AN ABSTRACT OF THE THESIS OF

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Title: PHYSIOLOGY OF RIPENING IN PEARS

Abstract approved: Dr. E. Hansen

Investigations were conducted to study in detail some biochemical and physiological aspects of ripening in pears. Anjou pears picked at different degrees of maturity were treated with Ethrel (2-chloroethane phosphonic acid), 4000 ppm, and ethylene, 500 ppm, for different lengths of time. Respiration rate was measured twice daily and changes in protein nitrogen, soluble pectin, flesh firmness and permeability were determined at frequent periodic intervals.

Treatment with Ethrel or for short periods of time with ethylene resulted in Anjou pears attaining full ripeness without a concomitant change in respiratory activity. Decrease in flesh firmness, increase in protein nitrogen and soluble pectin occurred, even though the fruit remained in the preclimacteric condition, as shown by a positive response in respiration to ethylene treatment when fully ripe. Fruits treated continuously with ethylene developed the climacteric rise. These fruits, however, did not ripen faster than fruits given short treatments which caused ripening but failed to induce a climacteric.

These data suggest that the mechanisms concerned with respiration and the initiation of ripening respond differently to ethylene and probably occupy different sites in the cell. Initiation of ripening apparently requires only a low initial concentration, while development of the climacteric rise in respiration requires the presence of a higher and possibly continuous amount. With increase in fruit maturity, ripening was initiated by shorter treatments. Samples picked at 61 and 73 percent of full maturity ripened after 24 hours' treatment while only 12 hours were required at 82 percent of maturity. When treated with Ethrel, which liberates ethylene when absorbed by the tissues, a climacteric rise in respiration developed only in fruits with more than 82 percent of full maturity. This would indicate that the ethylene producing capacity of Anjou pears increases with advance in maturity.

Initiation of ripening was accompanied by an increase in protein nitrogen and soluble pectin content. The maximum content of soluble pectin was reached at the fully-ripe stage, followed by a decline thereafter. Chlorophyllase activity in Bartlett pears was associated with the green to yellow color change and increased during ripening.

Permeability changes in cellular membranes during ripening were studied by leakage of electrolytes and water flux from fruit tissues. In both Anjou and Bartlett pears, ethylene treatment did cause changes in permeability but were detectable only after the changes in firmness and increase in protein nitrogen had occurred.

Physiology of Ripening in Pears

by

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PHYSIOLOGY OF RIPENING IN PEARS

INTRODUCTION

The climacteric rise in respiration has long been considered as an indispensable phenomenon during the ripening process in certain fruits. Many investigations have been conducted in an attempt to correlate the climacteric with various physiological and biochemical changes during ripening (11, 12, 60, 119). The onset of this rapid respiratory rise also is often used as an indicator of initiation of the ripening process (9, 67). Kidd and West (67, 68), Hansen (47, 49), Hulme (57, 60), Biale (9, 13), Pearson and Roberton (94), Millard et al. (86), Baker and Solomos (6), Bain and Mercer (4), and Burg and Burg (15, 18, 22) have all presented data attempting to interpret this phenomenon. However, the exact cause of this rapid increase in respiration still remains unexplained. Recently, the necessity of the climacteric in the ripening process of Anjou pears has been questioned by Hansen and Blanpied (50). They found that when treated with ethylene for short periods of time, ripening occurred without the development of the climacteric rise in respiration.

Most studies on fruit ripening have been concerned mainly with individual physiological changes, including enzymatic activity (32, 61, 100), permeability (7, 107, 111), protein nitrogen metabolism (46, 57), pectic substances (26, 31, 56), softening (45), and respiration (9). Few

comprehensive studies, however, have been made in which these factors have been considered together for the purpose of providing information on the overall organization and nature of the ripening process. This investigation was undertaken in an attempt to determine possible relationships between the various reactions involved, especially the significance of the climacteric rise in respiration in relation to the ensuing ripening reactions.

REVIEW OF LITERATURE

Considerable literature has been published concerning the conditions and reactions observed in fruits during ripening. The biochemical and physiological changes associated with climacteric rise in respiration and the role as well as the origin of ethylene in fruit ripening have attracted the attention of most postharvest physiologists during the past 40 years. Both the historical background and the present status of the research will be mentioned in this review, but emphasis will be on the physiological aspects of ripening, including the climacteric rise in respiration, ethylene action, permeability, and changes in proteins, enzymes, and pectin content.

Climacteric Rise in Respiration

Kidd and West (1922) observed a characteristic rise in the rate of respiration of Bramley's seedling apples after detachment from the tree, either just before or at the normal commercial harvesting time. In 1925, they stated (67):

> The onset of the senescence phase of the life cycle is marked by a relatively sudden alteration in the level of respiratory activity--and respiration increases of 50% to 150%. This striking phenomenon, which appears to mark the transition from growth to senescence, is here termed the 'Climacteric'.

Since that time this term has been widely accepted by fruit physiologists and horticulturists.

In 1960, Biale (9) classified the edible fruits as climacteric and non-climacteric on the basis of type of response obtained with ethylene treatment, and also described the physiological distinctions between the two groups. He stated that with a variety of fruits of the climacteric class, application of ethylene produced an early onset of the respiratory rise but significantly did not alter the peak values for oxygen uptake or CO_2 evolution. With non-climacteric fruits, the maximum values of O_2 uptake increased with increasing ethylene concentration in the range of 0.1 to 100 ppm. Biale (12) also demonstrated that non-climacteric fruits were able to respond to ethylene at all stages of maturity while in climacteric fruits no stimulation occurred after the onset of the rise in respiration.

Theories That Account for the Respiration Climacteric

Kidd and West (68) did not find any evidence that the rise in respiratory activity was accompanied by a corresponding change in the concentration of any of the estimated constituents, including starch, cane sugar, reducing sugar and total acids of the apple. They postulated as a tentative hypothesis that this rise was due to a change in state of the protoplasm.

Pearson and Robertson (94) from results obtained with whole fruits, tissue slices and particulates of Granny Smith apples, developed a hypothesis that the ATP/ADP ratio was the controlling factor

in respiration rate. They suggested that various synthetic processes were taking place at the time of the climacteric rise. When the demand was great on ATP for protein formation and for other reactions, the ratio would be low making adequate ADP available as phosphate acceptor for the increased oxidative activity of the Krebs cycle.

Millerd, Bonner and Biale (86) studied the climacteric in detached fruits of avocado and suggested that the link between oxidation and phosphorylation was broken at the time of the onset of the respiration climacteric by the appearance in the fruit of a soluble uncoupling agent which acted in a manner analogous to DNP. They considered ripening as a degenerative process involving a decrease in energy-yielding reactions.

With the mitochondrial system of the avocado, Romani and Biale (104) obtained results which were inconsistent with the uncoupling hypothesis. They found that the oxidative activity of mitochondria from preclimacteric fruit was higher than from fruit at the peak, and that esterification of inorganic phosphate proceeded actively in fruit at the climacteric peak.

Marks, Bernlohr and Varner (83) showed oxidative phosphorylation occurred also during ripening in tomatoes. The ripening was retarded when oxidative phosphorylation was inhibited by dinitrophenol at the climacteric peak, indicating that energy was required throughout the ripening process. Rowan, Pratt and Robertson (105)

also found that the climacteric rise in avocados was accompanied by an increase in total high energy phosphate when ripened after picking.

Tager and Biale (110) proposed the "shift in metabolic pathways" as an explanation for the differences observed between pre- and post-climacteric fruit. Two key enzymes, aldolase and pyruvic carboxylase, were absent in unripe but present in the ripe banana. However, Young (118) later extracted aldolase from the banana pulp tissue and found that the aldolase activity was the same in lyophilized preclimacteric and postclimacteric tissue.

Neal and Hulme (89) studied in detail the biochemical changes occurring during the climacteric in Bramley's Seedling and Cox's Orange Pippin apples and suggested that at least a part of the climacteric rise in respiration in detached apple fruit was due to the appearance of, or an increase in the activity of a system which decarboxylated malate to pyruvate and acetaldehyde without the direct participation of atmospheric oxygen.

Baker and Solomos (6) found that the content of fructose diphosphate increased by some twenty-fold, while that of phosphoenolpyruvate decreased initially 80% during the climacteric rise of CO_2 output in bananas. They thought the most probable cause of the rise in CO_2 output in bananas was a faster rate in metabolism of phosphoenolpyruvate, which produced extra ATP in the vicinity of the sugar phosphorylating and glycolytic enzymes. The extra ATP would thus

increase the content of fructose diphosphate and accelerate the rate of CO_2 output. However, Baker (5) recently working with apples, found no indication of any substantial increase in fructose diphosphate or decrease in phosphoenolpyruvate during the climacteric rise in CO_2 output and considered that the mechanism of the climacteric in apples could be different from that in bananas.

The other theories advanced to explain the series of biochemical changes which are known to be associated with the climacteric in fruits are changes in membrane permeability and increase in protein and enzymes. The literature pertaining to these aspects will be considered later.

Role of Ethylene in Ripening

At one time, there was considerable controversy concerning the role of ethylene in fruit ripening. Hansen (47, 49) and Kidd and West (69) found that young fruits produced small non-physiological quantities of ethylene while much greater amounts were produced at the onset of ripening. They proposed that ethylene might be an endogenous ripening hormone. In a comprehensive review, Porritt (95) cited numerous examples in support of this concept. On the contrary, Biale et al. (10, 13) reported that the ethylene production of certain climacteric type fruits did not reach a detectable level until after the

climacteric had started and concluded that ethylene was merely a by-product of the ripening process rather than a causal agent. Fidler (38) also suggested that, although the peak of ethylene production might occur during the climacteric period in apples, production of ethylene was not necessarily associated with ripening. At that time, the methods for measuring ethylene at physiologically active levels were not available.

The argument remained unsettled until 1962, when Burg and Burg (22) used the highly sensitive gas chromatographic instrument to determine the rate of ethylene production and the content of ethylene within a fruit. They demonstrated that stimulatory quantities of ethylene accumulated prior to the onset of the climacteric in several fruits and later published several papers (14, 17, 18) that strongly supported the original view of Hansen and Kidd and West.

Using the gas chromatographic method also, Lyons, McGlasson and Pratt (78) detected 0.04 ppm of ethylene in the cavity of cantaloupe ten days after pollination, and the ethylene concentration increased to 40 ppm at 40 days when the climacteric rise in respiration developed. Burg and Burg (22) found detectable amounts of ethylene in mango fruit a few hours after harvest, and the concentration increased to 0.05 ppm at the onset of the climacteric. Lyons and Pratt (80) determined that 0.08 ppm ethylene was present in tomato fruits ten days after pollination. The concentration increased ten fold to 0.8 ppm at 42 days

and it further increased to 400 times during ripening. Thus, it has been established for a number of fruits that ethylene is present in the tissue prior to the onset of the climacteric rise and acts as an endogenous triggering agent in the ripening process.

Mechanism of Ethylene Action

In addition to its role in fruit ripening (18, 22, 48, 69) ethylene has been shown to be an endogenous regulator of certain specific vegetative (20, 28, 42, 43, 65) and reproductive activities (16, 24). Abscission, bulb dormancy, epinasty, flowering, growth inhibition, and seed germination (15) are influenced by ethylene and almost without exception, there is a definite amount of the gas present in the tissues to cause a threshold response. Burg (15) listed the threshold value of ethylene for various processes (Table 1).

Table 1. Relative sensitivities of various processes to ethylene.

Response	Threshold (ppm)
Inhibition of hook opening	0.01
Stem swelling and inhibition of stem growth	0.01
Root swelling and inhibition of root growth	0.01
Epinasty	0.025-0.05
Inhibition of lateral IAA transport	0.03
Leaf fading	0.02
Abscission of explants	0.01
Fruit ripening	0.1-0.2

Abeles and Gahagan III (1) recently described some initial reactions regulated by ethylene, including alteration of membrane permeability (79), regulation of auxin activity (19, 87), and nucleic acid metabolism (53), induction of RNA and protein synthesis (2), and effects on the plant cell wall (28).

Burg and Burg (21) presented evidence that biological activity of ethylene required binding to a metal-containing receptor site. They further suggested that Zn might be the metal involved in the ethylene binding site, because Zn deficient tomato plants failed to respond to the gas. They also reported that CO_2 was a competitive inhibitor of ethylene and suggested that CO_2 delayed fruit ripening by displacing ethylene from its receptor site. Ethylene has been shown to cause changes in the permeability of lipoprotein membranes, as measured by increases in rates of mitochondrial swelling (79). The membrane was thought to regulate the respiration by controlling movement of the reactive components of oxidative systems, notably phosphate acceptors and donors.

Olson and Spencer (92), recently studied the effects of ethylene on mitochondria prepared from bean cotyledons. They concluded that ethylene affected an enzyme reacting with nucleotides and which was associated with mitochondrial volume changes. More recently they (93) suggested that the enzyme must be in its natural locale in the mitochondrial membrane where the gas could accumulate in relatively

high concentration.

The data of Holm and Abeles (54) on the mechanism of ethylene-induced abscission of bean explants and ripening of bananas suggest that ethylene acts by regulation and stimulation of the synthesis of ribosomal and messenger RNA.

Origin and Biosynthesis of Ethylene

Studies relating to the metabolic pathway and the site of ethylene formation in the fruit tissue have led to conflicting opinions. Spencer (109) originally reported the production of ethylene by mitochondria prepared from tomato fruits. Later, Spencer and his associate published a series of papers (84, 96, 97) and stated that a number of factors and conditions had to be carefully considered before ethylene could be produced from mitochondria. Meigh et al. (85), however, failed to detect ethylene evolution by mitochondria prepared from apple and tomato fruits. Recently Ku and Pratt (70) observed no ethylene production from a very active mitochondria preparation. Whether or not mitochondria directly produce ethylene remains unclear.

Shimokawa and Kasai (108) reported that subcellular particles of apple fruit catalyzed the formation of ethylene from pyruvate. Wang, Persyn and Krackov (112) speculated that the conversion of glucose to ethylene followed the pathway of glycolysis. Burg and

Burg (17), however, concluded that the biosynthetic source of ethylene was not to be found in intermediates of glycolysis, acid metabolism or the direct oxidation pathways of sugars. Lieberman et al. (74) postulated two pathways for the biosynthesis of ethylene in plant tissue. One involves the breakdown of peroxidized linolenic acid or its methyl ester, the other is associated with the degradation of methionine. Later, Lieberman and Kunish (73) suggested that propanal, a product of the decomposition of peroxidized linolenate, was a very effective precursor of ethylene in the copper-catalyzed ethylene-forming model system.

