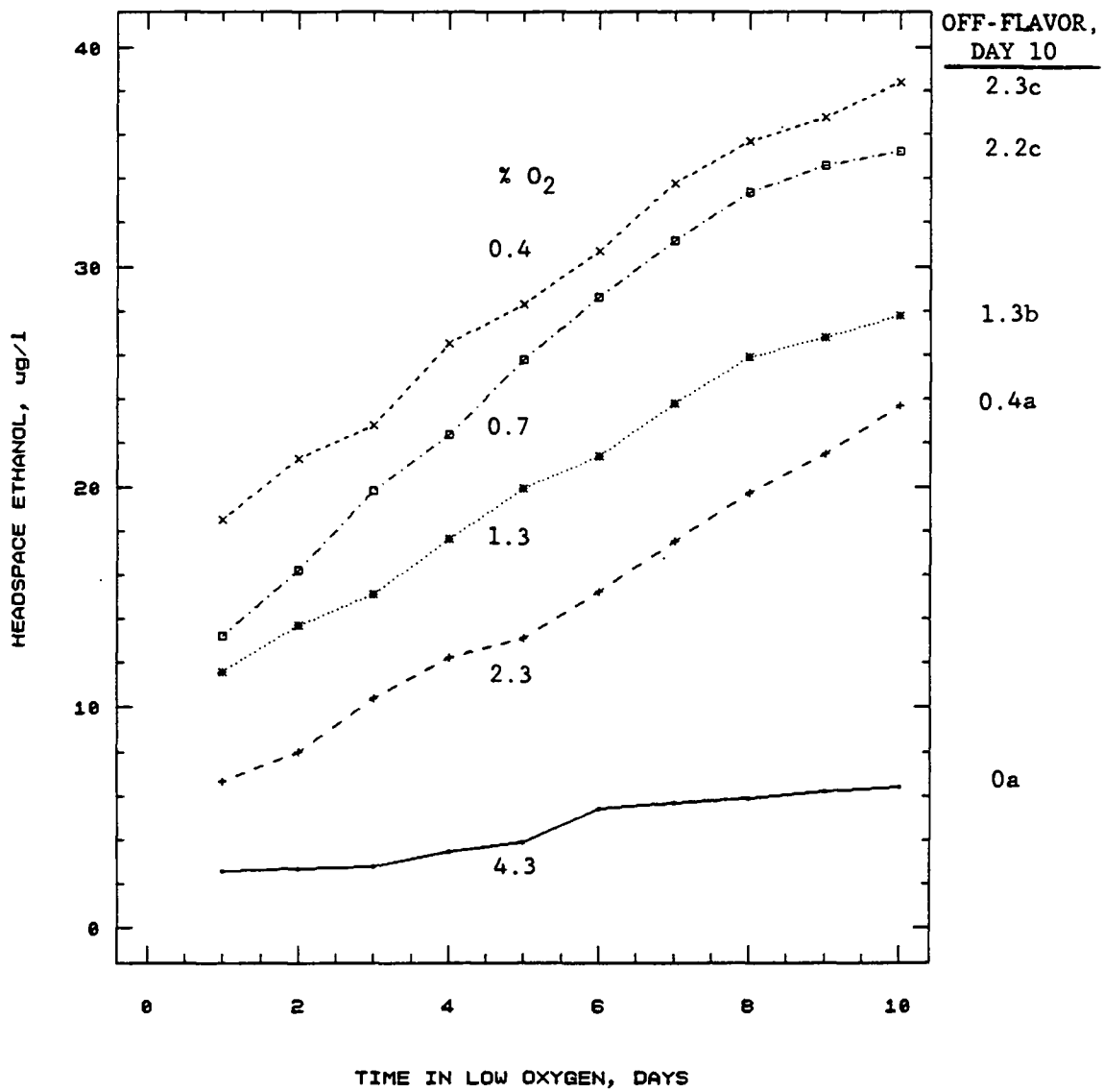

Fig. VI.6. Headspace ethanol of 'Amity' red raspberries in 0°C storage with continuous flow (10 ml/min) low-O_2 atmospheres; and off-flavor scores (0 to 8, 0 = no off flavor) after 10 days. Each point represents the mean of 2 observations for ethanol and 10 observations for off-flavor scores by the sensory panel. Mean separation by Duncan's multiple range test, 5% level.

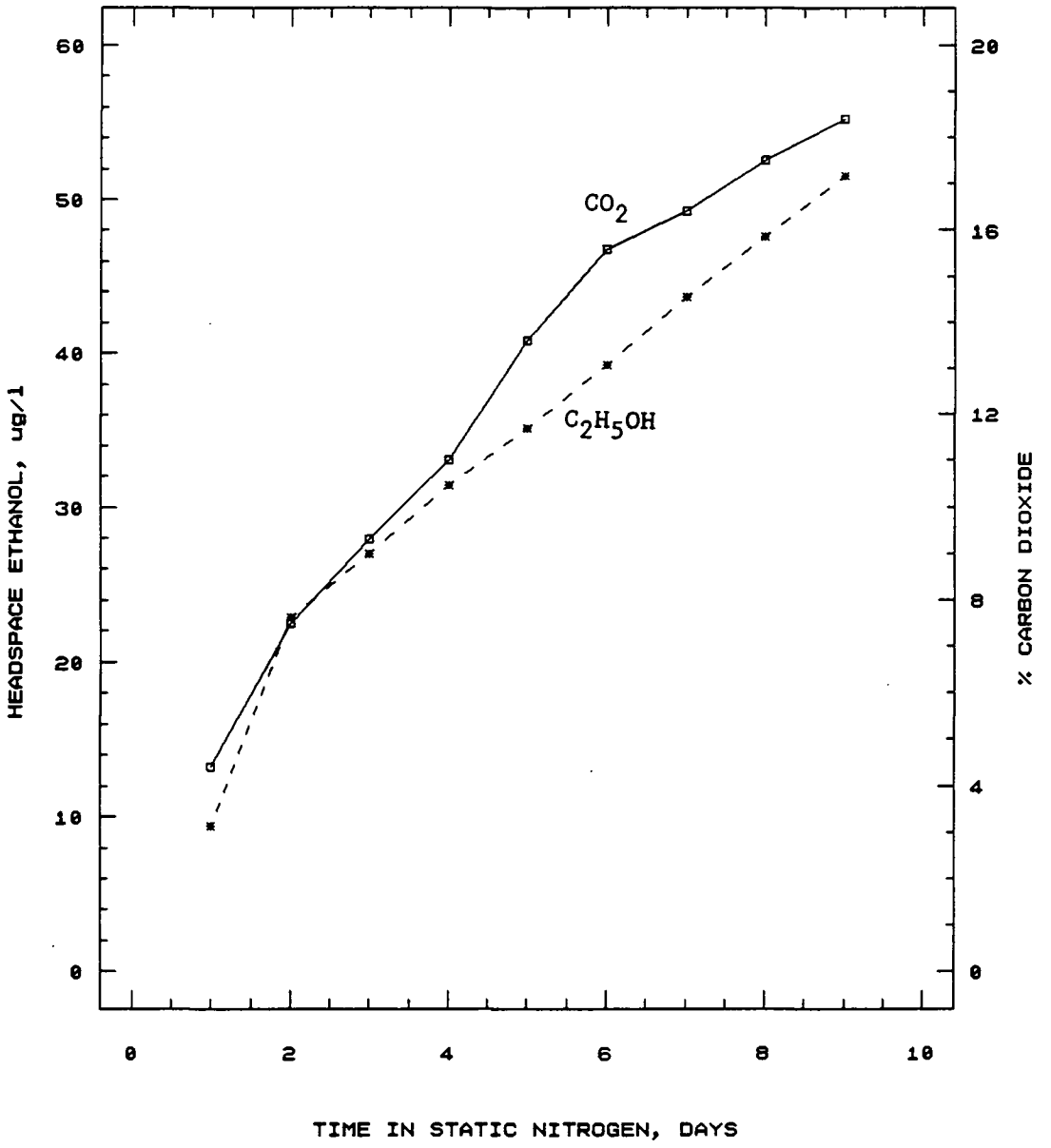

Fig. VI.7. Accumulated headspace ethanol and CO_2 concentrations of 'Marion' blackberries in static N_2 at 0°C. Each point represents the mean of 4 observations.

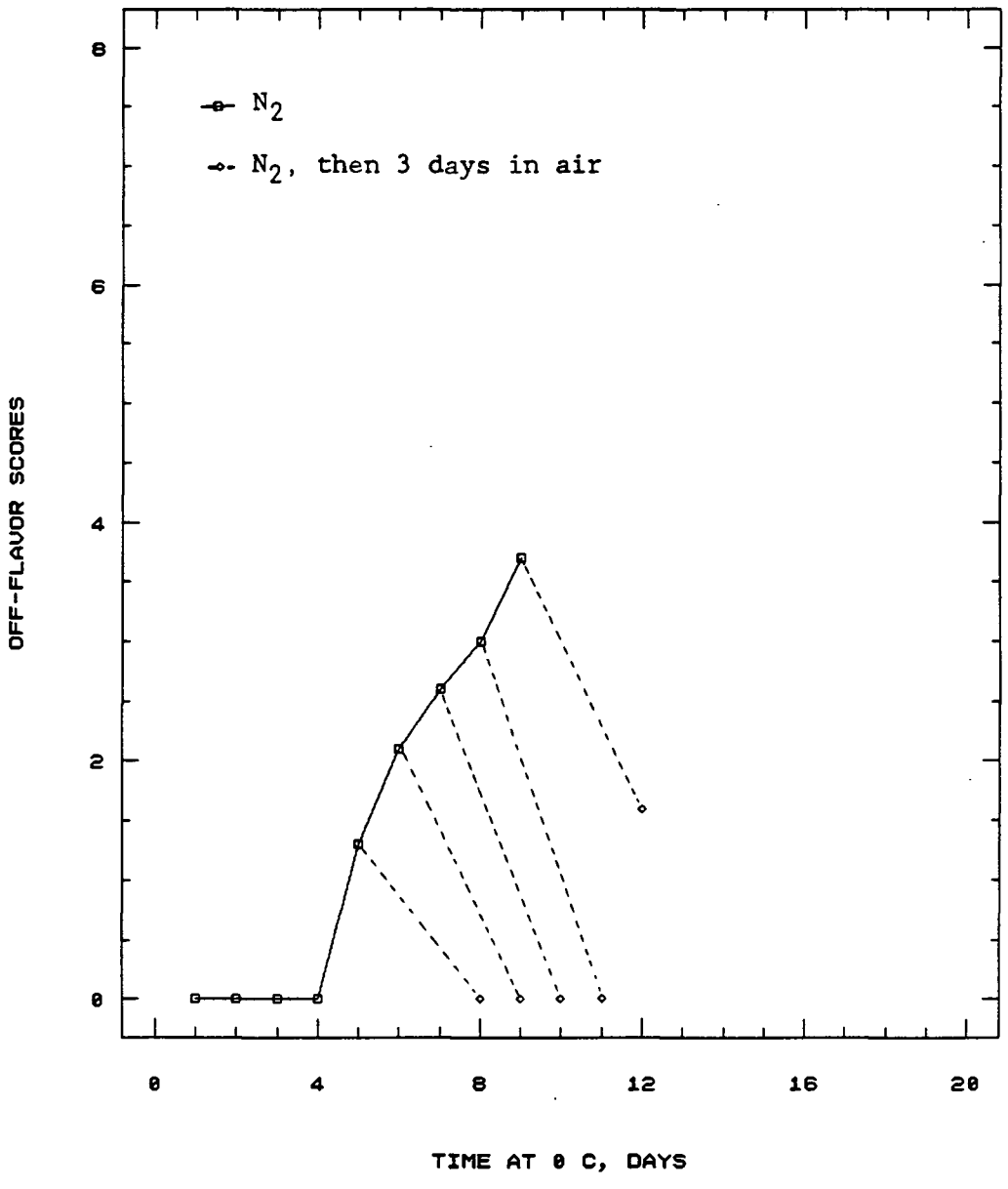

Fig. VI.8. Off-flavor (scale 0 to 8, 0 = no off flavor) development of 'Marion' blackberries in static N_2 at 0°C and partial reversal by transfer to air storage for 3 days. Each point represents the mean of 10 observations.