Recently, Yang (117) demonstrated that ethylene could be rapidly formed aerobically from methional by an enzyme system consisting of horseradish peroxidase, Mn, and sulfite ions, and a monohydric phenol. Mapson and Wardale (82) found that two enzymes were necessary for the production of ethylene from methional. The first enzyme, generating hydrogen peroxide, appears to be similar to the fungal glucose oxidase. The second enzyme, in the presence of cofactors and peroxide generated by the first enzyme, cleaves methional to ethylene.

Association of the Climacteric with Ripening

The climacteric rise in respiration has been considered as inseparable from the ripening process. However, recent evidence

has shown that sometimes the ripening process could still take place without the climacteric or vice versa.

In their studies of tomatoes, Dostal and Leopold (33) found that ethylene stimulation of color development was prevented by treatment with GA, but ethylene stimulation of respiration was not.

Frenkel, Klein and Dilley (39) found that fruit ripening and ethylene synthesis were inhibited when protein synthesis was blocked by treatment with cycloheximide, but the respiratory climacteric was not inhibited.

Hansen and Blanpied (50) reported that Anjou pear fruits treated with ethylene for 48 hours ripened as rapidly as those treated continuously, while the climacteric failed to develop.

Permeability Changes in Ripening Fruits

Sacher (106, 107) studied the changes in free space of banana tissue during ripening and concluded that the initiation of membrane permeability changes were associated with the climacteric in banana. It was also demonstrated for cantaloupe (111) and avocado (8) that an increase in leakage of solutes preceded the onset of the climacteric. Baur and Workman (7) observed that the increase in membrane permeability occurred simultaneously with the increase in respiration and concluded that increased permeability could be one of many changes initiated in fruit during the climacteric rise. Young and Biale (119)

concluded from studies of ^{32}P-uptake by disks of the avocado that neither uncoupling nor acceptor control could account for the onset of the respiratory rise. Changes in permeability appeared to play an important role in fruit metabolism during the climacteric.

From observations with the electron microscope, Bain and Mercer (4) observed that changes in the inner structure of the chloroplasts had already occurred by the time the climacteric rise had begun in Bartlett pears. They suggested that this change in cytoplasmic organization, by altering permeability of cells and location of enzymes, could lead to changes in the availability of either cofactors or substrates for respiration. However, Burg et al. (23) demonstrated that membrane permeability remains constant during banana ripening and the increased leakage was simply a manifestation of the increase in endogenous levels of sugars.

Rhodes et al. (40, 41, 60, 99, 101) recently presented a series of papers describing how the aging of disks of peel from pre-climacteric apples resulted in the development of several systems, each reaching a maximum level after different periods of aging. The first to rise to a maximum was lipid synthesis (2-4 hours) followed by protein synthesis (8 hours) and ethylene evolution (6-8 hours) and lastly, malate decarboxylate (16-24 hours). They proposed the following sequence of events to explain these phenomena; removal of peel and preparation of disks initiates a de-repressor; m-RNA is formed and causes the

synthesis of enzymes possibly including lipoxidase and fatty acid producing systems concerned with ethylene biosynthesis; ethylene was then produced and acts on a further portion of DNA leading to the formation of m-RNA associated with other enzyme systems such as malic enzyme. Uptake studies showed that none of the changes in these systems could be due solely to changes in the permeability of the tissue over the climacteric period.

Changes in Protein and Enzymes during Ripening

Hulme (57) showed that, coinciding with the climacteric rise in respiration in detached apples, there was a rise in the net protein content of the fruit. Hansen (46) found also that pears behave similarly. Hulme (55) examined nine varieties of apples, including early, medium and late season types, and found that there was always a shift in the equilibrium between nonprotein and protein nitrogen in favor of protein over the period of the climacteric. A net increase in protein nitrogen during ripening has also been shown in the fruits of avocado and tomato by Rowan, Pratt, and Robertson (105).

However, Pearson and Robertson (94) found no rise in protein in apples until late in the climacteric phase. Sacher (106) concluded that a net increase in protein nitrogen did not develop in the banana fruit during ripening. Richmond and Biale (103) have shown that in avocados, there was a period of increased protein synthesis during

the early stages of the climacteric, but there was no direct relationship between protein synthesis and increased O_2 uptake throughout the climacteric period. The failure to observe a net increase in protein nitrogen does not necessarily mean that no new enzymes were formed, since there could be a turnover in protein, involving enzyme synthesis without an actual increase in protein nitrogen.

Hulme (58) demonstrated an increase in the ratio of rate of respiration to protein content (R/P) and suggested that a change in the pattern of protein to a more enzymatically active type might occur during the climacteric rise. Li and Hansen (72) demonstrated that the failure of Bartlett pear fruits to ripen normally after prolonged low temperature storage was related to decreased capacity for protein synthesis. Frenkel, Klein and Dilley (39) recently concluded that protein synthesis was required for normal ripening, and the proteins synthesized early in the ripening process were, in fact, enzymes required for ripening.

Indeed, during the past few years increases in the activity of several mitochondrial (51, 62, 71) and soluble enzyme systems (32, 61, 100) have been reported. Hulme and Wooltorton (61) and Dilly (32) found that malic enzyme activity increased during the respiratory climacteric of apple fruits and suggested that it was the result of an increased quantity of the enzyme. Richmond and Biale (102) showed that in the early stages of the climacteric in avocados, there

was a stimulation in the rate of synthesis of ribosomal and messenger RNA. A marked increase in total RNA also was observed during the climacteric rise in apples by Looney and Patterson (76). Rhodes and Wooltorton (100) demonstrated that the activities of four hydrolytic enzymes, chlorophyllase, lipase, ribonuclease, and acid phosphatase increased over the climacteric period in apple fruits.

There have been reports of increases of activity of other enzymes during ripening, including lipoxidase (116) and pyruvic carboxylase (59) in apple, catalase (37) in pear, aldolase and carboxylase (110) in banana.

Chlorophyllase Activity in Ripening Fruits

Chlorophyllase was discovered by Willstätter and Stoll in 1910 (115). They stated that the enzyme functioned in chlorophyll synthesis as well as degradation. Chiba et al. (29) studied the relation between chlorophyll content and the hydrolytic activity of chlorophyllase activity in Chlorella protothecoides and found that an increase in chlorophyllase activity was parallel to that in chlorophyll content during the development of green coloration in the bleached cells. The activity sharply declined and a parallel disappearance of chlorophyll also occurred during bleaching of the green cells. This suggested that chlorophyllase has a biosynthetic role. Noack (90) on the other hand, suggested that chlorophyllase has a function in the

removal of the phytol chain and from the chlorophyll molecule to yield chlorophyllide. Holden (52) showed that in a fat solvent such as petroleum ether, the chlorophyllide molecule was much less soluble than chlorophyll. They also showed that by shaking in petroleum ether and acetone-water solution, chlorophyll could be separated from chlorophyllide quantitatively. Ogura and Takamiya (91) studied the chlorophyllase in tea leaves and found that the partially purified enzyme was stable in heat treatment and the optimum conditions for enzyme activity were pH 6.5 and an acetone concentration of 50%. Rhode and Wooltorton (100) recently found that in the ripening fruit the increase in chlorophyllase activity begins before the climacteric rise and continues beyond the climacteric peak.

Changes in Pectic Substances during Ripening

An increase in water soluble pectin substances and a decrease in protopectin content in the deciduous fruit tissue during ripening was first reported in 1848 by Fremy as cited by Esau et al. (36). Since that time, the content and role of pectin in many fruits have been investigated extensively by Carre' (26), Kertesz (66), Hulme (56) and Joslyn (63). The following percentage increases were reported for soluble pectins between the green and ripe stages: 200 for Elberta peach (3), 250 for banana (30), 1250 for Jonathan apple (44), and 1900 for Worcester Pearmain pear (35).

The most comprehensive study of pectin changes in pears during growth on the tree and during storage and ripening is that of Weurman (113). He showed that there was a rapid decrease in protopectin when pears were ripened at room temperature and the decrease in hardness of the fruit tended to accompany fall in protopectin. Hansen (45) found that the increase in soluble pectin after picking and during ripening closely paralleled the increase in softening of the pear fruit.

The data of Date and Hansen (31) illustrated the different behavior of different varieties of pears. They found that the capacity of Bartlett pear and Bosc to hydrolyze protopectin during ripening at warm temperatures progressively declines with length of storage. At the end of the storage period, the Bartlett pears failed to soften to "melting ripe" when removed to ripening temperatures, suggesting that this might be associated with inactivation of protopectinase. The Anjou pears retained their ability to convert protopectin to soluble pectin and to soften on ripening to the end of the storage period. Emmert (35) studied pectic changes occurring in pears at certain low temperatures and concluded that the lack of keeping quality was due mainly to a rapid rate of degradation of the pectic compounds.

Reeves (98) believed that pectic changes during maturation and ripening involved more than a simple change from water insoluble to water soluble fractions. Degree of esterification, molecular chain length, spatial configuration and complexity of side chain

structure influenced the solubility and gelatinous properties. Kertesz (66) was the first to provide evidence that softening was accompanied by a decrease in degree of esterification and degree of polymerization of extracted pectins. McCready and McComb (81) found that the degree of esterification with methyl groups was shown 86% in unripe pears and below 40% in ripe fruits.

A number of enzymes have been investigated in an attempt to determine the mechanisms of conversion of pectin in fruits in relation to ripening. The hydrolysis of methyl groups is brought about by pectin methyl esterase (or pectase) and the chain splitting by pectinase (or polygalacturonase) (11). Joslyn and Sedky (64) attributed the decrease of pectin in pulp of apple and citrus fruit to the action of pectinase. The pectase activity in pears was investigated by Weurman (114) by measuring the increase in viscosity of a solution of pear pectin added to enzymic extracts. He detected the presence of pectinase in pears in the ripe stage but not in unripe or overripe fruits. McCready and McComb (81) observed no polygalacturonase action in unripe pears, but found considerable activity in ripe pear fruits. They thought that pectic enzymes came in closer contact with the pectic substances during ripening.

MATERIALS AND METHODS

Variety, Source and Maturity of Fruits

The Anjou and Bartlett pear varieties used in these experiments were obtained from single source blocks at Mid-Columbia Experiment Station, Hood River, Oregon. Fruits were picked at several maturities to satisfy the requirements of individual experiments. The Anjou and Bartlett samples were taken during the 1967 and 1968 seasons. Samplings taken of the Anjou variety ranged from 61% to 100% of maturity. For the Bartlett variety, the maturity range was from 82% to 100%.

Pre-treatment Handling

After removal from the tree, fruits were placed in brown paper bags and transported to the University laboratories at Corvallis. This required a time lapse of approximately five hours at temperatures of 20° C ± 1° C. Immediately upon arrival at the University, the samples were transferred to a cold storage room and held at 1° C for approximately 12 hours. Prior to conducting the several experiments, fruits were allowed to equilibrate with the temperature at which the experiments were carried out.

Identification Pertinent to Experiments

The several experiments involved ripening and respiration, chlorophyll and chlorophyllase, and permeability studies. A table (Table 2) is presented to aid in identifying and providing information pertinent to each experiment.

Table 2. Identification of experiments.

Study	Experimental designation			Maturity of samples	
	Year	Variety	Experiment number	Percent	Days from full bloom
Ripening and Respiration	1968	A	1	61	91
	1968	A	2	73	109
	1968	A	3	82	123
	1968	A	4	100	150
Chlorophyll and Chlorophyllase	1968	B	5	100	125
Permeability	1967	B	6	82	103
	1967	B	7	89	111
	1967	A	8	94	141
	1968	A	9	61	91

A = Anjou
B = Bartlett

Treatments

Ethylene

A sufficient number of fruits were collected on each harvest date to provide samples for determining respiratory rate and daily chemical analyses. For respiration studies duplicate ten to 15 fruit

samples, depending on size, were put in three-gallon jars and sealed with screw-type lids containing inlet and outlet tubes. For chemical analyses, duplicate lots were placed in five-gallon glass jars. From each of these, five fruits were taken for chemical analyses. These jars were also fitted with lids containing inlet and outlet tubes. The jars containing the respiration and chemical analyses samples were connected in tandem so each would receive the same rate of ethylene treatment. All experiments were conducted in a room maintained at 20 ° C.

Aeration was maintained at 200 ml per minute with fresh air drawn from outdoors. Ethylene was metered into the air stream from a Sigma flexible tubing pump to provide a concentration of 200 ppm for Bartletts and 500 ppm for Anjous. The apparatus is pictured in Figure 1.

Bartlett fruits were primarily used for the studies of changes in chlorophyllase activity, because of their conspicuous color change during the ripening process. Five fruits from each treatment were analyzed daily to compare the chlorophyll content and chlorophyllase activity of ethylene treated and untreated fruits.