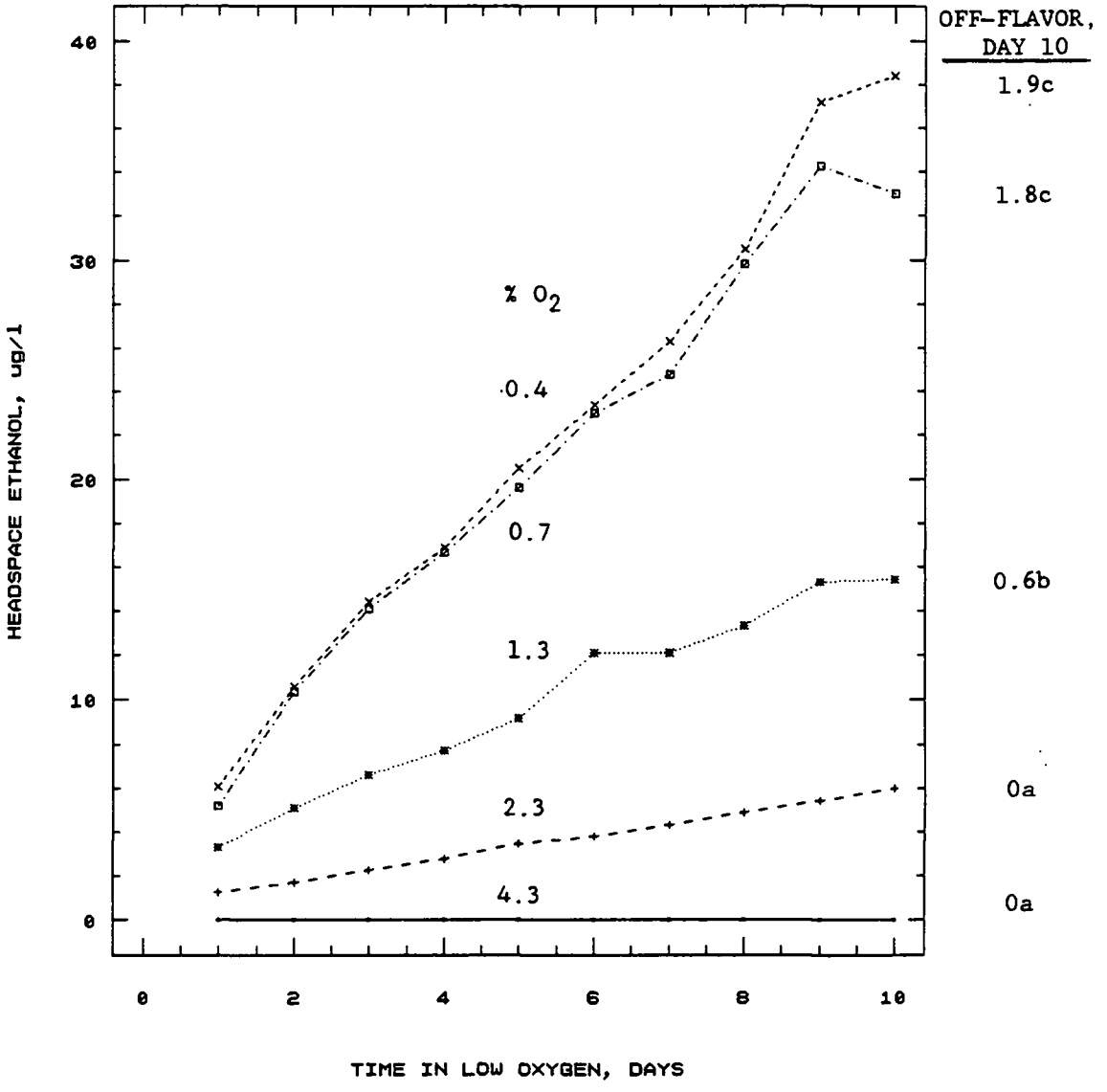

Fig. VI.9. Headspace ethanol of 'Marion' blackberries in 0°C storage with continuous flow (10 ml/min) low-O_2 atmospheres; and off-flavor scores (0 to 8, 0 = no off flavor) after 10 days. Each point represents the mean of 2 observations for ethanol and 10 observations for off-flavor scores by the sensory panel. Mean separation by Duncan's multiple range test, 5% level.

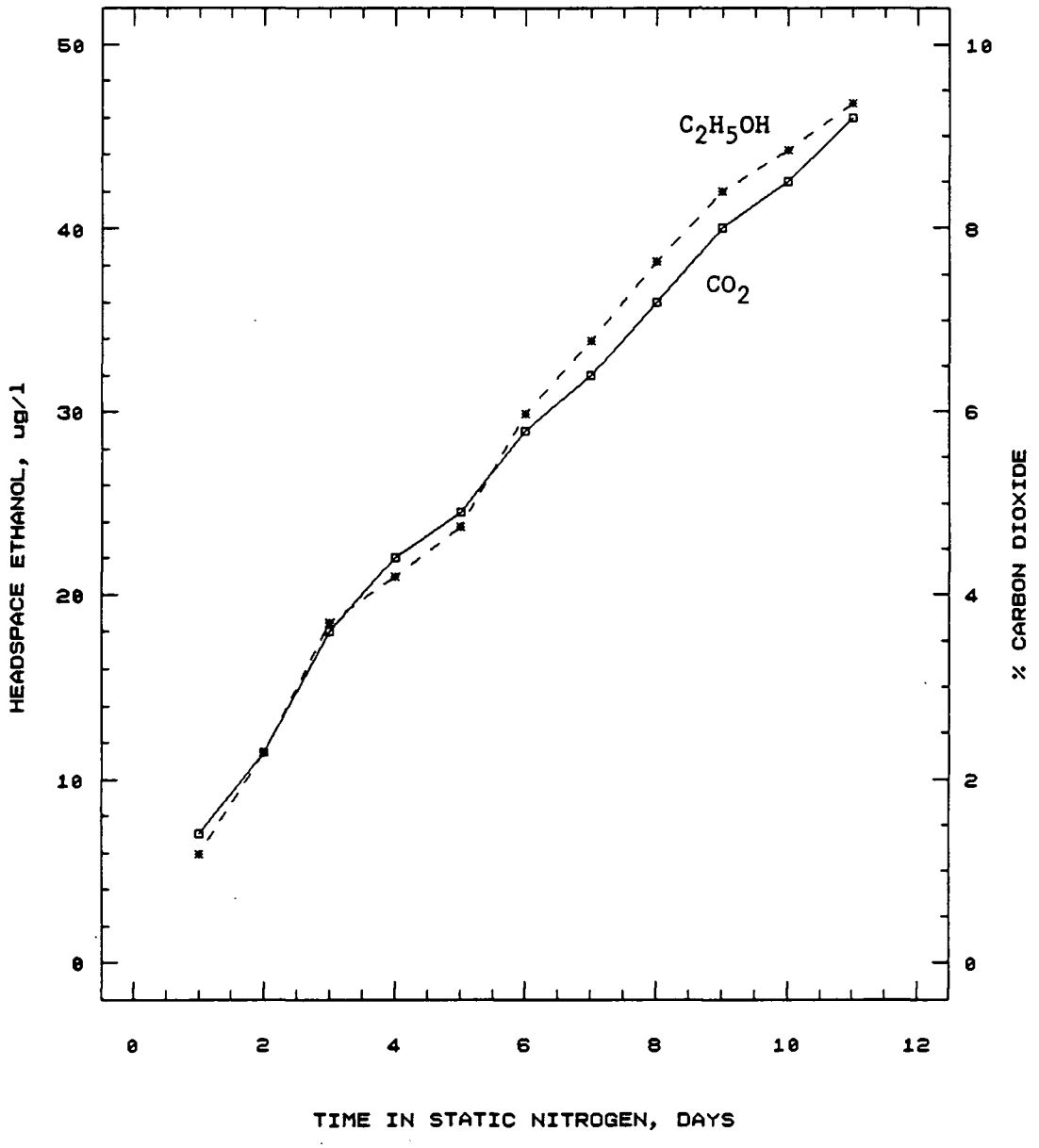

Fig. VI.10. Accumulated headspace ethanol and CO_2 concentrations of 'Italian' plums in static N_2 at 0°C. Each point represents the mean of 4 observations.

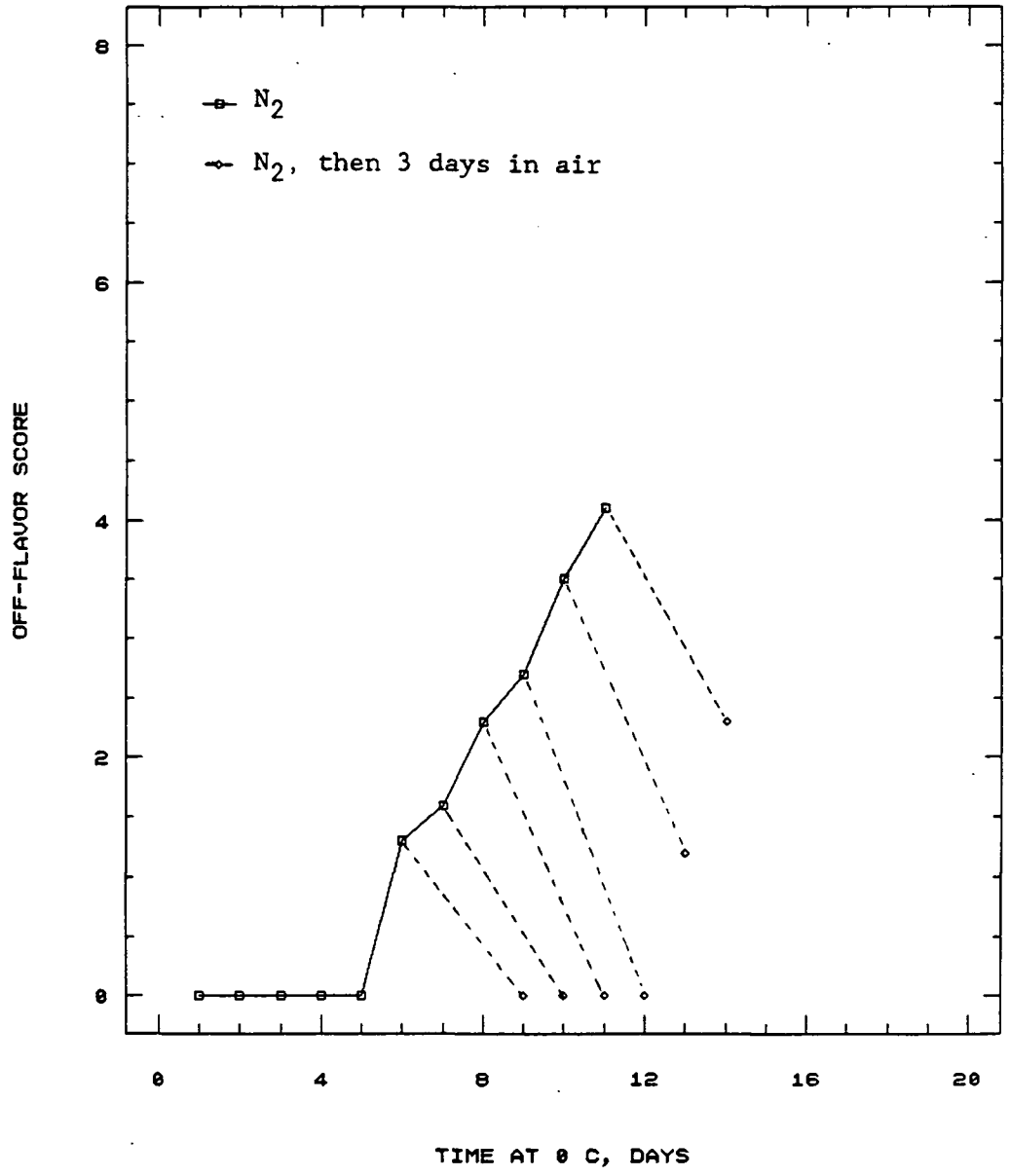

Fig. VI.11. Off-flavor (scale 0 to 8, 0 = no off flavor) development of 'Italian' plums in static N_2 at 0°C, and partial reversal by transfer to air storage for 3 days. Each point represents the mean of 10 observations.