Ethrel (2-chloroethane phosphonic acid)

Preliminary experiments with Ethrel indicated that a concentration of 4000 ppm was most effective in stimulating ripening of pears;

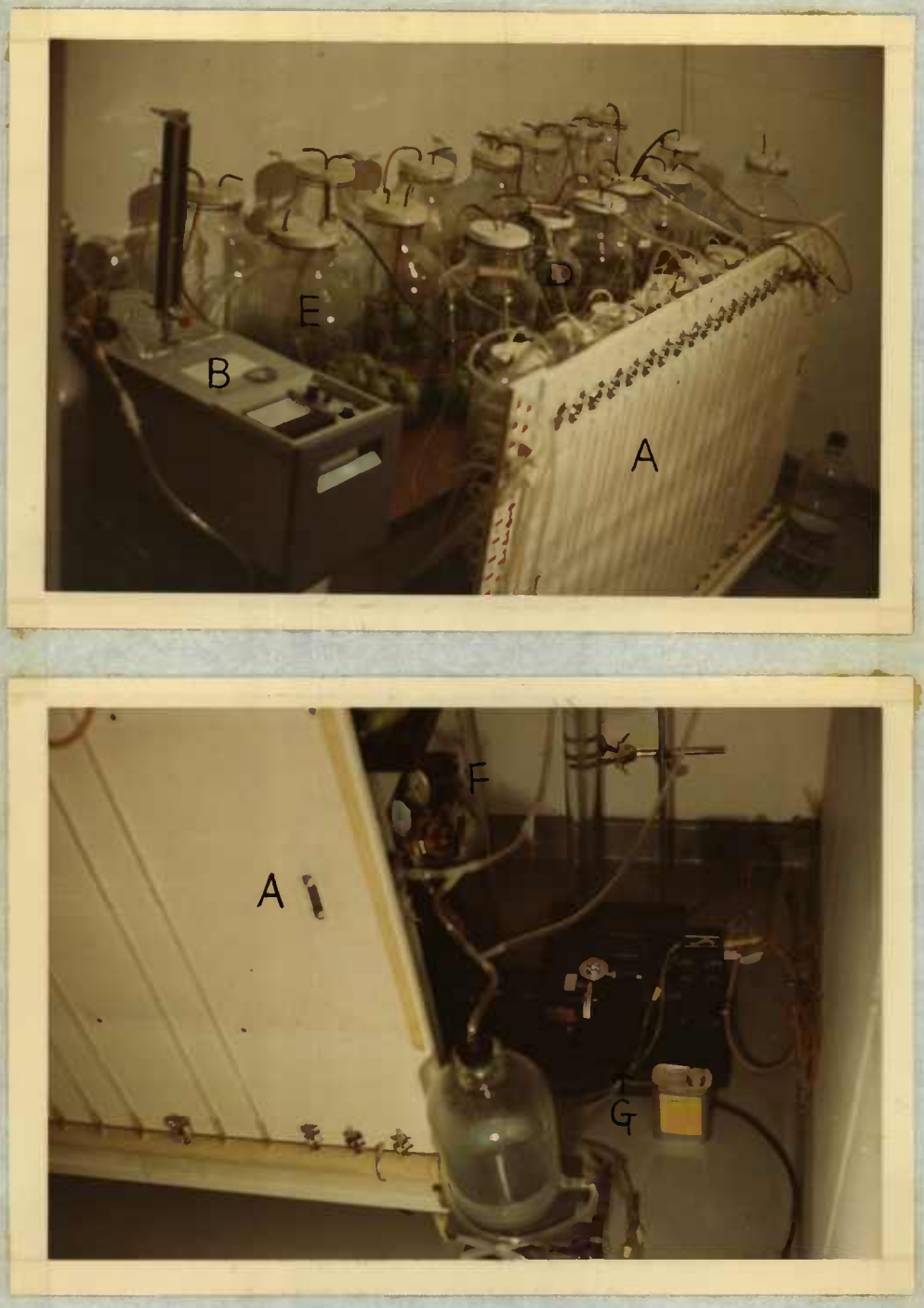
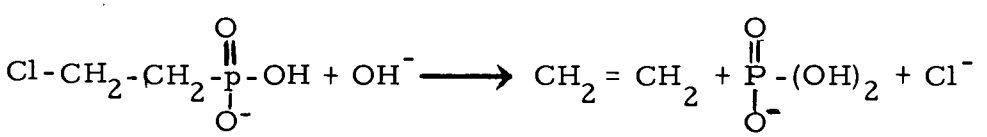


Figure 1. Apparatus used to measure fruit respiration. (A) flow board, (B) Beckman CO_2 infrared analyzer, (C) flow meter, (D) respiration chamber, (E) Jars containing fruits for chemical analyses, (F) Ethylene cylinder, (G) Sigma flexible tubing pump.

therefore, this concentration was used and treatment applied by dipping the fruit into the solution for 30 seconds, unless otherwise stated.

The mode of action of 2-chloroethane phosphonic acid is related to its ability to release ethylene in plant tissues. The acid undergoes a chemical decomposition which can best be described as a base catalyzed elimination reaction, as illustrated:



The design of the individual experiments is outlined as follows:

Experiment 1968 A1:

a. Control, aerated with ethylene free air.

b. Initial 48-hour treatment, followed by aeration with ethylene free air for 12 days.

c. Initial 48-hour treatment + retreatment for 12 hours on 11th day.

d. Continuous ethylene treatment for 14 days.

e. Ethrel treatment.

Experiment 1968 A2:

a. Control.

b. Initial 24-hour treatment + first retreatment on 8th day for 12 hours; + second retreatment on 13th day for 12

hours.

c. Initial 36-hour treatment + first retreatment on 8th day for 12 hours; + second retreatment on 13th day for 12 hours.

d. Initial 48-hour treatment + first retreatment on 8th day for 12 hours; + second retreatment on 13th day for 12 hours.

e. Continuous ethylene treatment for 16 days.

f. Ethrel treatment.

Experiment 1968 A3:

a. Control.

b. Initial 6-hour treatment + retreatment for 12 hours on 9th days.

c. Initial 12-hour treatment + retreatment for 12 hours on 9th days.

d. Initial 18-hour treatment + retreatment for 12 hours on 9th days.

e. Initial 24-hour treatment + retreatment for 12 hours on 9th days.

f. Continuous ethylene treatment for 11 days.

g. Ethrel treatment.

Experiment 1968A4:

a. Control.

b. Initial 6-hour treatment + retreatment for 12 hours on 8th day.

c. Initial 12-hour treatment + retreatment for 12 hours on 8th day.

d. Initial 18-hour treatment + retreatment for 12 hours on 8th day.

e. Initial 24-hour treatment + retreatment for 12 hours on 8th day.

f. Continuous ethylene treatment for 10 days.

g. Ethrel treatment.

Experiment 1968 B5:

a. Control.

b. Continuous ethylene treatment for 8 days.

Experiment 1967 B6:

a. Control.

b. Continuous ethylene treatment for 7 days.

Experiment 1967 B7:

a. Control.

b. Continuous ethylene treatment for 8 days.

Experiment 1967 A8:

a. Control.

b. Continuous ethylene treatment for 11 days.

Experiment 1968 A9:

a. Control.

b. Continuous ethylene treatment for 14 days.

c. Ethrel treatment.

Determination of Chemical and Physiological Changes during Ripening

Pressure Test

Flesh firmness was measured by a Balauf 10 or 30 lb tester with a 5/16" point. Measurements were made daily on five fruits from each treatment, using two punches on each pared fruit.

Respiration

Two methods were used for determining the rate of respiration.

Absorption and Titration Method.

(a) Preparation of absorber

Fifty ml 0.3 N KOH was pipetted into a dry absorber mounted on a burette stand. A sintered glass gas disperser tube was inserted into each absorber and connected to a manometer. The manometer was then connected to the jar containing the pears. Two drops of n-butyl alcohol were added to each tube to decrease surface tension

and increase foaming. The rate of air flow was adjusted to 200 ml per minute. Determinations extended from one to three hours, depending upon the rate of respiration.

(b) Titration

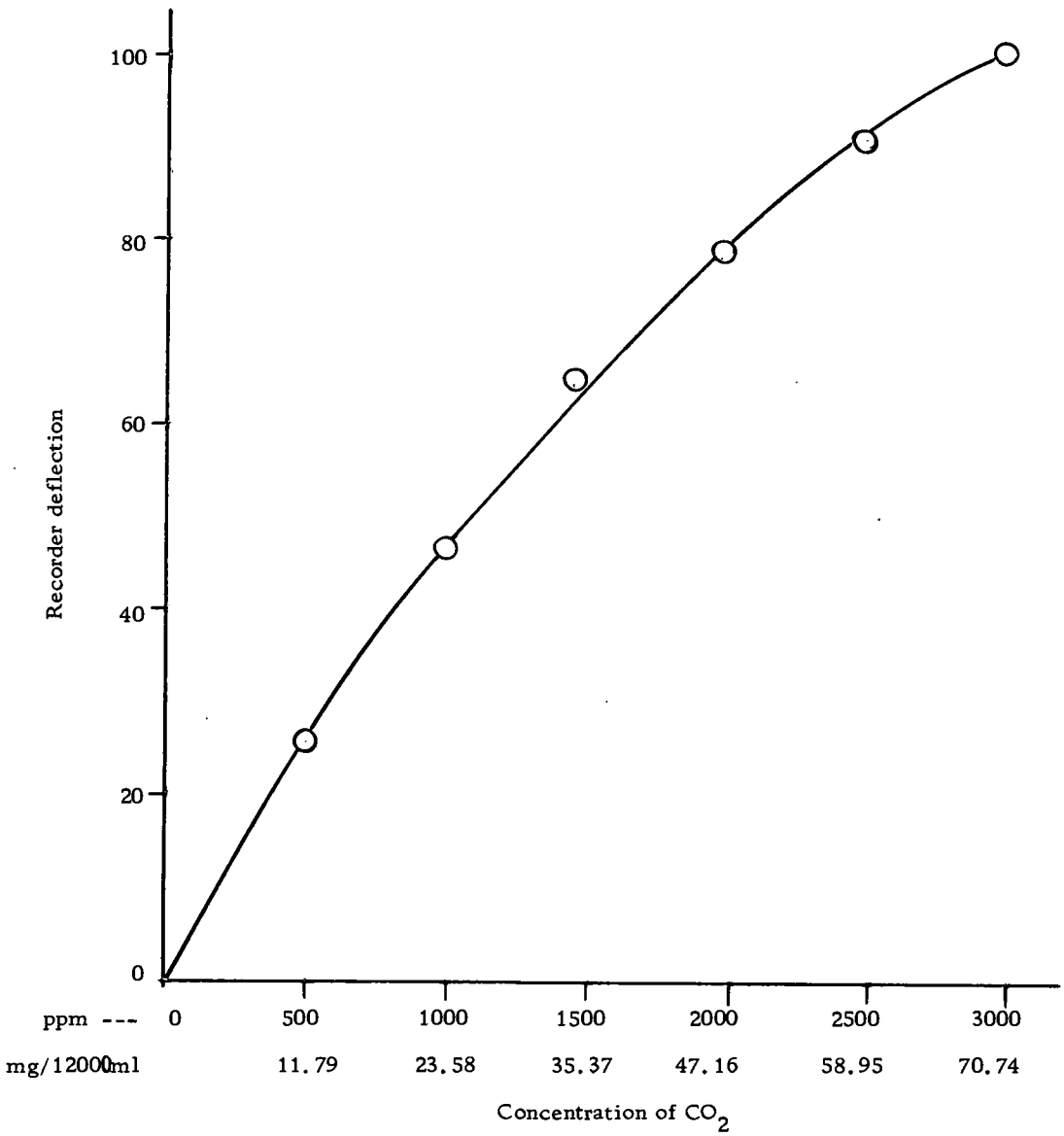
After one to three hours, the manometer and the absorber were disconnected from the jar containing the fruit. The gas disperser was removed from the absorber and the tube sealed with a rubber stopper. Fifty ml H_2O, ten ml 20% $BaCl_2$ solution and five drops thymolphthalein indicator (0.5% in 50% ethanol) were added to a 250 ml Erlenmeyer flask, followed by ten ml of KOH from the absorber. The solution was then titrated with a 0.1 N HCl, while being agitated with a magnetic stirrer.

(c) Calculation

$$\text{mg } CO_2/\text{hr}/\text{kg} = \frac{\text{ml HCl used} \times 10 \times 50 \times A^*}{\text{weight of fruits (Kg)} \times \text{time (hr)} \times 10 \times \text{blank (ml HCl)}}$$

$$^*A \text{ mg } CO_2 = \text{one ml KOH}$$

CO_2 Infrared Analyzer. Respiration rate was determined twice daily with a Beckman Model 215 A Infrared analyzer. Rate of respiration in mg CO_2/kg-hr was calculated from the galvanometer deflection according to the data in Figure 2 and Table 3.



Factor to convert scale readings (ppm) to mg/12000 ml = x 0.02358

Figure 2. Standard curve for conversion of Beckman infrared spectrophotometer recorder deflections to concentration of CO_2.

Table 3. Beckman infrared spectrophotometer scale deflections in $MgCO_2$/12 1.

Def.	$MgCO_2$/12 1	Def.	$MgCO_2$/12 1	Def.	$MgCO_2$/12 1
1	.28	35	16.51	69	38.55
2	.70	36	17.10	70	39.38
3	1.18	37	17.57	71	40.20
4	1.77	38	18.04	72	41.03
5	2.12	39	18.75	73	41.85
6	2.64	40	19.45	74	42.68
7	2.95	41	20.04	75	43.62
8	3.54	42	20.63	76	44.45
9	4.01	43	21.22	77	45.39
10	4.36	44	21.81	78	46.22
11	4.83	45	22.40	79	47.16
12	5.31	46	22.99	80	48.06
13	5.78	47	23.58	81	49.00
14	6.20	48	24.17	82	49.99
15	6.72	49	24.76	83	50.98
16	7.19	50	25.35	84	51.99
17	7.55	51	25.94	85	53.06
18	8.02	52	26.65	86	53.93
19	8.49	53	27.35	87	54.23
20	8.96	54	27.99	88	56.00
21	9.43	55	28.58	89	57.06
22	9.90	56	29.24	90	58.24
23	10.38	57	29.95	91	59.30
24	10.85	58	30.65	92	60.25
25	11.32	59	31.24	93	61.43
26	11.79	60	31.83	94	62.72
27	12.26	61	32.54	95	63.95
28	12.73	62	33.13	96	65.13
29	13.20	63	33.88	97	66.31
30	13.68	64	34.66	98	67.79
31	14.15	65	35.37	99	69.25
32	14.74	66	36.08	100	70.74
33	15.33	67	36.90		
34	15.92	68	37.73		

Preparation of Samples for Chemical Analyses

Wedge shaped sectors were cut from each of five fruits per sample after paring to provide a total weight in excess of 100 grams. The sectors were then ground through a food chopper and thoroughly mixed with a spoon to prevent separation of juice and pulp.

Duplicate 50 gram samples were weighed into tared 400 ml Pyrex beakers, covered with 220 ml 95% ethanol and then heated to boiling on an electric hot plate. After cooling, the samples were transferred to a Waring blendor and ground for three minutes at high speed. The sample was then filtered through Whatman #1 filter paper into 500 ml volumetric flasks. The residues were washed four to five times with 80% ethanol and finally transferred to weighing dishes and dried at 70° C to a constant weight. These dried residues were used for determination of pectin and protein nitrogen after grinding in a Wiley mill through a 40-mesh screen.

Protein Nitrogen

One gm aliquots of the alcohol insoluble residues were weighed on an analytical balance. Protein nitrogen was then determined by the Kjeldahl Gunning Arnold method (88).

Soluble Pectin

The calcium pectate method of Carré and Haynes (27) was modified for use with pear residues to determine the content of soluble pectin.

A one gram aliquot of the alcohol insoluble residue was transferred to a 600 ml beaker, 100 ml distilled water added and stirred until the mixture was evenly dispersed. After ten minutes, with occasional stirring the residue was filtered. The filtrate was saved and the residue was washed back into the beaker. The water extraction was repeated, using another 100 ml distilled water. Both filtrates were combined and 100 ml 0.1 N NaOH added. After one hour 50 ml of 1 N acetic acid were added, followed by 50 ml of 1 M $CaCl_2$. The contents were allowed to stand for an additional hour then heated to boiling for a few minutes and filtered through a large fluted filter. The precipitated soluble pectin was washed with boiling water and washing continued until the filtrate gave no test for chloride with silver nitrate. The residue was washed back into the beaker, boiled filtered, and re-tested with silver nitrate to make sure there was not any trace of chloride. The gelatinous residue was then transferred to a crucible, and finally dried at 100 ° C to constant weight.

Chlorophyllase and Chlorophyll

The method described by Looney and Patterson (77) was used to measure the chlorophyllase activity. The activity of chlorophyllase was expressed as absorbance which was the difference between incubated samples and the non-incubated control. This is based on the degradation of chlorophyll molecule by the action of chlorophyllase and yields chlorophyllide. The chlorophyllide is much less soluble than the chlorophyll in petroleum ether, so that a difference in absorbance at O. D. 665 mμ between before incubation and after incubation will give the activity of chlorophyllase.

The chlorophyll content was expressed as absorbance at O. D. 665 mμ of the petroleum ether extract of the non-incubated fraction in the enzyme assay.

Permeability

Two cylinders of tissue were removed from each of five fruits with a 13 mm cork borer, a disk two mm in thickness was then removed about four mm below the epidermis from each cylinder by use of a slicing tool made from two razor blades. Changes in permeability in fruit from the different treatments was measured by two methods. In the first method, change in permeability was related to the change in water content of the disks; in the second method as the

change in conductivity in the solution covering the tissue.

(1) Method of Von Abrams and Pratt (111).

The ten disks obtained for each treatment were placed in a 250 ml beaker and carefully rinsed three times with deionized water to free from cell debris. Then the disks were blotted and weighed. After weighing, they were placed in 150 ml of deionized water. Weighing continued at one hour intervals, covering with fresh deionized water after each weighing. The experiment was conducted in a 20 ° C constant temperature room. The changes in weight each hour were expressed as percentage of the initial weight.

(2) Method of Baur and Workman (7).

The tissue disks were excised and handled the same way as in method one. After being rinsed with deionized water, ten disks from each treatment were placed in 300 ml freshly deionized water. The water was gently agitated by a stream of air bubbles in place of magnetic stirring to avoid temperature increase from the heat generated by the motors. The changes in membrane permeability in pear tissue were determined by measuring the rate of ion leakage from tissue disks into deionized water. The changes of the rate of ion leakage with time was measured at 20 ° C in terms of resistance with a Leeds and Northrup electrolytic conductivity bridge at one hour intervals. The disks remained in the water after each measurement. The measured resistance was converted to specific resistance by multiplying by

the cell constant 0.099. The specific conductance is the reciprocal of specific resistance. Specific conductance is therefore expressed as μmhos.

RESULTS

Effects of Ethrel, Different Lengths of Ethylene Treatment and Maturity on Respiration, Firmness, Protein Nitrogen and Soluble Pectin

Experiment 1968 A1: Anjou pears used for this experiment were picked 91 days from full bloom which was 61% of full maturity. Average weight per fruit was 54.8 gram.

As shown in Figure 3, the initial rates of respiration of the five lots of fruits ranged from 30 to 40 mg/kg-hr. The ethylene treated samples showed a sharp increase in respiration to approximately 80 mg. This initial rapid increase following ethylene treatment is not the true climacteric and appears to be a characteristic reaction in fruits treated with ethylene at immature stages of development (46, 69). The cause is not known. Following the initial stimulation, the rates of respiration declined to minimum values on the fourth day, then gradually increased to the climacteric peak seven days later. The same trend was shown by the samples receiving the 48-hour initial treatment and those treated continuously.

Fruit maintained in an ethylene free atmosphere showed a steady gradual decline in rate of respiration throughout the 14 days of the experiment. The Ethrel-treated sample also decreased in respiratory activity for four days then maintained a steady rate above the level of the control. Respiratory activity doubled in this lot of

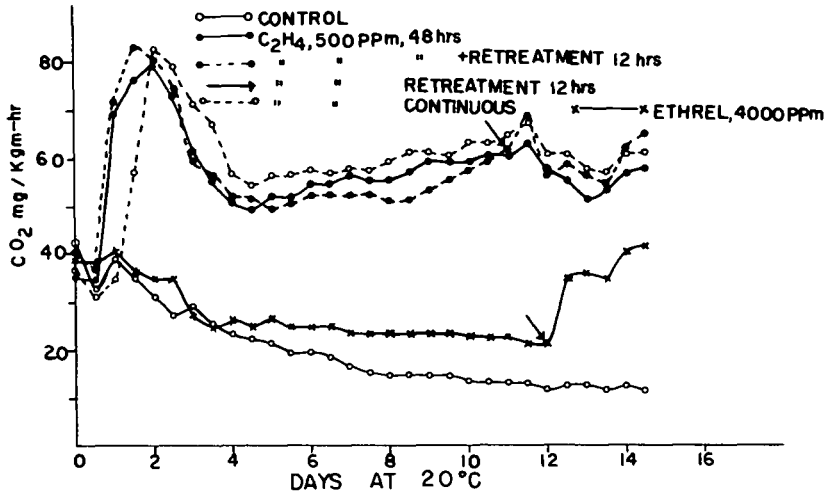


FIGURE 3. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT IN RESPIRATION RATE OF ANJOU PEARS.
PICKED JULY 10, 1968

(61% of full maturity)

fruit when treated with ethylene for 12 hours on the twelfth day, indicating that it was still in a pre-climacteric condition. The sample receiving the initial 48-hour ethylene treatment, however, showed only a slight and probably insignificant increase in respiration when retreated on the same day.

All fruits softened at about the same rate except the untreated ones (Figure 4). Untreated fruits remained firm after 14 days at 20° C but fruits treated with ethylene continuously or treated for 48 hours decreased in firmness from 28.5 lbs to about six lbs in nine days (Table 4). Those fruits which were treated with Ethrel decreased from 28.5 lbs to seven lbs in ten days even though the respiration rate remained low and was apparently pre-climacteric in stage of development.

Protein nitrogen in the fruit maintained in an ethylene-free atmosphere showed no increase in concentration during the course of the experiment (Figure 5). The sample in which ethylene treatment was terminated after 48 hours increased in protein nitrogen content similarly to the fruit receiving continuous ethylene treatment (Table 5). The Ethrel-treated pears also increased in protein, even though respiration remained at a low level.

Soluble pectin (Figure 6) increased after three days ethylene treatment and reached its maximum at the ninth day when treated with ethylene continuously. After an initial treatment for 48 hours,

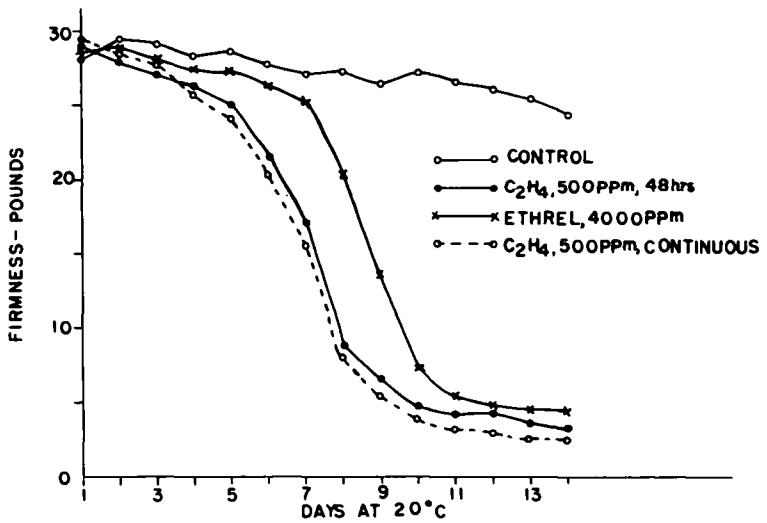


FIGURE 4. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON FIRMNESS OF ANJOU PEARS. PICKED JULY 10, 1968.

(61% of full maturity)

Table 4. Effect of ethylene and Ethrel treatment on firmness of Anjou pears harvested at 61% of full maturity.

Date	Days ripened	Pressure test (lbs)			
		Untreated	Ethylene 48 hours	Ethylene continuous	Ethrel
7-11-68	1	28.3	28.7	28.8	28.5
12	2	28.9	28.3	28.5	28.6
13	3	28.7	27.3	27.6	27.8
14	4	28.1	26.1	25.8	27.4
15	5	28.3	25.0	24.2	27.4
16	6	27.8	21.4	20.2	26.1
17	7	27.0	16.8	15.6	25.0
18	8	27.1	8.6	8.1	20.3
19	9	26.5	6.0	5.6	13.5
20	10	27.2	4.8	4.2	7.3
21	11	26.7	4.2	3.7	5.3
22	12	26.2	4.4	3.1	4.5
23	13	25.4	3.6	2.5	4.4
24	14	24.2	3.3	2.5	4.1

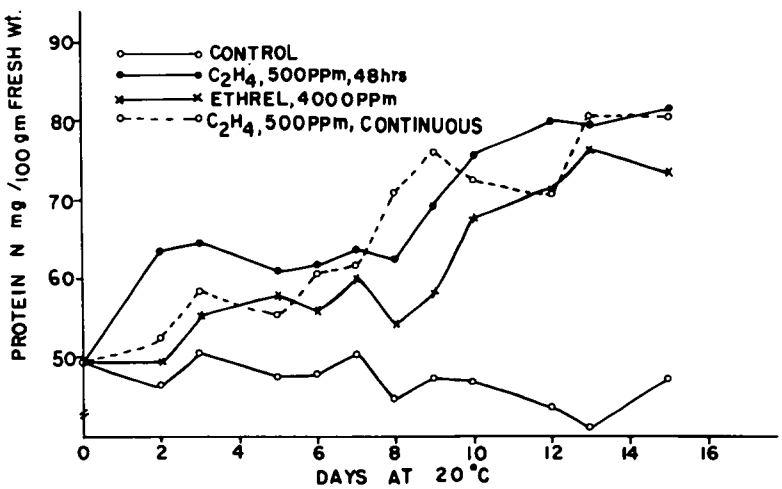


FIGURE 5. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON PROTEIN NITROGEN OF ANJOU PEARS. PICKED JULY 10, 1968.

(61% of full maturity)

Table 5. Effect of ethylene and Ethrel treatment on protein nitrogen content of Anjou pears harvested at 61% of full maturity.

Date	Days ripened	mg protein nitrogen per 100 gm fresh weight			
		Untreated	Ethylene continuous	Ethylene 48-hour	Ethrel
7-10-68	0	49.8	49.8	49.8	49.8
12	2	46.3	52.1	63.7	49.8
13	3	50.1	58.1	64.8	55.1
15	5	47.2	55.1	61.0	57.6
16	6	47.6	60.2	61.9	55.7
17	7	50.4	61.6	64.0	60.4
18	8	44.5	70.6	62.5	54.0
19	9	47.1	75.9	69.3	58.3
20	10	46.9	72.1	75.6	67.8
22	12	43.3	70.4	80.0	70.5
23	13	40.8	80.2	79.7	76.4
25	15	47.4	80.7	81.5	73.6

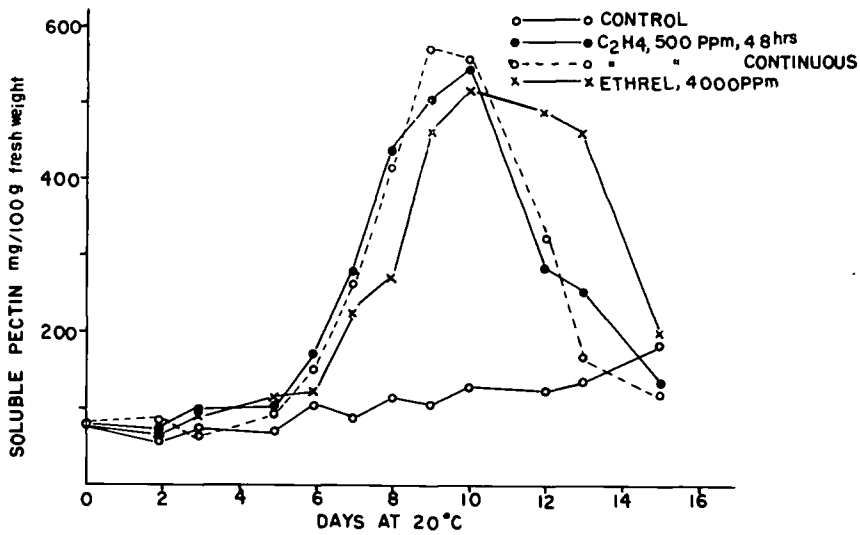


FIGURE 6. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON SOLUBLE PECTIN OF ANJOU PEARS PICKED JULY 10, 1968.

(61% of full maturity)

soluble pectin showed a rapid increase from 0.101 to 0.548 gm/100 g fresh weight from fifth day to tenth day (Table 6). In both of the above treatments, the content of soluble pectin declined when the fruits became over ripe. This may be explained by the conversion of soluble pectin to galacturonic acid as further ripening occurs. The soluble pectin content of Ethrel treated fruits increased to 0.513 gm/100 g fresh weight showing that ripening was proceeding without an increase in respiration rate. The soluble pectin in untreated fruits remained more or less constant at a low level throughout the experiment.

Experiment 1968 A2: Anjou pears used for this experiment were picked 109 days from full bloom which was 73% of full maturity. Average weight per fruit was 83.6 gm. Since an initial 48-hour ethylene treatment in the former experiment was sufficient to stimulate not only a rise in respiration but also increases in protein nitrogen, soluble pectin as well as a decrease in firmness, ethylene treatments of 36 and 24 hours were added in this experiment to determine if shorter treatments would produce similar stimulation.