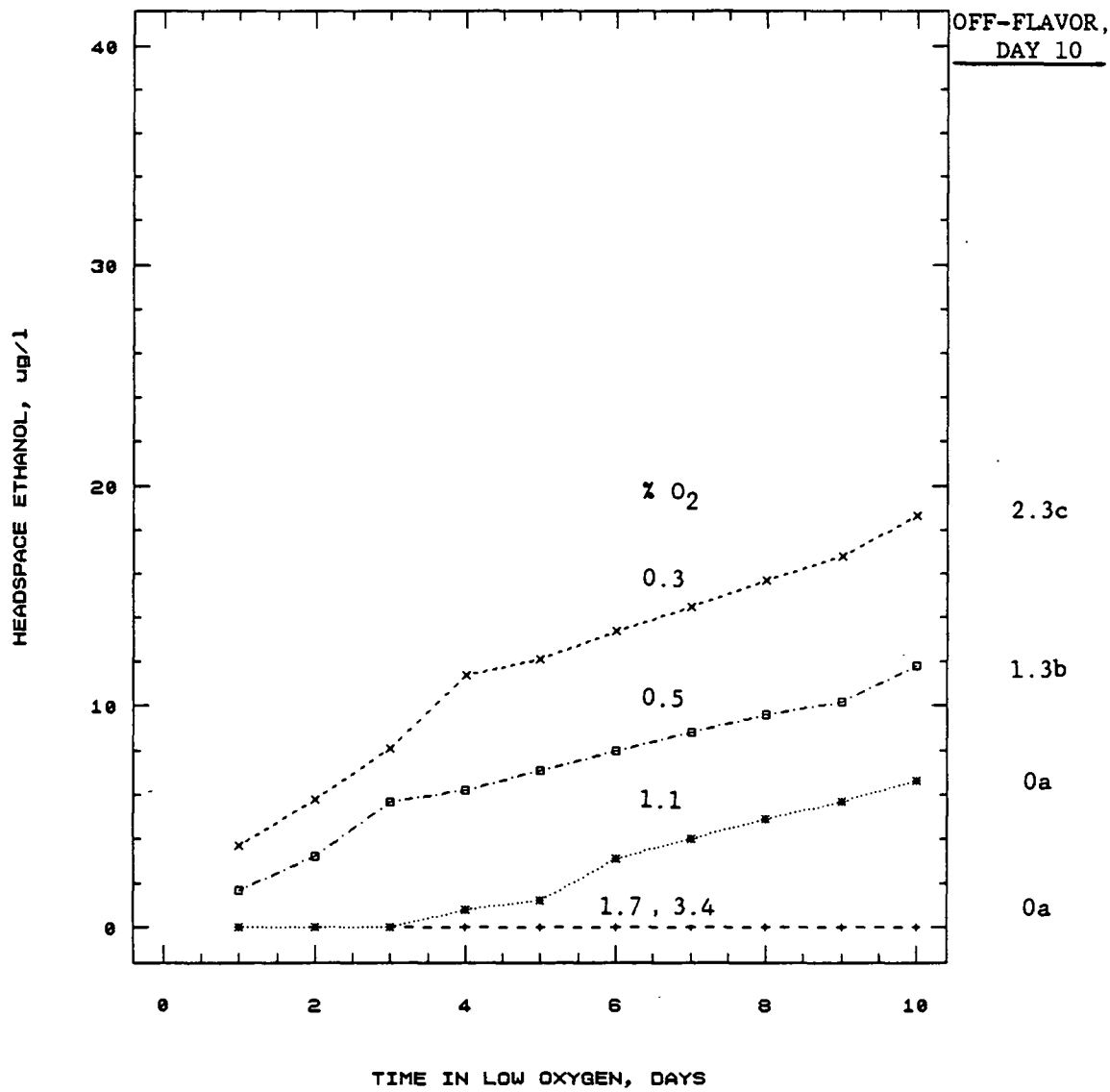

Fig. VI.12. Headspace ethanol of 'Italian' plums in 0°C storage with continuous flow (10 ml/min) low-O_2 atmospheres; and off-flavor scores (0 to 8, 0 = no off flavor) after 10 days. Each point represents the mean of 2 observations for ethanol and 10 observations for off-flavor scores by the sensory panel. Mean separation by Duncan's multiple range test, 5% level.

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CONCLUSIONS

Major contributions of this thesis research are as follows:

1. Under static N_2 (anaerobic) conditions at 0°C, these fruits can be held for the following periods of time before developing off-flavors.

	days	
'Marion' blackberries	4	(8)
'Italian' plums	5	(9)
'Amity' red raspberries	7	(8)
'Blue Jay' blueberries	9	(16)
'Bartlett' pears	11	(22)
'Golden Delicious' apples	20	(22)
'Granny Smith' apples	beyond 25	
'Anjou' pears	beyond 25	

The numbers in parentheses are the days in anaerobic conditions which allowed off-flavors to be reversed to an acceptable level (less than a score of 1) when the fruits were held in air for 3 (berries and plums) and 7 days (apples and pears).

2. Complete mortality of apple maggot eggs and larvae in mature 'Golden Delicious' apples was accomplished after holding infested fruits in air at 0°C for 35 days, or in static N_2 at 0° and 20°C for 24 and 7 days, respectively.

3. Anaerobically stored apples and pears which had developed off-flavors, completely or partially lost those off-flavors after

return to air for 7 days, yet retained almost all of the accumulated acetaldehyde and ethanol.

4. Therefore, acetaldehyde and ethanol are not themselves responsible for off-flavors. However, metabolites or some other compounds as yet unidentified must be the off-flavors.

5. Exposure of apples and pears to air after holding in anaerobic (static N_2) conditions did not change pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities.

6. Twenty-four days of exposure to as low as 0.3% O_2 at 0°C in a flow-through system did not cause any adverse effects on 'Granny Smith' apples and 'Anjou' pears.

7. 'Blue Jay' blueberries, 'Amity' red raspberries, and 'Marion' blackberries held in $\leq$1.3% O_2 and 'Italian' plums held in $\leq$0.5% O_2 at 0°C in a flow-through system for 10 days developed slight off-flavors which dissipated after 3 days in air.

Since 'Anjou' pears and 'Golden Delicious' apples did not develop off-flavors up to 25 days in static N_2 at 0°C, this treatment could be used to kill apple maggot which had 100% mortality in 24 days. However, it is important to realize the differences in varietal responses because the same treatment would not be safe for 'Golden Delicious' apples and 'Bartlett' pears which developed unacceptable off-flavors when the storage periods were beyond 22 days. One would also expect that other quarantined insect pests would need to be evaluated relative to their tolerance to anaerobic conditions. If a storage period is not a matter of concern, refrigerated storage at 0°C for 45 days still

remains an effective and safe treatment for fruits that can tolerate low temperature stress. A combination of 0.3% O_2 and 1.4% CO_2 can be used as a quarantine procedure in 'Granny Smith' apples and 'Anjou' pears for controlling any quarantined insects, provided complete insect mortality is accomplished in 24 days or less.

Among the small fruits examined, blueberries were the most tolerant to anaerobic off-flavors at 0°C, followed by raspberries and blackberries. Plums could tolerate such conditions for 5 days. Small fruits could be stored in $\geq$1.3% O_2, and plums in $\geq$0.5% O_2 at 0°C for 10 days without developing unacceptable off-flavors. When the potential for reversing or dissipating off-flavors is considered, they could possibly be held beyond 10 days. This information is useful to modified atmosphere packaging, or to researchers who wish to further investigate the effects of anaerobic conditions and low-O_2 atmospheres on other quarantined insects in case such needs arise in the future, particularly in fruits destined for the international market.

CHAPTER VIII

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APPENDICES

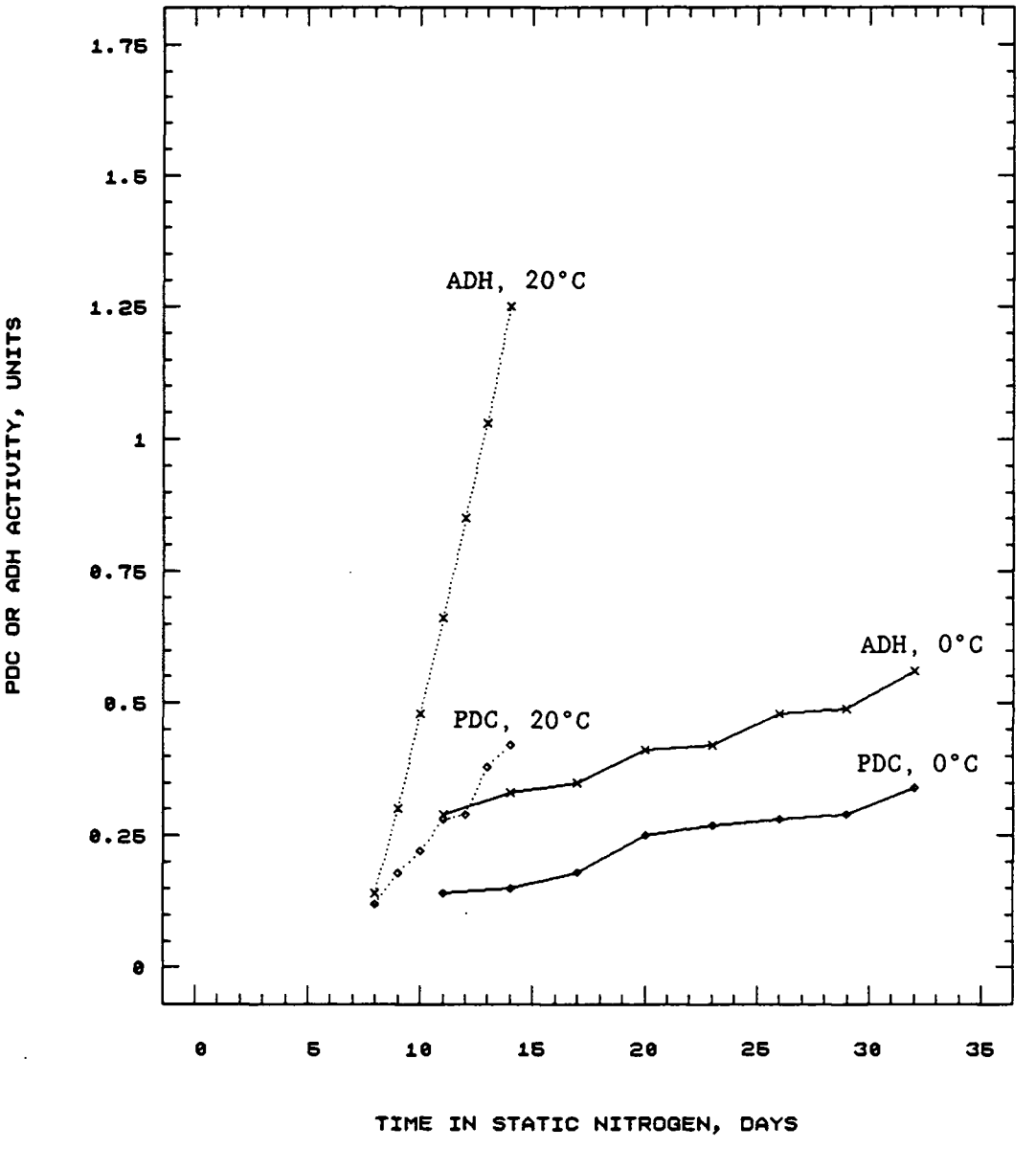

Fig. A.1. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Golden Delicious' apples under static N_2 at 0° and 20°C, followed by 7 days in air. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

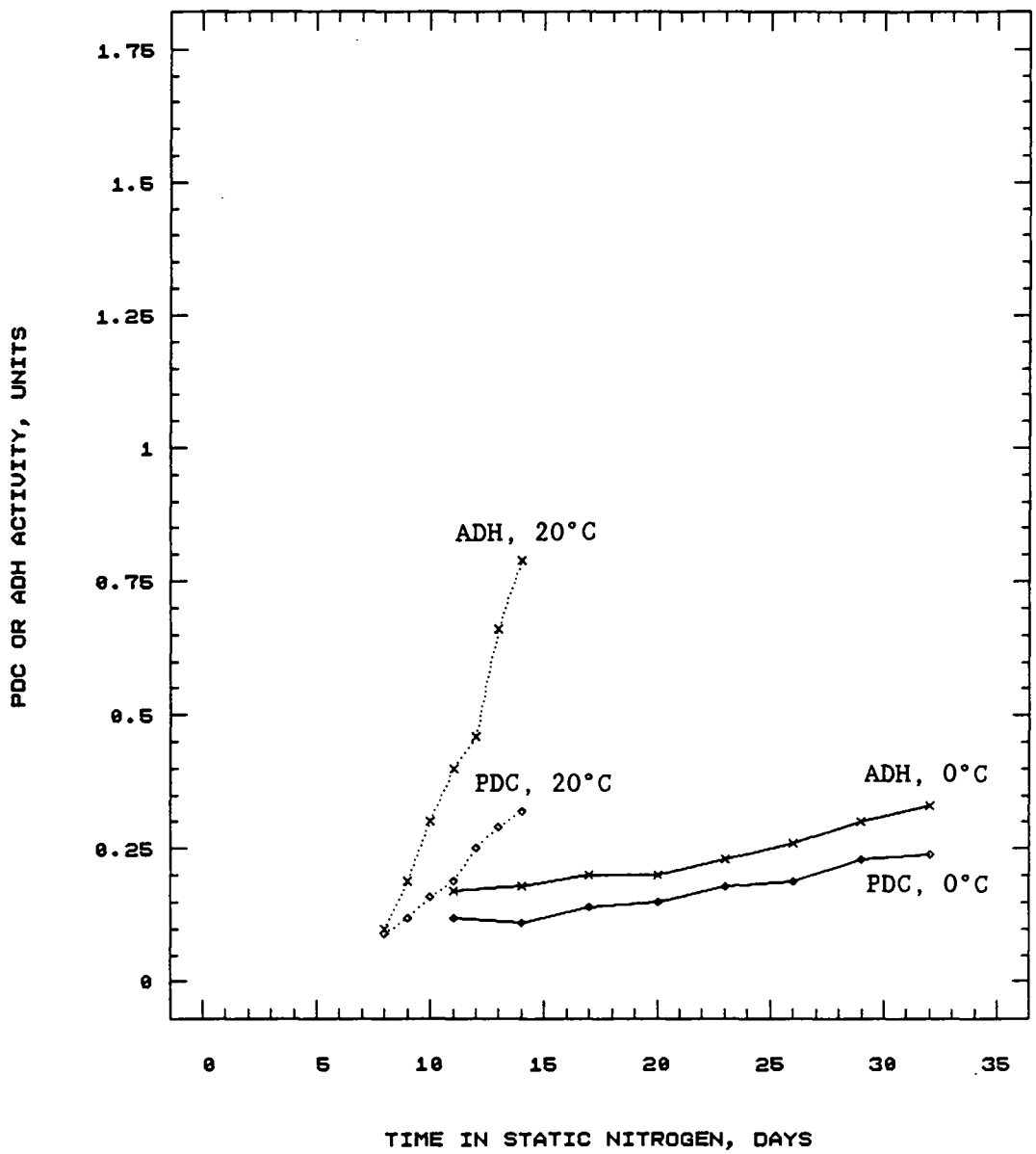

Fig. A.2. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Granny Smith' apples under static N_2 at 0° and 20°C, followed by 7 days in air. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

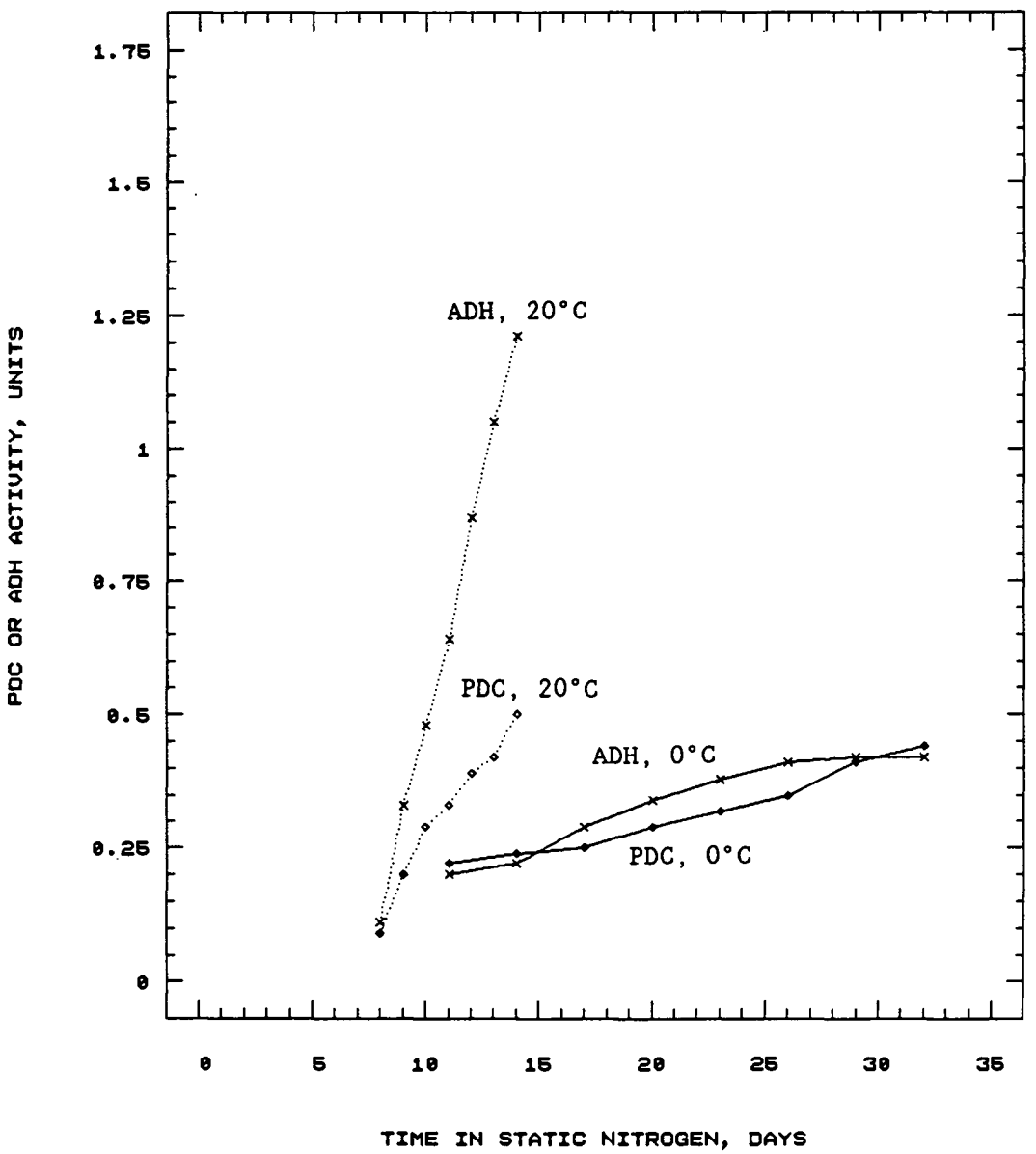

Fig. A.3. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Anjou' pears under static N_2 at 0° and 20°C, followed by 7 days in air. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

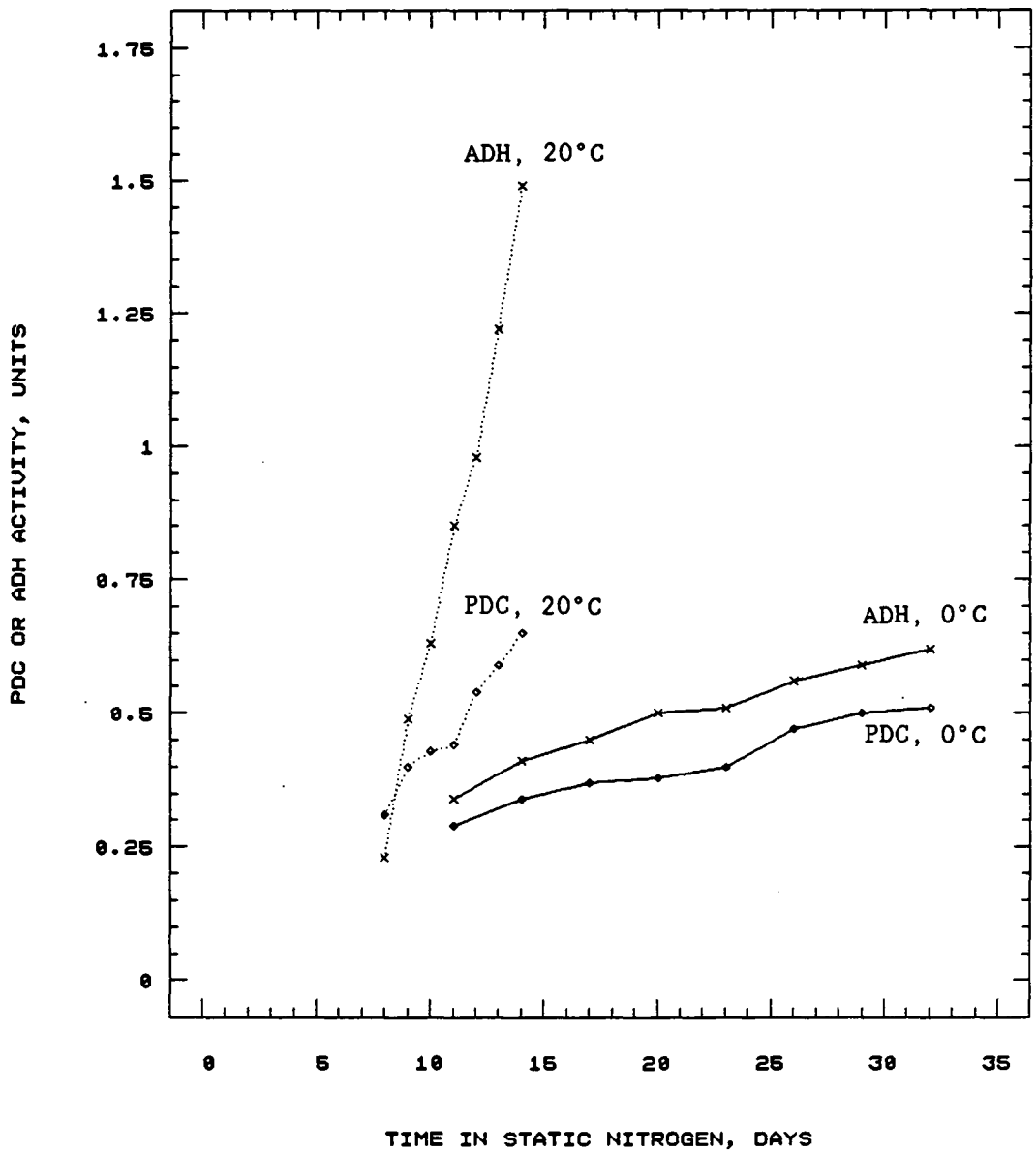

Fig. A.4. Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) activities in 'Bartlett' pears under static N_2 at 0° and 20°C, followed by 7 days in air. A unit is equivalent to 1 umol NADH oxidized/min/mg protein. Each point represents the mean of 3 observations.