The respiration rates are shown in Figure 7. As in the previous experiment, respiration of the ethylene treated fruits approximately doubled on the second day following treatment and then decreased. The 48-hour and continously treated samples thereafter increased to the climacteric maximum on the tenth day. After the

Table 6. Effect of ethylene and Ethrel treatment on soluble pectin content of Anjou pears harvested at 61% of full maturity.

Date	Days ripened	mg soluble pectin per 100 gm fresh weight			
		Untreated	Ethylene continuous	Ethylene 48-hour	Ethrel
7-10-68	0	81	81	81	81
12	2	63	77	70	66
13	3	75	65	97	97
15	5	73	98	101	102
16	6	109	151	173	122
17	7	87	268	279	225
18	8	113	412	439	270
19	9	104	571	509	460
20	10	129	559	548	513
22	12	126	323	282	486
23	13	135	167	252	458
25	15	181	124	136	196

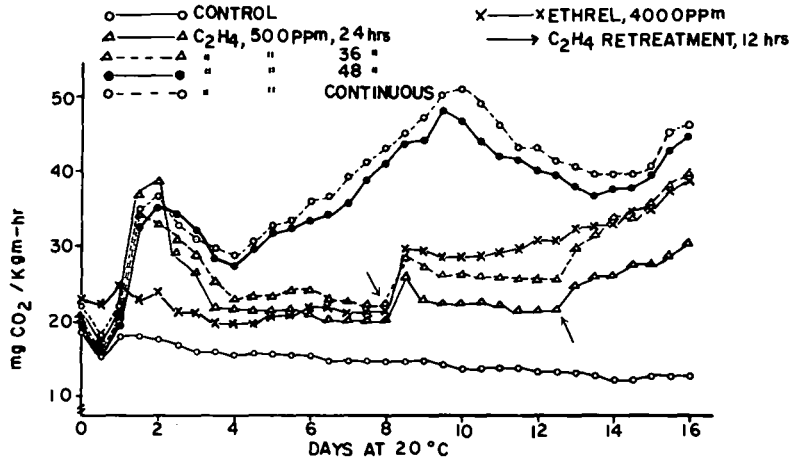


FIGURE 7. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON RESPIRATION RATE OF ANJOU PEARS PICKED JULY 28, 1968.
(73% of full maturity)

initial stimulation, the respiration rates of those fruits treated for 36 hours and 24 hours decreased after transfer to ethylene-free air. In order to determine whether they were still in the pre-climacteric stage, the two lots were retreated with ethylene for 12 hours on the eighth day. In both cases, the respiration rates were markedly increased, indicating a pre-climacteric stage. Similarly, fruits treated with Ethrel showed a low respiration rate until retreated with ethylene on the eighth day. A second retreatment was made at the twelfth day and there was still a slight stimulation of the 24- and 36-hour treated fruits. The respiration of untreated fruits remained low throughout the experiment.

Anjou pears softened only slightly after 12 days at 20° C in an ethylene-free atmosphere (Figure 8), while those treated for 24 and 48 hours and continuously decreased from 25.7 to 5.9 lbs, 4.8 lbs, and 4.2 lbs, respectively, in seven days (Table 7). Fruits treated with Ethrel also decreased in firmness from 25.5 lbs to 5.7 lbs in seven days. The data show that the fruits were fully ripe, even though the climacteric failed to develop.

Fruits treated with Ethrel or with ethylene for 24 hours, 48 hours, or continuously, increased in protein nitrogen content from 35.7 mg/100 gm fresh weight to 55.9, 54.6, 53.4, 58.7 mg/100 gm fresh weight, respectively (Table 8), while fruits kept in an ethylene-free atmosphere showed little increase in protein nitrogen.

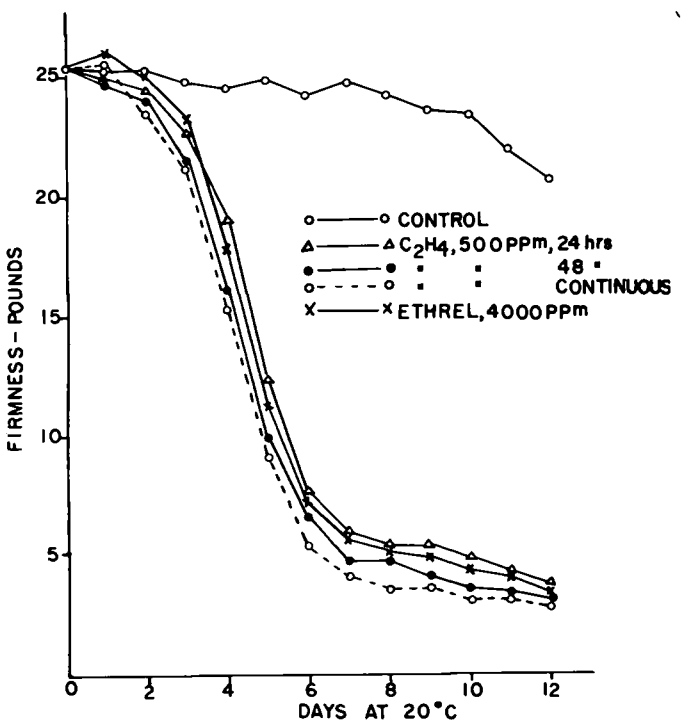


FIGURE 8. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON FIRMNESS OF ANJOU PEARS PICKED JULY 28, 1968.

(73% of full maturity)

Table 7. Effect of Ethrel and different lengths of ethylene treatment on firmness of Anjou pears picked at 73% of full maturity.

Date	Days ripened	Pressure test (lbs)				
		Untreated	Ethylene	Ethylene	Ethylene	Ethrel
7-28-68	0	25.4	25.7	25.7	25.5	25.5
29	1	25.6	25.2	25.1	25.3	25.6
30	2	25.3	24.3	24.0	23.5	24.9
31	3	24.9	22.5	21.5	21.2	23.0
8- 1-68	4	24.5	18.7	16.1	15.4	17.7
2	5	24.7	12.3	9.9	9.0	11.2
3	6	24.2	7.5	6.7	5.5	7.0
4	7	24.6	5.9	4.8	4.2	5.7
5	8	24.2	5.4	4.8	3.7	5.1
6	9	23.6	5.5	4.2	3.8	4.9
7	10	23.1	4.9	3.6	3.3	4.3
8	11	21.7	4.2	3.7	3.3	4.2
9	12	20.4	3.8	3.2	3.2	3.6

Table 8. Effect of Ethrel and different lengths of ethylene treatment on protein nitrogen content of Anjou pears picked at 73% of full maturity.

Date	Days ripened	mg protein nitrogen per 100 gm fresh weight				
		Untreated	Ethylene continuous	Ethylene 48-hour	Ethylene 24-hour	Ethrel
7-28-68	0	35.7	35.7	35.7	35.7	35.7
30	2	36.5	41.8	39.4	40.6	41.4
8-1-68	4	39.4	41.0	38.7	41.2	39.2
4	7	37.7	43.0	41.8	45.6	38.7
6	9	36.6	48.5	42.0	43.9	36.2
8	11	34.5	52.6	44.6	52.4	44.9
10	13	38.2	59.5	52.9	57.2	52.1
12	15	41.4	58.7	53.4	54.6	55.9

Soluble pectins in fruits treated with Ethrel or with ethylene for 24 hours, or 48 hours, or continuously, increased rapidly after two days at 20° C (Table 9), while untreated fruits showed only a slight increase after 16 days. The overall changes in protein nitrogen and soluble pectin are shown in Figures 9 and 10. These data provide additional evidence that the ripening processes proceed, in the absence of development of the climacteric.

Experiment 1968 A3: Anjou pears used for this experiment were picked 123 days from full bloom, which was 82% of full maturity. Average weight per fruit was 115.4 g. In order to find the critical length of time of ethylene treatment required for induction of the softening process, the treatments with ethylene were reduced to 24, 18, 12, and 6 hours.

The respiration rates are shown in Figure 11. As in the two previous experiments, the ethylene treatment produced a rapid initial rise in respiration followed by a decline. In this experiment, only the fruit continuously treated with ethylene developed the typical climacteric pattern, while respiratory activity of the 6, 12, 18, 24 hours and Ethrel treated samples declined to a low level and maintained a more or less steady state, until stimulated by retreatment on the ninth day. The respiration rate remained low and steady in fruits kept in an ethylene-free atmosphere.

The experiment was terminated on the eleventh day and the

Table 9. Effect of Ethrel and different lengths of ethylene treatment on soluble pectin of Anjou pears picked at 73% of full maturity.

Date	Days ripened	mg soluble pectin per 100 gm fresh weight				
		Untreated	Ethylene continuous	Ethylene 48-hour	Ethylene 24-hour	Ethrel
7-28-68	0	98	98	98	98	98
30	2	107	84	112	60	74
8- 1-68	4	118	186	223	181	157
4	7	168	585	554	457	401
6	9	147	575	558	486	562
8	11	244	437	448	595	477
10	13	215	262	296	473	388
12	15	320	154	171	278	270

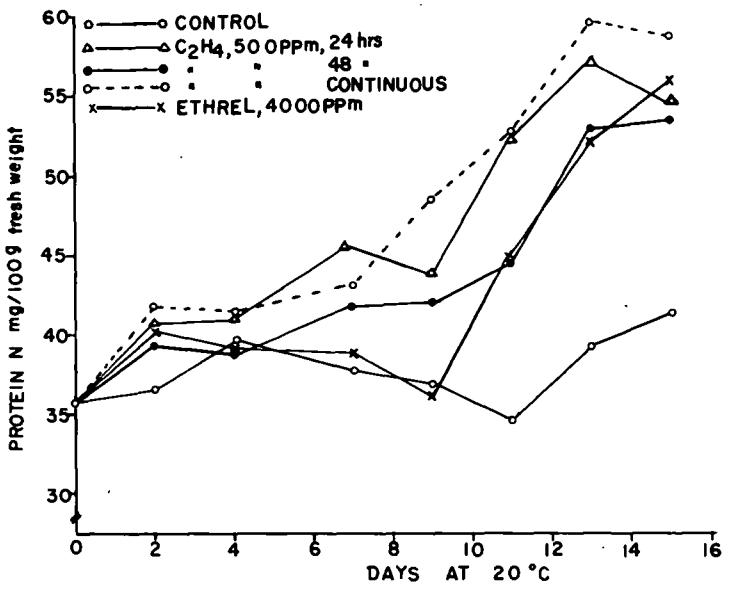


FIGURE 9. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON PROTEIN NITROGEN OF ANJOU PEARS PICKED JULY 28, 1968.
(73% of full maturity)

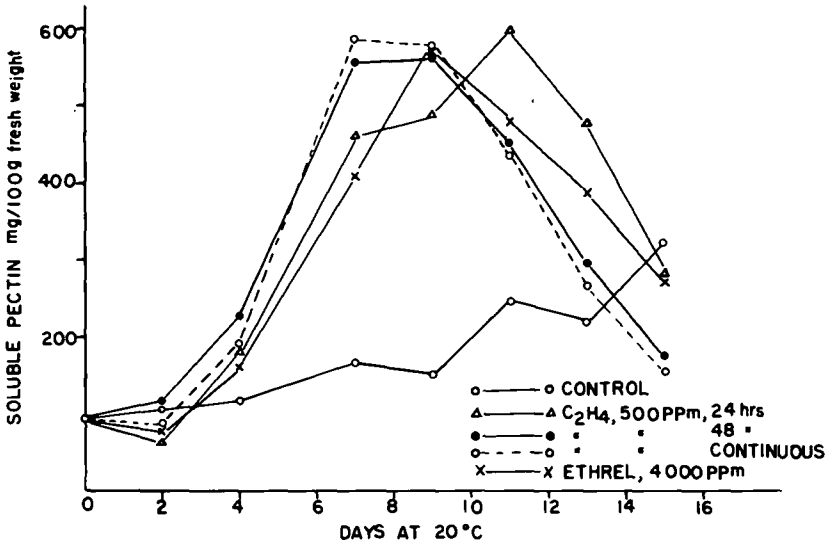


FIGURE 10. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON SOLUBLE PECTIN OF ANJOU PEARS PICKED JULY 28, 1968.

(73% of full maturity)

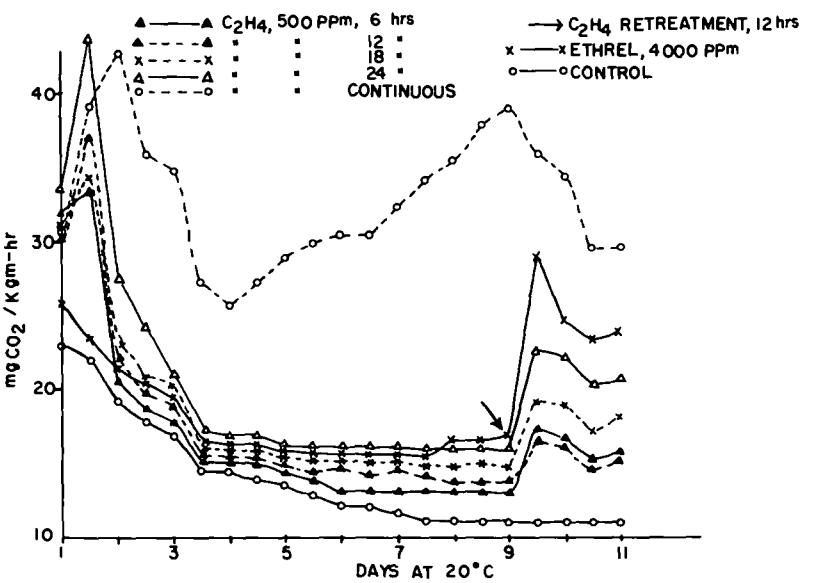


FIGURE 11. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON RESPIRATION RATE OF ANJOU PEARS PICKED AUG. 27, 1968.

(82% of full maturity)

fruits were tested for firmness. The fruits had a pressure test of 22.6 lbs at the beginning of the experiment. At the termination the pressure tests for the untreated, Ethrel treated, and samples treated with ethylene for 6 hours, 12 hours, 18 hours, 24 hours and continuously were 20.3, 4.4, 18.9, 18.2, 15.5, 3.6 and 2.8 lbs, respectively.

Experiment 1968 A4: Anjou pears used for this experiment were picked at the commercially mature stage which was 150 days after full bloom. Average weight per fruit was 167.9 g. The experimental design was the same as in 1968 A3. The purpose was to determine how fully mature pears responded to treatment as compared to immature fruit used in the previous experiments.

As shown in Figure 12a, several distinct differences in respiration are evident. The initial stimulation following treatment was low compared to that obtained in previous experiments. The initial stimulation was followed by a decline. The continuously treated sample developed the typical climacteric rise to a maximum on the ninth day. Contrary to the previous experiments, respiration of the Ethrel-treated pears showed a similar pattern instead of maintaining a low level rate. The samples treated from six to 24 hours with ethylene also showed a steady rise after the initial stimulation but at a slower rate than in the continuously and Ethrel treated fruits.

Pressure tests (Table 10) made on the eighth and tenth days

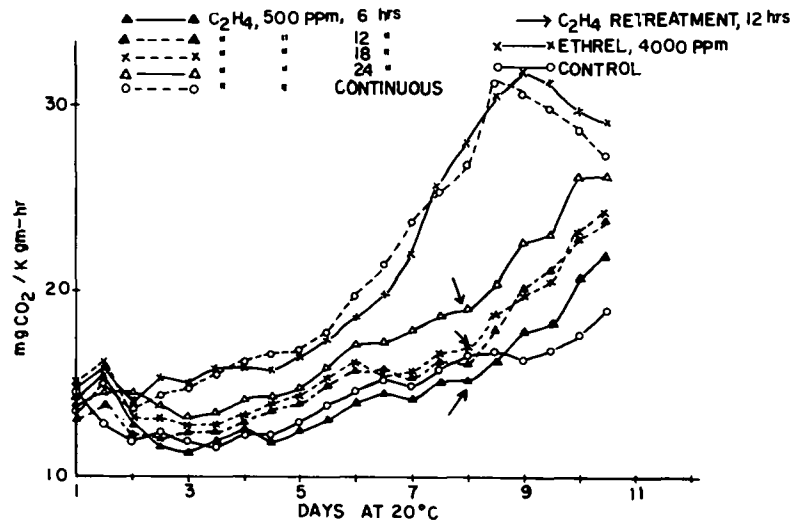


FIGURE 12. EFFECT OF ETHREL AND DIFFERENT LENGTHS OF ETHYLENE TREATMENT ON RESPIRATION RATE OF ANJOU PEARS PICKED SEPT. 24, 1968.

(fully mature)

Table 10. Pressure test of the Anjou pears harvested at the commercial maturity and treated with ethylene and Ethrel.

	8th day	10th day
Control	13.2 lbs	7.7 lbs
6 hours treated	12.7 lbs.	5.4 lbs
12 hours treated	6.5 lbs.	3.3 lbs
18 hours treated	4.8 lbs	3.1 lbs
24 hours treated	3.5 lbs	2.8 lbs
continue treated	3.0 lbs	2.2 lbs
Ethrel	3.2 lbs	2.2 lbs
Initial	16.5 lbs	

showed that the untreated fruits and those treated for six hours did not soften appreciably until after the eighth day, when the pressure test had decreased from 16.5 to 13.2 and 12.7 pounds, respectively. The 12, 18 and 24 hours treated as well as the Ethrel and continuously treated samples had softened appreciably by the eighth day and were fully ripe on the tenth day.

The concentrations of protein nitrogen which were determined at the beginning and the end of the experiment are presented in Table 11. The initial protein nitrogen content not only was lower than in previous samples but showed less increase during ripening.

In normal conditions, pears ripen only after harvesting. Pear fruits remain firm as long as they are attached to the tree, and the ripening process will not proceed until after harvest. The rate of respiration declines to a minimum shortly after harvesting and then rises rapidly to a maximum value of the climacteric peak. The duration of the pre-climacteric minimum is dependent upon external conditions and the maturity of the fruit itself.

The respiration of the Anjou pears picked at the immature stage (61% maturity) showed a continuous decline (Figure 12b) which flattened and turned to a steady state when the fruits were picked at 73% maturity. A slow increase in respiration was observed as fruits were harvested at the mature stage and kept in air at 20° C.

When treated continuously with ethylene, the respiration rate of

Table 11. Protein nitrogen content of the Anjou pears harvested at commercially mature stage.

Sample	Date	Day ripened	mg protein nitrogen per 100 g fresh weight
Initial	9-24-68	1	23.08
Ethylene 6-hour	10- 3-68	10	26.83
Ethylene 12-hour	10- 3-68	10	26.00
Ethylene 18-hour	10- 3-68	10	27.03
Ethylene 24 hour	10- 3-68	10	29.23
Ethylene continuous	10- 3-68	10	29.21
Ethrel	10- 3-68	10	32.54
Untreated	10- 3-68	10	26.03

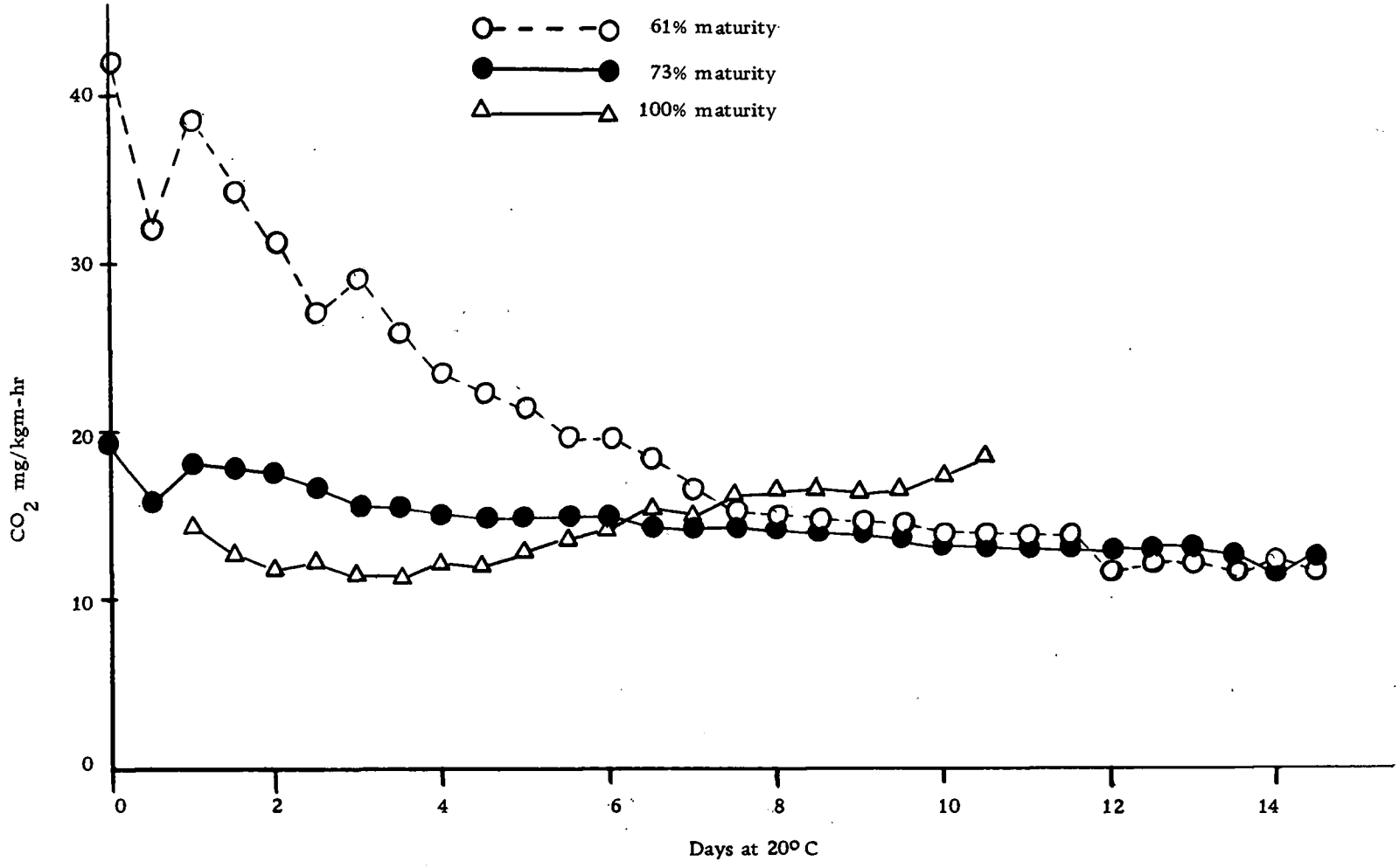


Figure 12b. Respiration rates of Anjou pears harvested at different degrees of maturity.

immature Anjou pears showed an immediate sharp increase. As the fruits approached maturity, this initial stimulation was reduced (Figure 12c). The time required to reach the climacteric maximum decreased with the increase in maturation. In immature fruits, it required 11 days to reach the peak, while in 82% and 100% mature fruits, only 10 and eight days, respectively, were required. A decrease in peak value was also observed as fruits approached maturity. The peak value was as high as 66.8 mg of CO_2 per kg hour in immature Anjou, while it decreased to 51.1 mg when fruits were picked at 73% maturity. The peak value further decreased to 31.1 mg as the fruits were harvested at full maturity.

From the experiments with Ethrel it is interesting to note that fruits with different degrees of maturity showed different responses to treatment. This is evident in Figure 12d. In earlier pickings, the respiration rates of the Ethrel treated Anjou pears did not increase until retreated by ethylene on the eighth and twelfth days. However, a climacteric rise developed in the mature fruits following treatment.

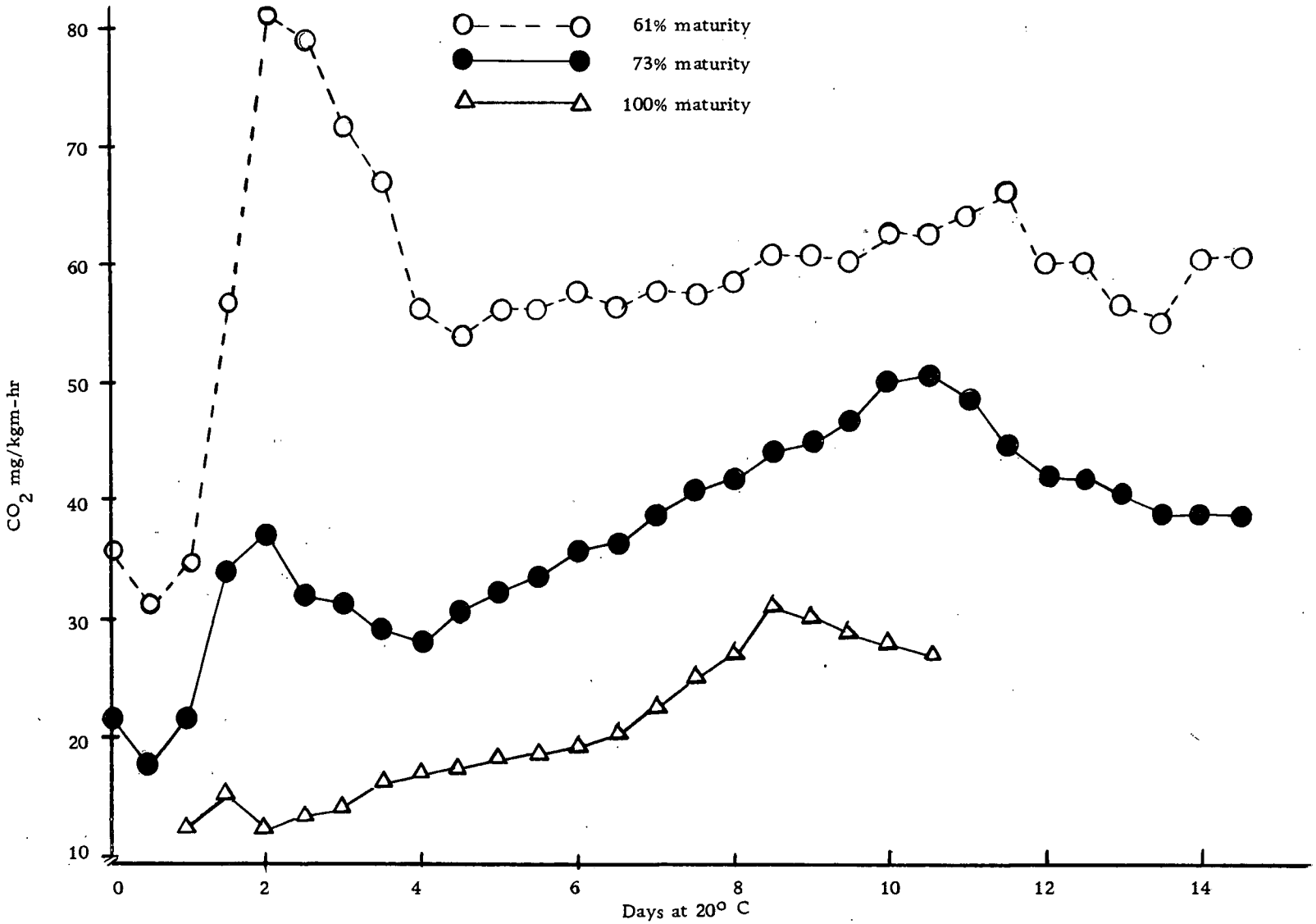


Figure 12c. Respiration rate of continuous ethylene treated Anjou pears harvested at different degrees of maturity.