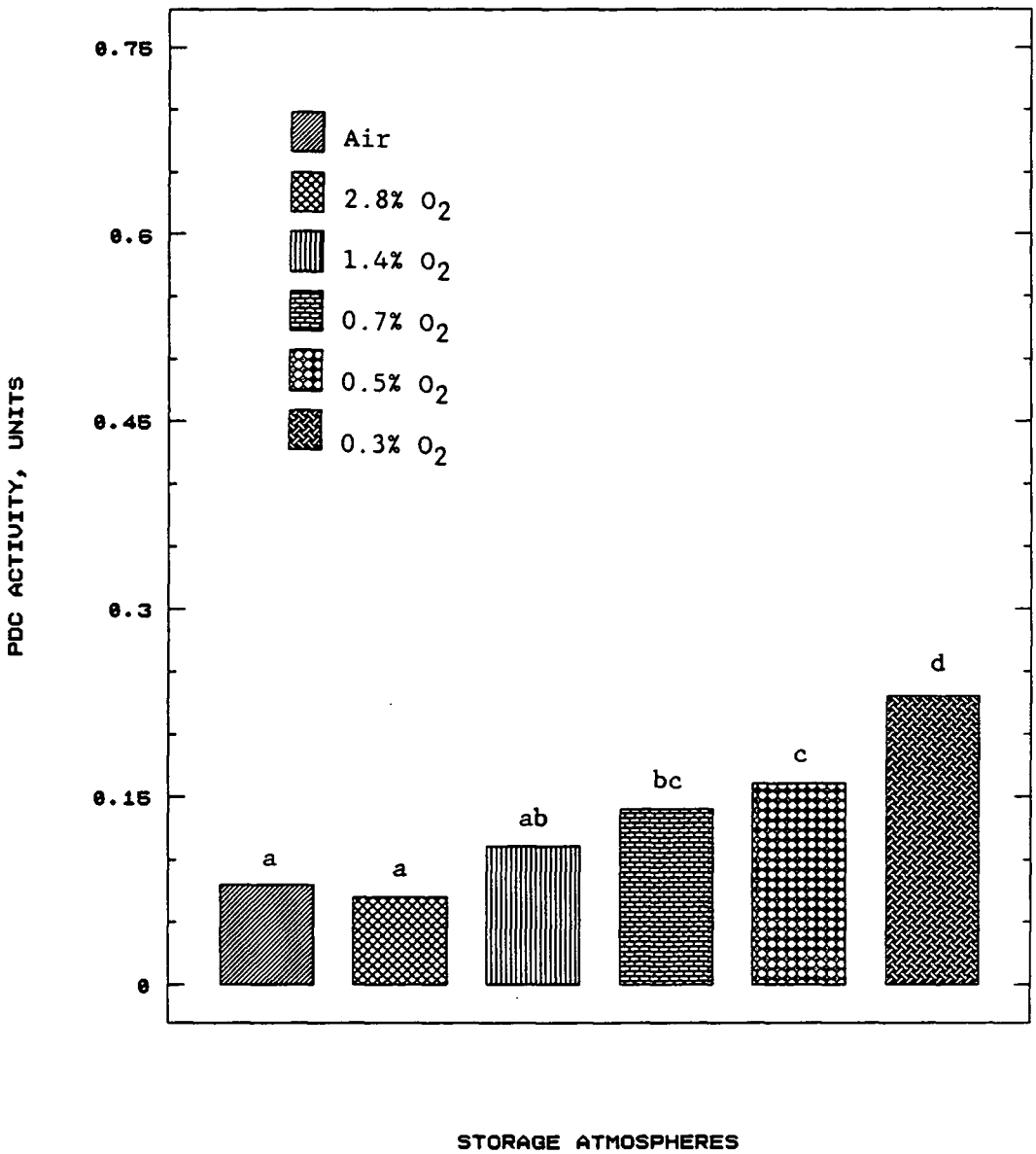

Fig. B.1. Pyruvate decarboxylase (PDC) activity of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

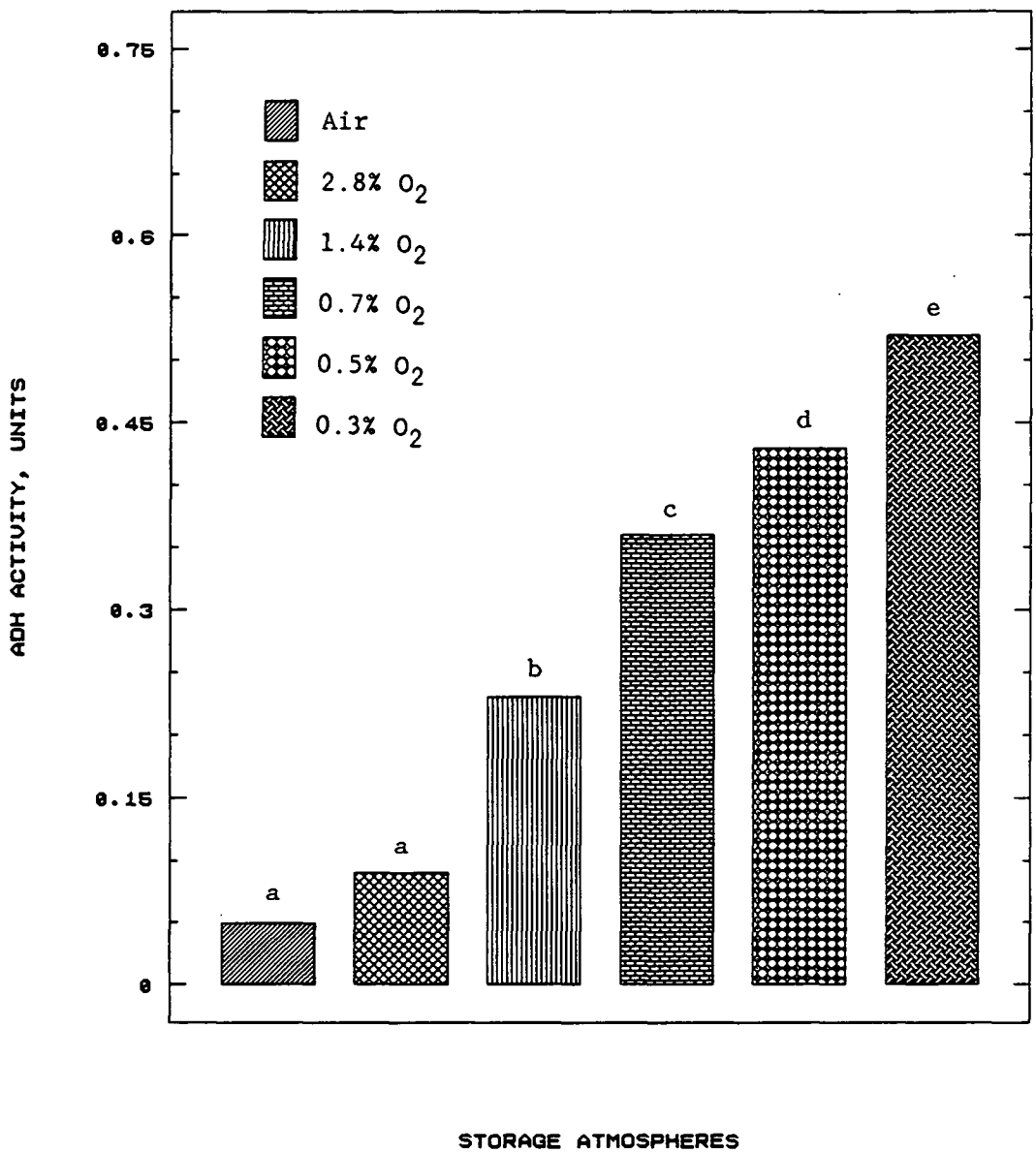

Fig. B.2. Alcohol dehydrogenase (ADH) activity of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

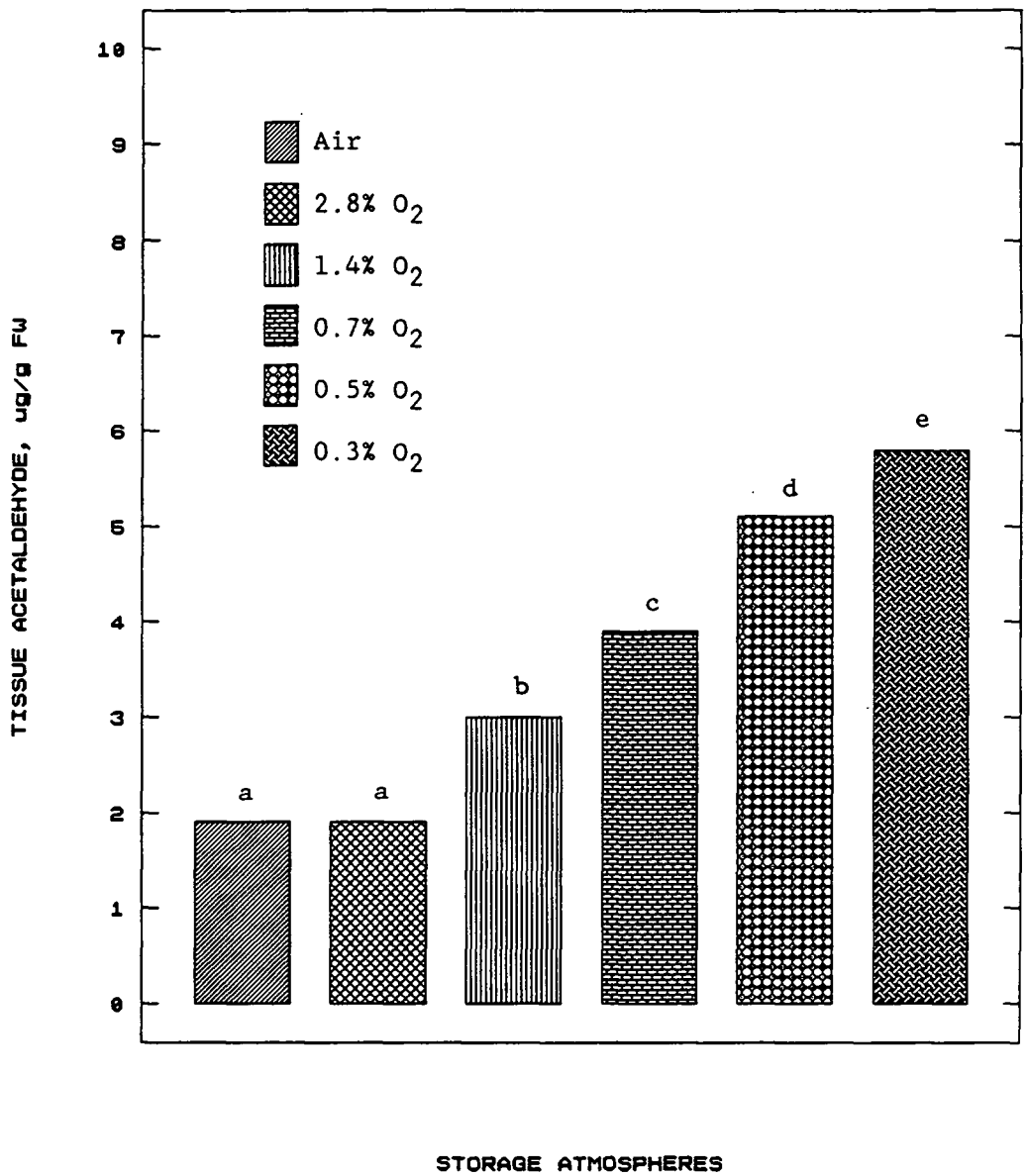

Fig. B.3. Tissue acetaldehyde of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