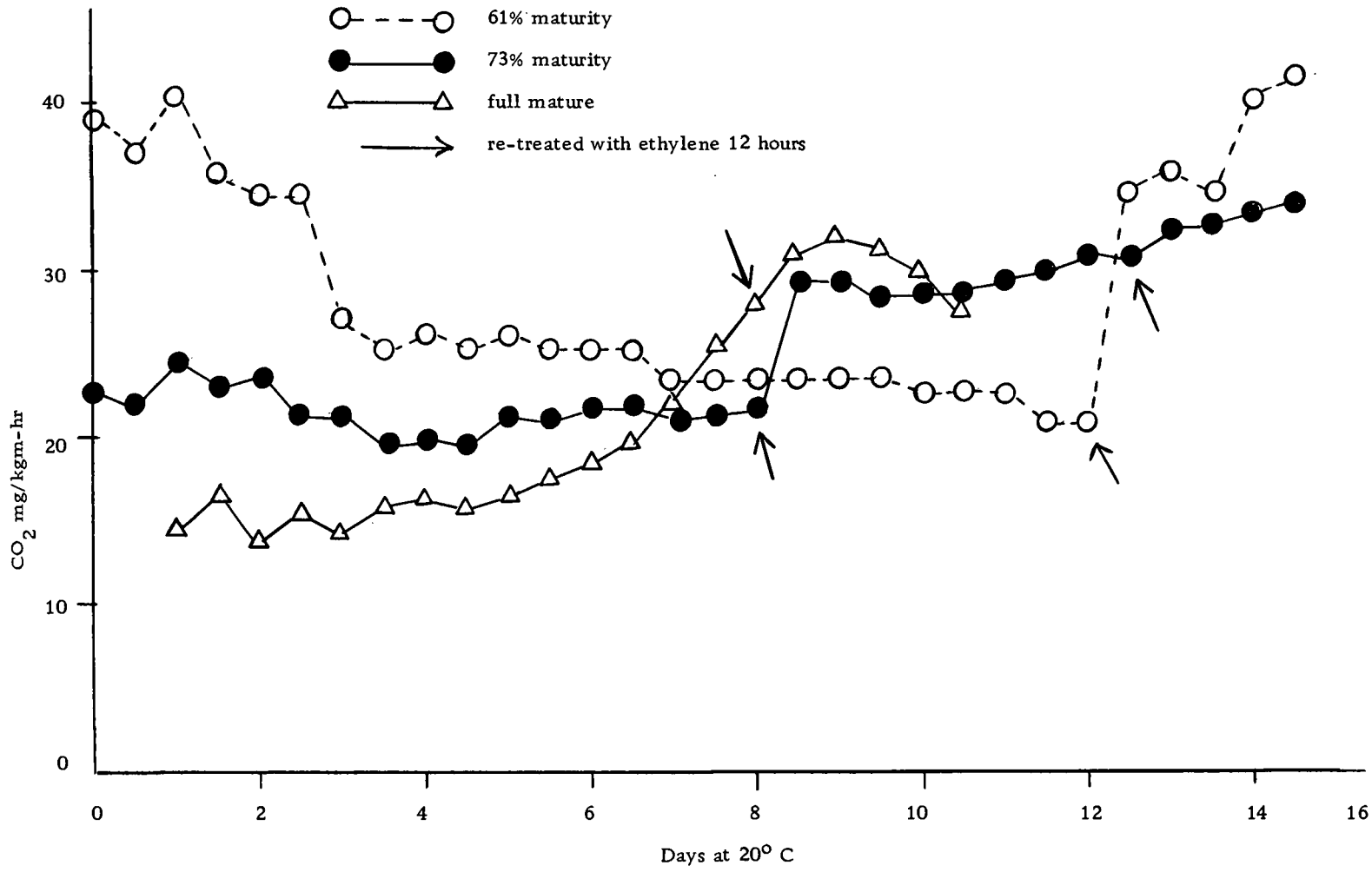


Figure 12d. Respiration rate of Ethrel treated Anjou pears harvested at three stages of maturity.

Changes in Chlorophyll Content and Chlorophyllase Activity During Ripening of Ethylene Treated and Untreated Fruits

Experiment 1968 B5: Bartlett pears were used in this experiment because a distinct color change from green to yellow develops during ripening. The method of Looney and Patterson (90) was used for determining chlorophyllase activity. The activity of chlorophyllase was expressed as absorbance which was the difference between incubated samples and the non-incubated control. Because the endogenous chlorophyll was catabolized during ripening, it was necessary to add exogenous chlorophyll prior to the end of the experiment to prevent lack of substrate from limiting the reaction. Enough chlorophyll extracted from spinach was added to bring the absorbance above 0.4 O.D. units at 665 mμ. This addition was made before the division of the filtrate into a control fraction and a fraction to be incubated. The fruits were kept at 20° C and the respiration rate, firmness, chlorophyll content and chlorophyllase activity were analyzed daily.

The data show that the chlorophyllase activity increased rapidly after two days of ethylene treatment (Figure 13). At the same time, the content of chlorophyll declined (Figure 13). The respiration rate showed a slow climacteric rise (Figure 14) as the fruits began to soften (Figure 15). The activity of chlorophyllase decreased when the fruits were fully ripe and yellow in color. The colors of

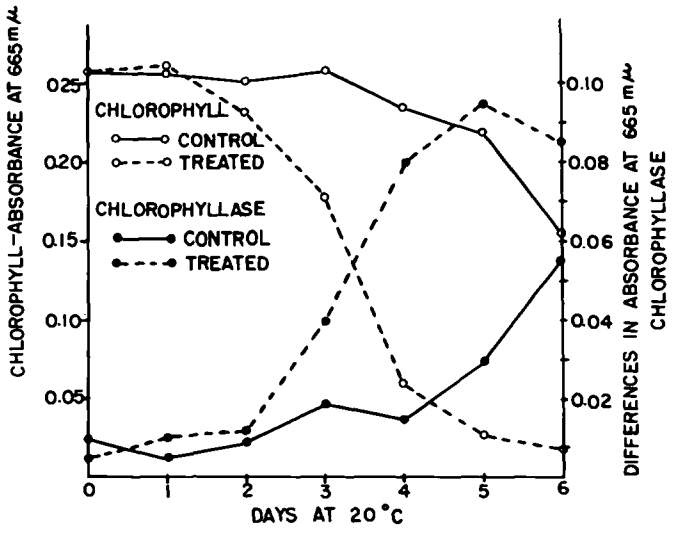


FIGURE 13. CHLOROPHYLLASE ACTIVITY AND CHLOROPHYLL CONTENT IN ETHYLENE TREATED AND UNTREATED BARTLETT PEARS DURING RIPENING AT 20°C. PICKED AUG. 7, 1968.

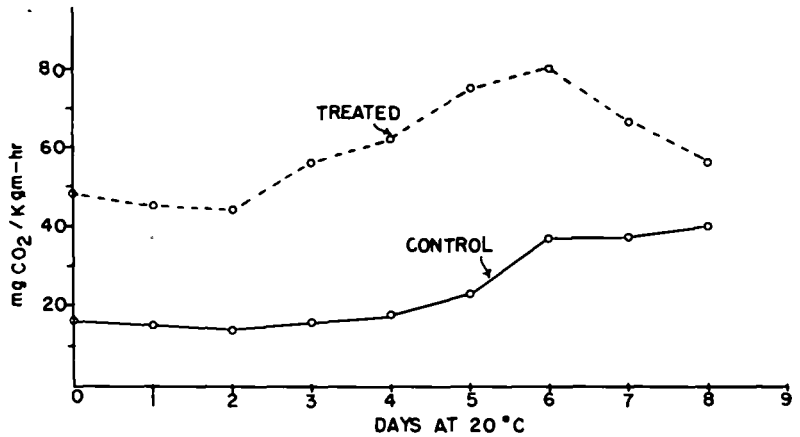


FIGURE 14. EFFECT OF ETHYLENE ON RESPIRATION RATE OF BARTLETT PEARS AT 20°C. PICKED AUG. 7, 1968.

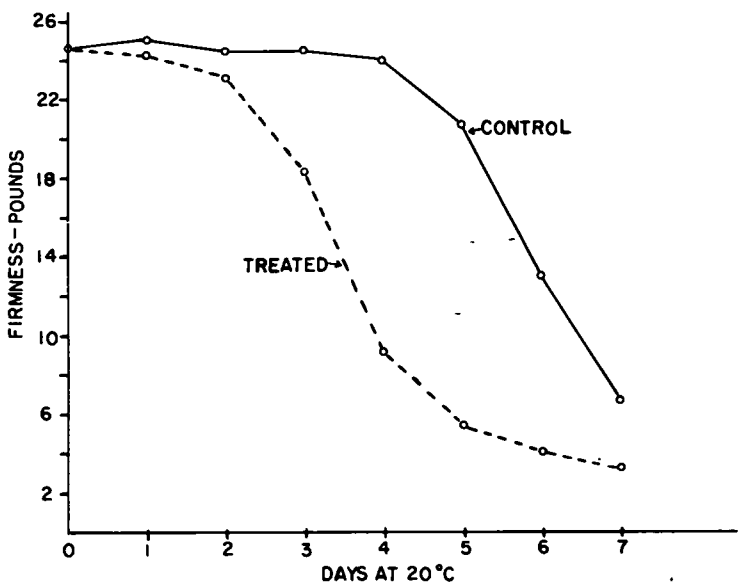


FIGURE 15. EFFECT OF ETHYLENE TREATMENT ON SOFTENING OF BARTLETT PEARS AT 20°C PICKED AUG. 7, 1968.

ethylene-treated and untreated fruits at the end of the experiment are shown in Figure 16.

Changes in Permeability During Ripening

Experiment 1967 B6: Bartlett pears used in this experiment were harvested 22 days prior to the commercial harvest date in the 1967 season. The firmness, respiration rate and permeability were measured daily at 20° C. Permeability change was expressed as the hourly change in weight of excised tissue disks as a percentage of the initial weight. The weight change of the tissue disks in deionized water was predominantly a change in water content of the tissue. This change in water content would indicate change in permeability.

In ethylene treated fruits there was an increase in water efflux on the third day of the treatment (Table 12, Figure 17) which was about one day after the fruits started to soften (Figure 18) and the respiration rate started to increase (Figure 19).

Experiment 1967 B7: The Bartlett pears used in this experiment were picked 14 days before the commercial harvest date in the 1967 season. On the third day of the experiment, the ethylene treated fruit showed an increase in water influx in the first two hours, followed by a strongly accelerated efflux (Figure 20). On the fourth and fifth days, the increase in water efflux from ethylene treated tissue disks was even more prominent (Table 13). However, no changes in

Figure 16. Color differences in untreated (1) and ethylene-treated (2) Bartlett pears after seven days at 20° C.

Table 12. Permeability[a] of ethylene treated and untreated Bartlett pears picked on August 1, 1967.

Hours after excision	Days stored at 20° C								
	1	2		3		4		5	
	Initial	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	108.87	107.71	107.91	107.59	105.47	111.48	111.23	109.07	108.78
2	108.30	107.60	108.53	106.78	103.13	112.84	111.54	109.49	106.34
3	109.36	107.39	106.76	105.65	101.42	112.63	109.55	109.07	102.76
4	108.17	105.14	104.99	105.02	100.57	110.54	107.76	-	-
5	107.45	103.53	102.81	104.11	98.43	108.56	105.67	-	-
6	107.71	100.01	100.32	103.94	97.36	107.41	101.99	-	-

[a]Change in weight each hour expressed as percentage of initial weight.

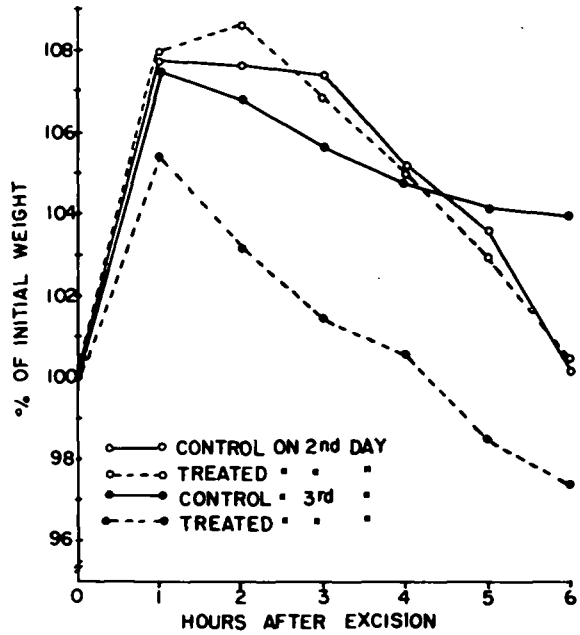


FIGURE 17. EFFECT OF ETHYLENE ON PERMEABILITY OF BARTLETT PEAR TISSUE DURING RIPENING AT 20°C. PICKED AUG. 1, 1967.

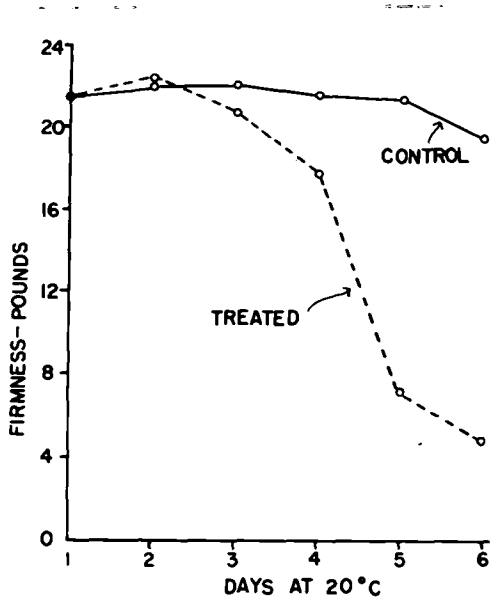


FIGURE 18. EFFECT OF ETHYLENE TREATMENT ON SOFTENING OF BARTLETT PEARS AT 20°C PICKED AUG.1 1967.

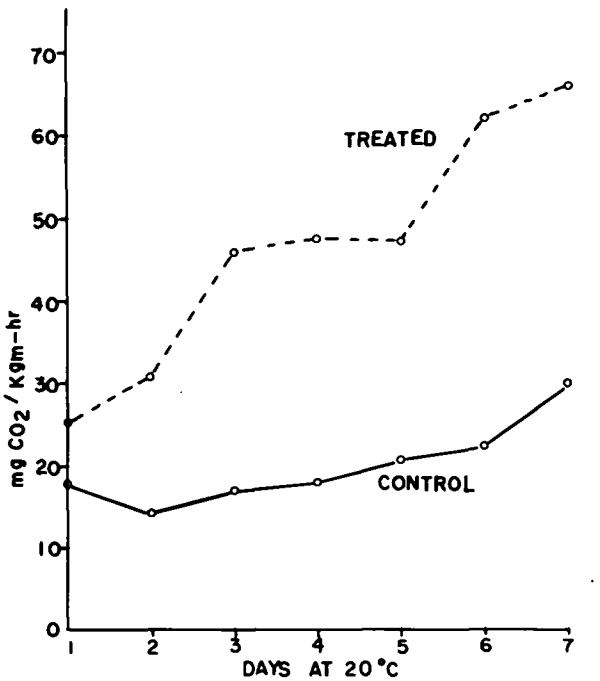


FIGURE 19. EFFECT OF ETHYLENE ON RESPIRATION RATE OF BARTLETT PEARS AT 20°C. PICKED AUG. 1, 1967.