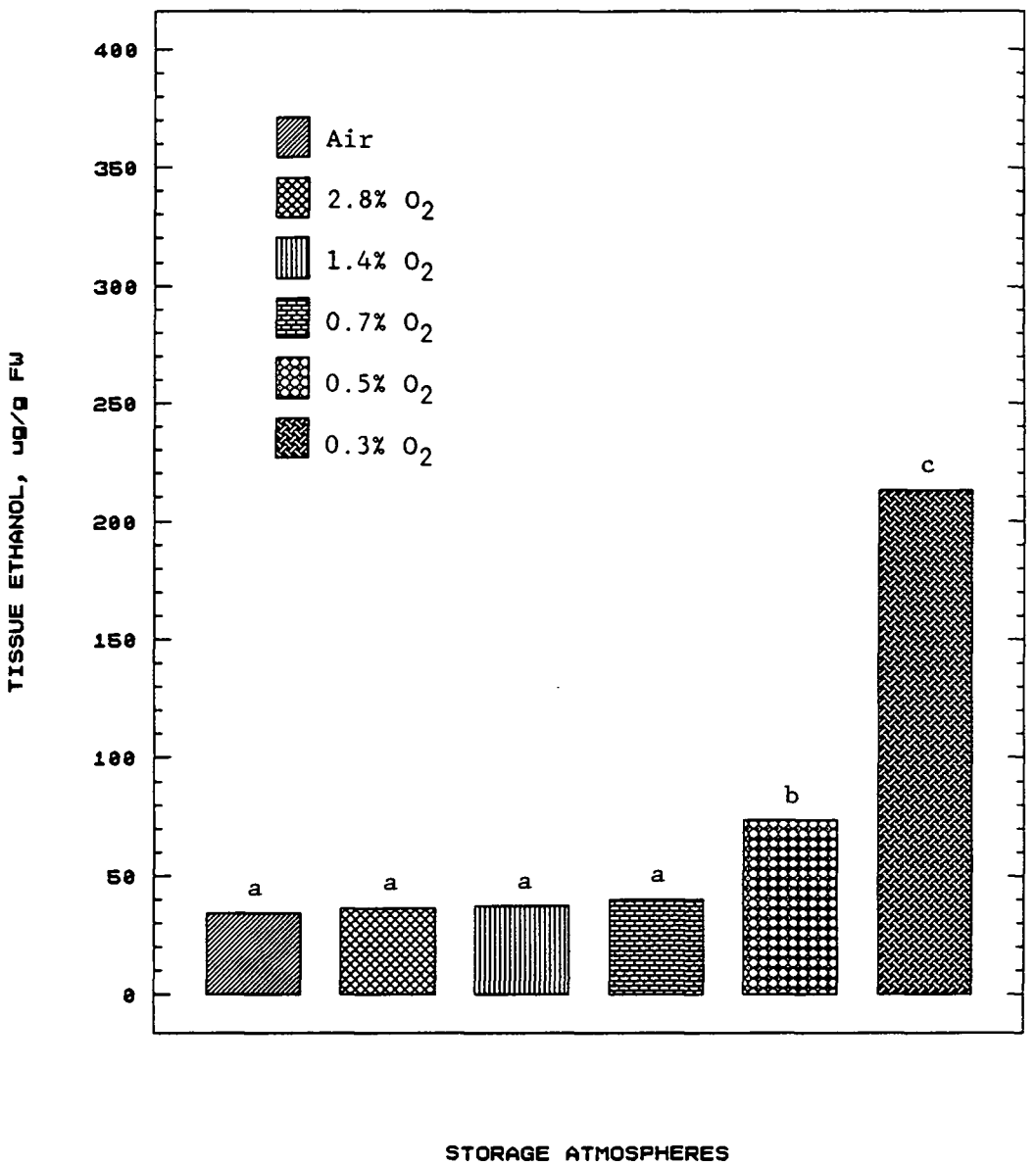

Fig. B.4. Tissue ethanol of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

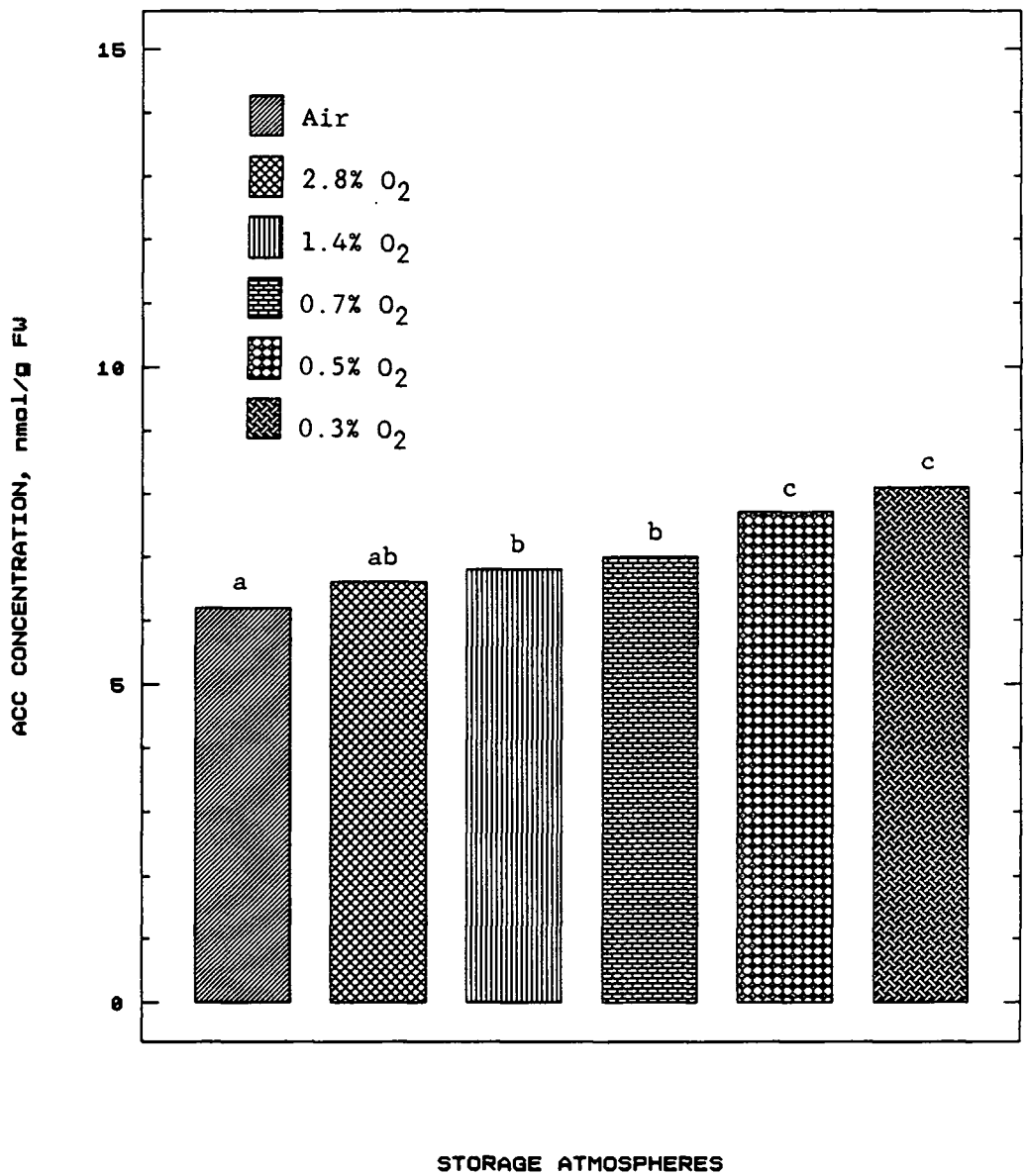

Fig. B.5. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

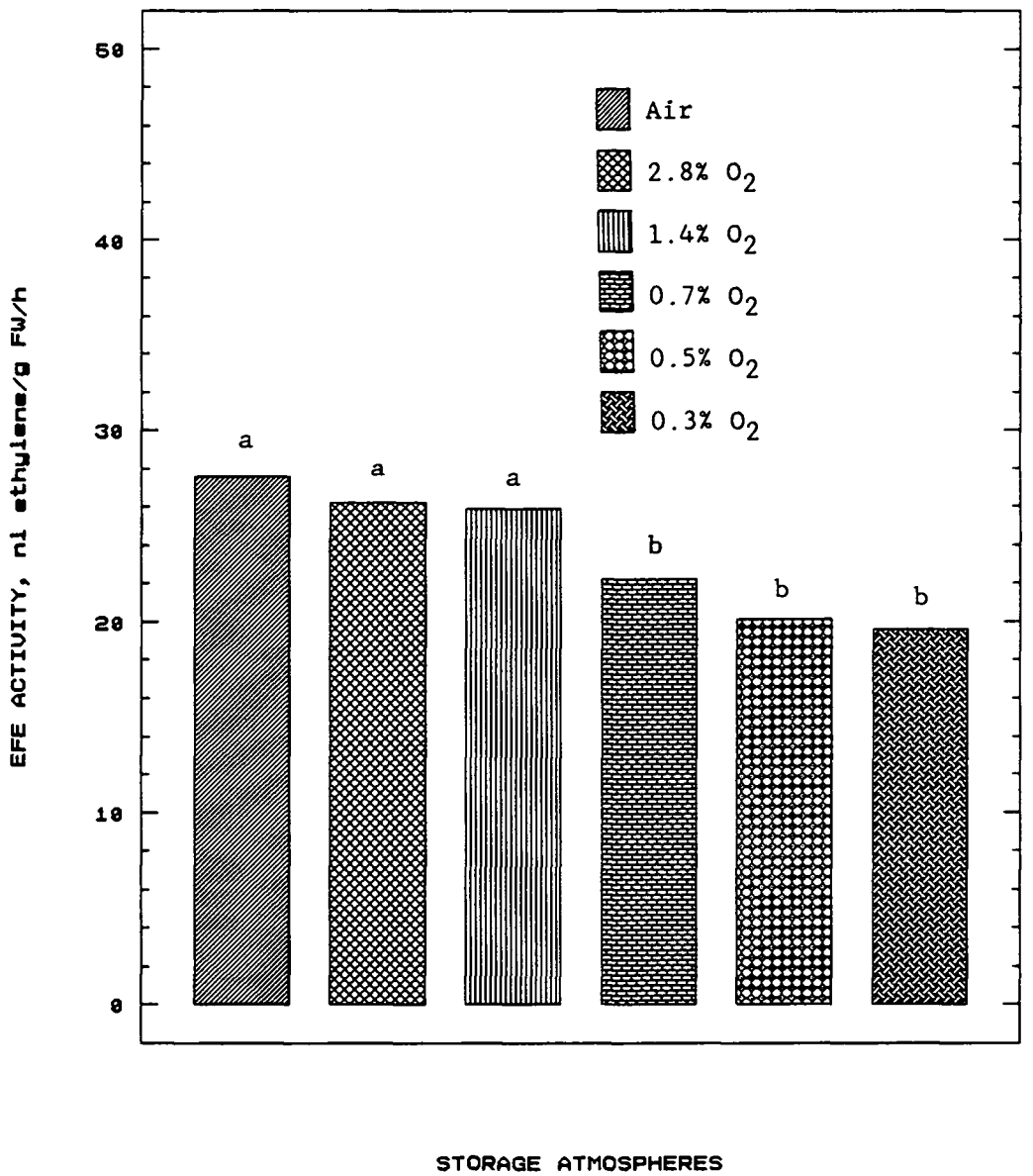

Fig. B.6. Ethylene forming enzyme (EFE) activity of 'Granny Smith' apples after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

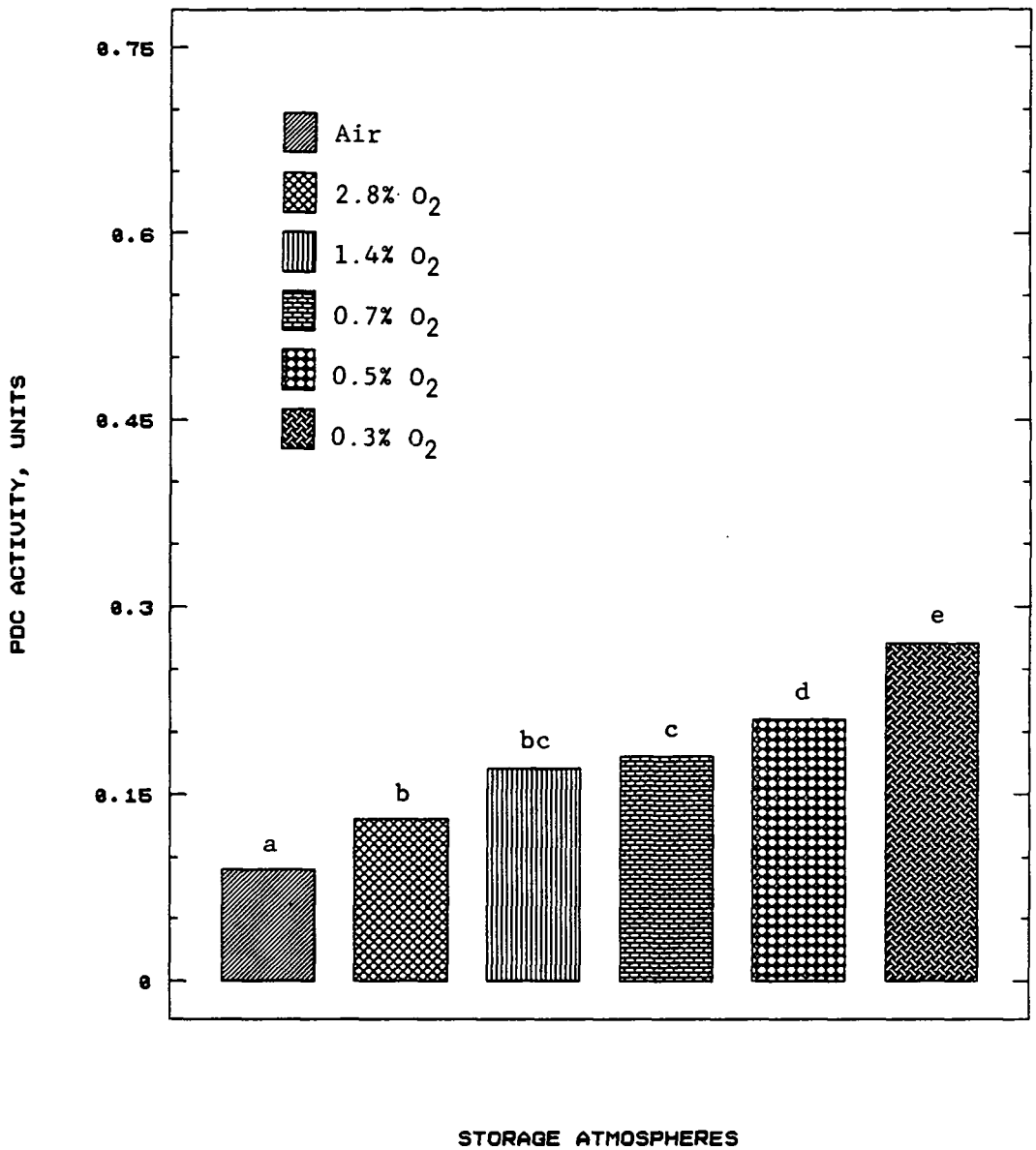

Fig. B.7. Pyruvate decarboxylase (PDC) activity of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

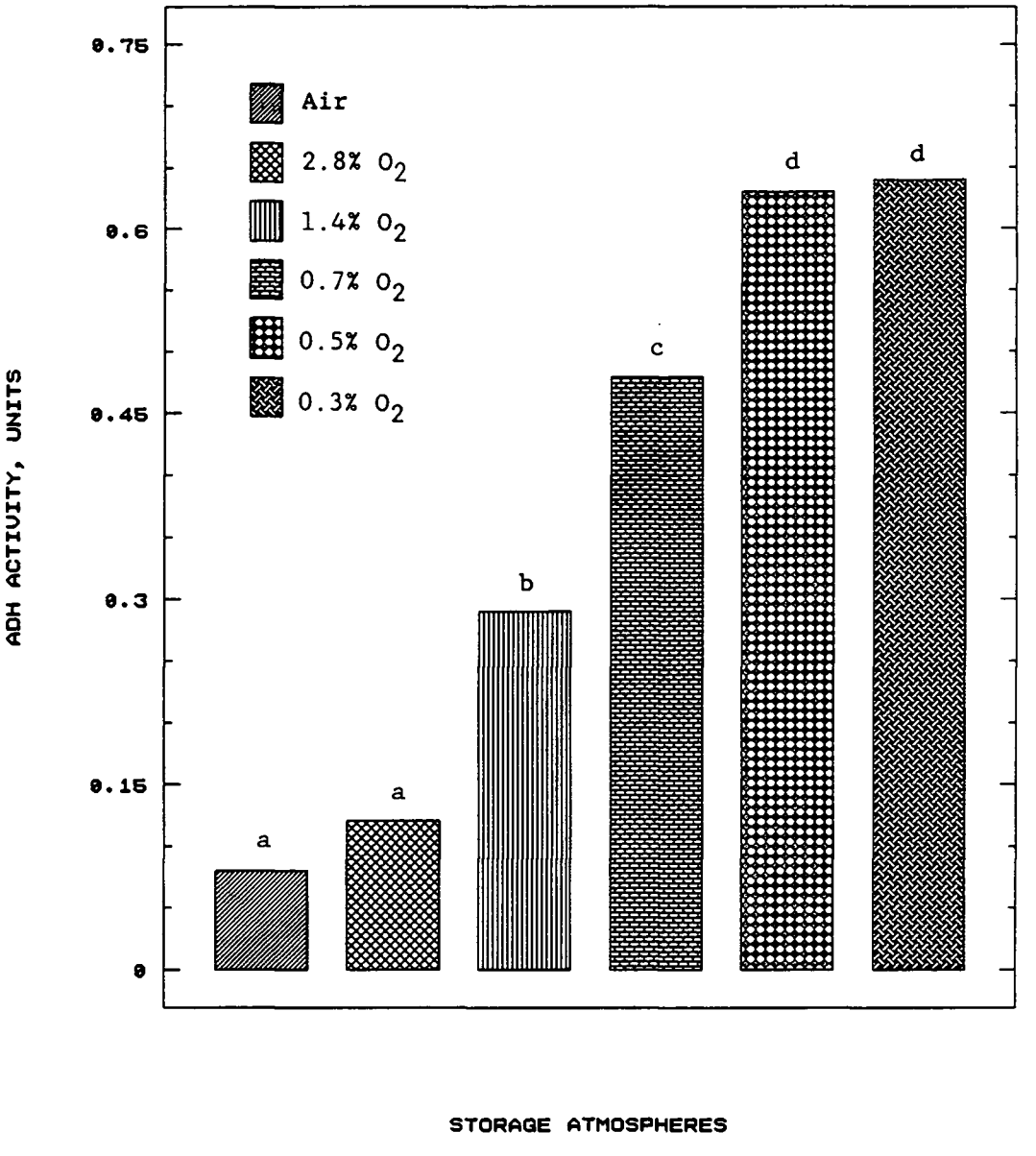

Fig. B.8. Alcohol dehydrogenase (ADH) activity of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

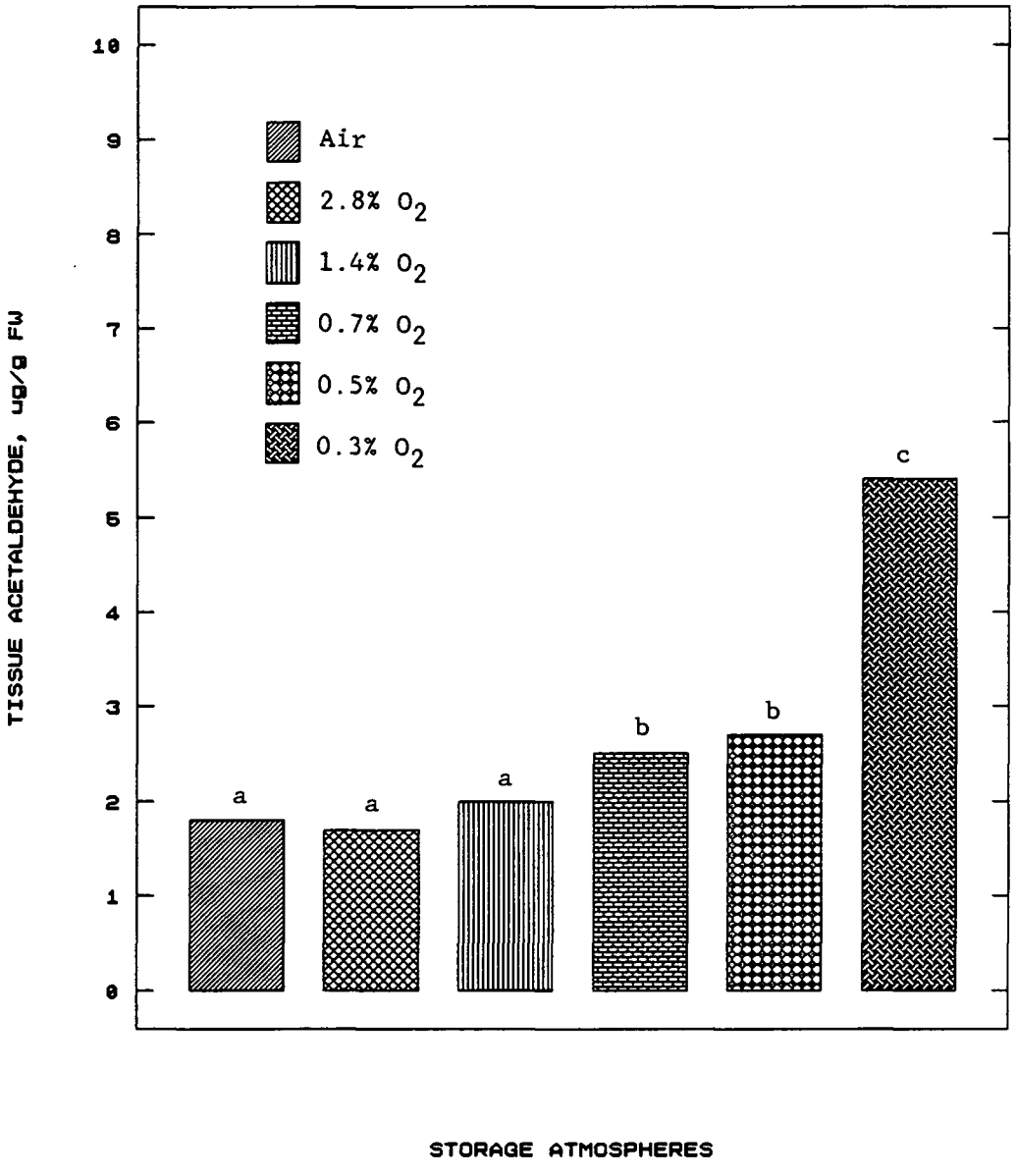

Fig. B.9. Tissue acetaldehyde of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

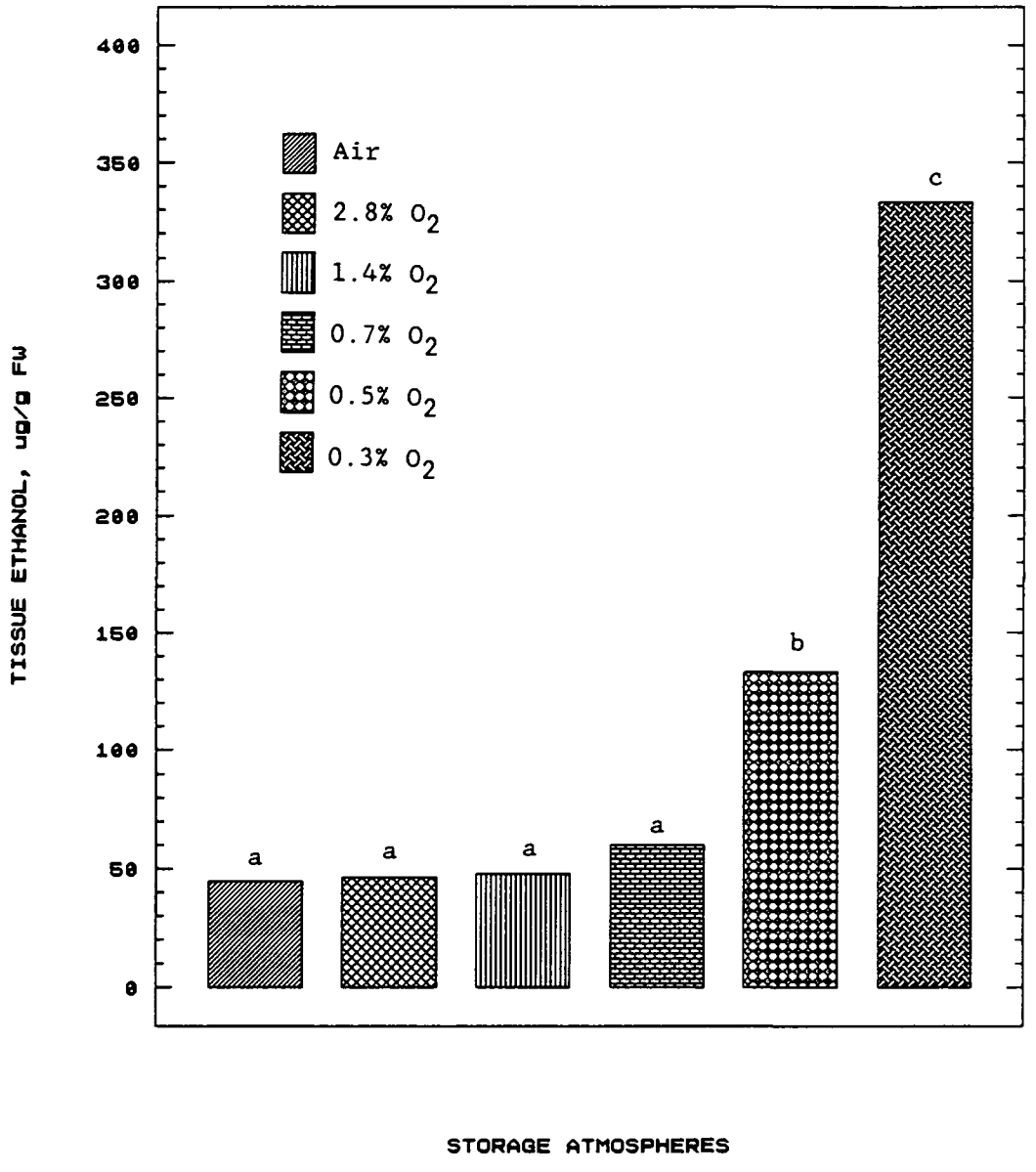

Fig. B.10. Tissue ethanol of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

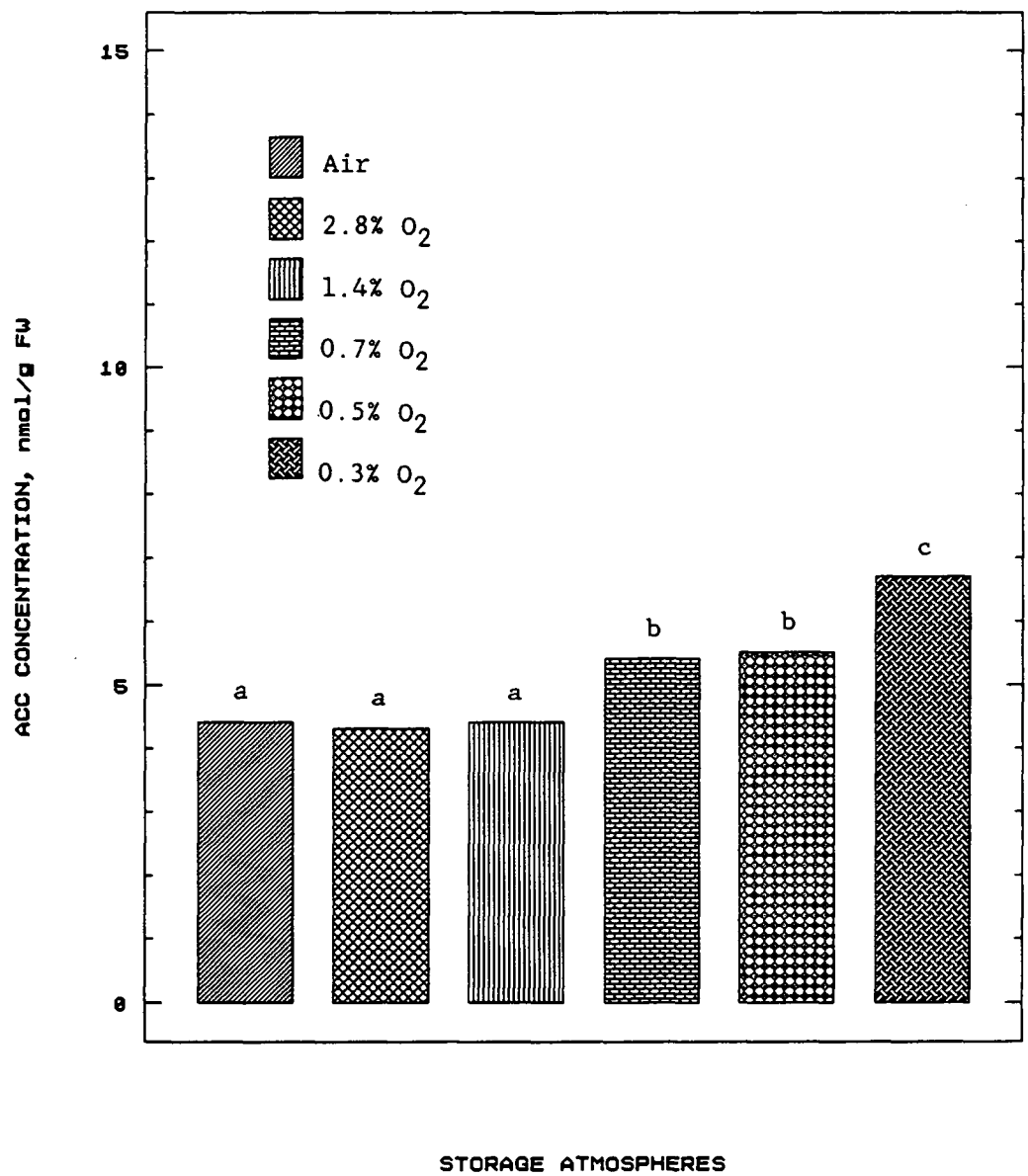

Fig. B.11. 1-Aminocyclopropane-1-carboxylic acid (ACC) concentration of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.

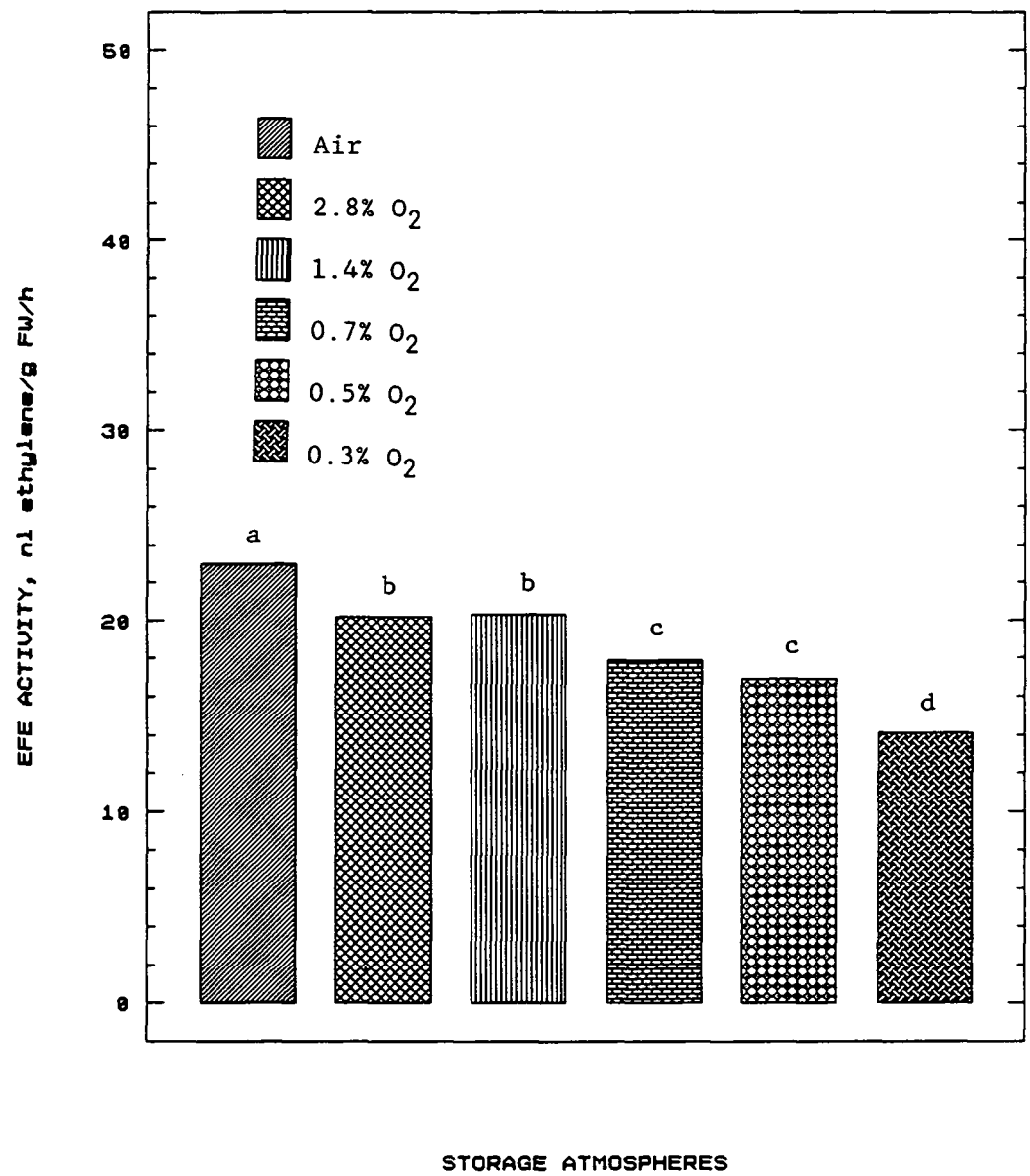

Fig. B.12. Ethylene forming enzyme (EFE) activity of 'Anjou' pears after 24 days in air or controlled atmosphere storage at 0°C, followed by 7 days in air. Each bar represents the mean of 4 observations. Mean separation by Duncan's multiple range test, 5% level.