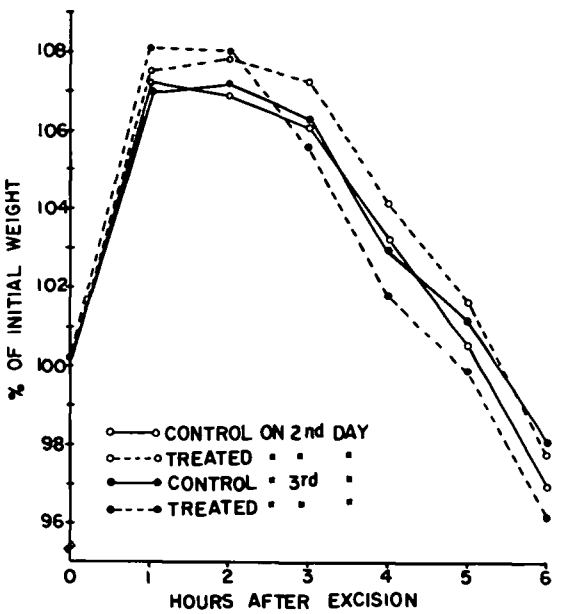


FIGURE 20. EFFECT OF ETHYLENE ON PERMEABILITY OF BARTLETT PEAR TISSUE DURING RIPENING AT 20°C. PICKED AUG. 7, 1967.

Table 13. Permeability[a] of ethylene treated and untreated Bartlett pears picked on August 7, 1967.

Hours after excision	Days stored at 20° C								
	1	2		3		4		5	
	Initial	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	102.63	107.09	107.41	107.05	108.06	107.92	107.73	108.71	107.41
2	101.82	106.99	107.83	107.16	107.85	108.13	107.94	108.08	106.37
3	100.10	106.05	107.20	106.22	105.62	107.61	106.12	106.27	104.17
4	97.57	103.13	104.07	102.90	101.76	105.73	102.79	104.14	100.73
5	95.55	100.42	101.57	101.03	99.89	105.10	101.07	103.08	99.37
6	95.14	96.87	97.70	97.92	96.18	104.17	97.96	101.34	97.28

[a]Change in weight each hour expressed as percentage of initial weight.

permeability were detected until about one day after the fruits began to soften (Figure 21) and two days after the onset of the climacteric rise (Figure 22).

Experiment 1967 A8: Anjou pears used in this experiment were picked nine days prior to the commercial harvest date in the 1967 season. The increase in water efflux caused by ethylene treatment was not apparent until the fourth day (Table 14, Figure 23). The time of increase in permeability in ethylene treated fruit was one day after the onset of the climacteric (Figure 24) and two days after the decrease in firmness (Figure 25).

Experiment 1968 A9: Anjou pears used in this experiment were harvested at the same time as those in experiment 1968 A1. The method of Baur and Workman (7) was used to determine the rate of ion leakage.

In the first three days, the specific conductance for untreated, continuous ethylene and Ethrel treated fruits showed more or less similar trends (Table 15). On the fourth day, the specific conductance of the continuous ethylene and Ethrel treated fruits after six hours was greatly increased from 54.05 μm hos on the third day to 92.36 and 91.45 μmhos, respectively, on the fourth day (Figure 26). The changes in respiration rate, firmness, protein nitrogen and soluble pectin were the same as those in experiment 2 A1.

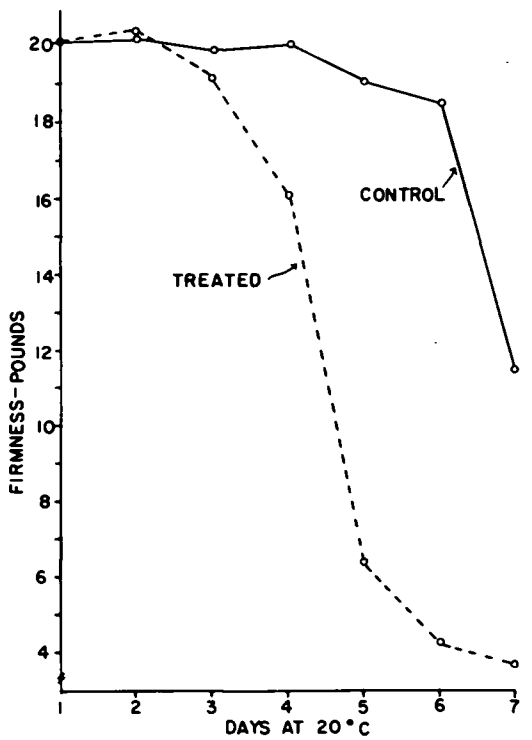


FIGURE 21. EFFECT OF ETHYLENE TREATMENT ON SOFTENING OF BARTLETT PEARS AT 20°C PICKED AUG.7, 1967.

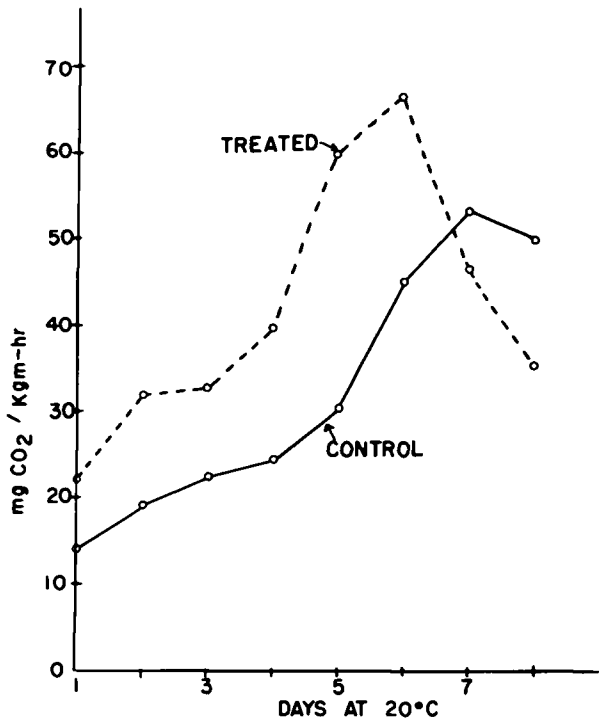


FIGURE 22. EFFECT OF ETHYLENE ON RESPIRATION RATE OF BARTLETT PEARS AT 20°C. PICKED AUG.7, 1967.

Table 14. Permeability[a] of ethylene treated and untreated Anjou pears picked on September 5, 1967.

Hours after excision	Days stored at 20° C										
	1	2		3		4		5		6	
	Initial	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
0	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
1	101.31	102.27	103.30	103.56	102.93	102.90	101.00	105.49	105.13	107.39	106.70
2	97.82	98.64	100.00	98.00	98.65	100.22	97.37	102.20	101.78	106.28	104.24
3	93.68	95.46	96.70	94.65	95.72	98.88	95.19	100.00	98.82	100.67	98.98
4	90.63	93.58	93.86	91.31	93.47	94.21	90.68	96.04	93.30	96.54	94.75
5	87.41	90.49	91.53	85.97	87.84	90.85	83.24	90.99	89.06	93.05	91.52
6	83.35	87.53	89.21	82.07	83.65	87.53	80.69	87.47	85.27	89.62	87.36

[a]Change in weight each hour expressed as percentage of initial weight.

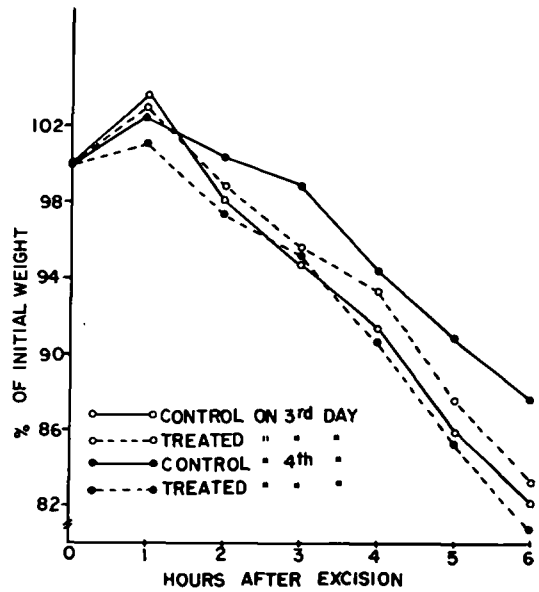


FIGURE 23. EFFECT OF ETHYLENE ON PERMEABILITY OF ANJOU PEAR TISSUE DURING RIPENING AT 20 °C. PICKED SEPT. 5, 1967.

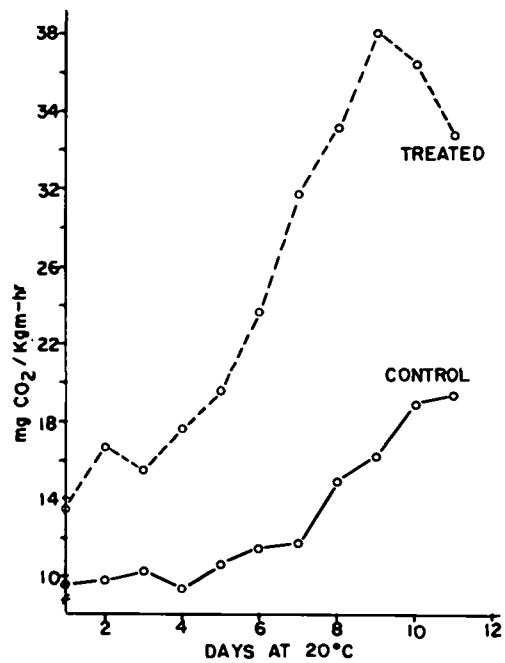


FIGURE 24. EFFECT OF ETHYLENE TREATMENT ON RESPIRATION RATE OF ANJOU PEARS AT 20°C. PICKED SEPT. 5, 1967.

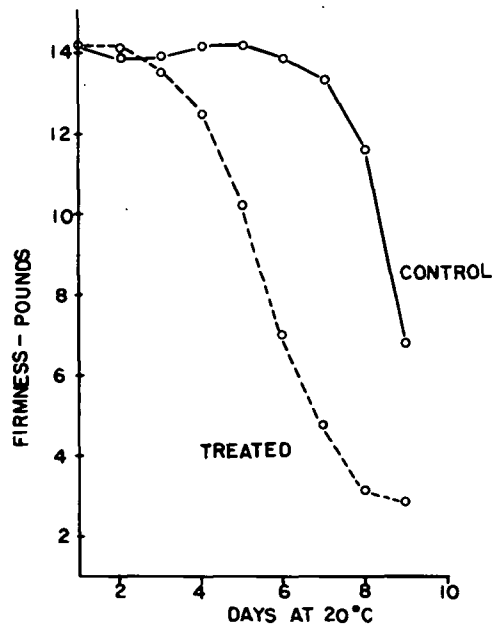


FIGURE 25. EFFECT OF ETHYLENE TREATMENT ON SOFTENING OF ANJOU PEARS AT 20°C. PICKED SEPT. 5, 1967.

Table 15. Rate of leakage of electrolytes from untreated, ethylene-treated, Ethrel-treated pear tissues, measured in conductivity (μmhos) of bathing solution.

Hours after excision	Days at 20° C														
	1			2			3			4			5		
	Control	Ethylene	Ethrel	Control	Ethylene	Ethrel	Control	Ethylene	Ethrel	Control	Ethylene	Ethrel	Control	Ethylene	Ethrel
1	27.02	21.73	22.72	21.27	18.51	17.85	25.00	23.80	23.80	29.41	43.47	41.66	35.71	47.61	45.45
2	33.33	27.77	31.25	30.30	27.77	27.02	33.33	33.33	32.25	35.71	62.50	52.63	50.00	76.92	66.66
3	35.71	34.48	34.48	35.71	32.25	30.30	43.47	40.00	38.46	45.45	76.92	66.66	62.50	90.90	86.95
4	38.46	37.03	38.46	41.66	40.00	38.46	47.61	43.47	45.45	52.63	86.95	80.00	68.96	95.23	94.78
5	41.66	38.46	41.66	47.61	45.45	43.47	50.00	46.95	47.61	58.82	90.90	88.47	76.92	105.26	104.71
6	45.45	43.47	45.45	48.78	47.61	47.61	50.26	50.26	50.26	62.50	92.36	91.45	83.33	117.64	117.64

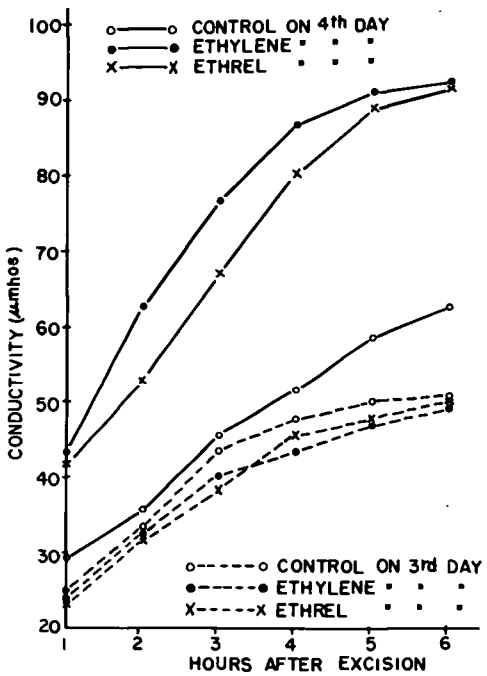


FIGURE 26. RATE OF ION LEAKAGE FROM ETHYLENE-TREATED, ETHREL-TREATED AND UNTREATED ANJOU PEARS DURING RIPENING AT 20°C. PICKED JULY 10, 1968.

DISCUSSION

On the basis of the results obtained in these experiments, ripening of pears requires initiation or induction by ethylene but can occur independently of the climacteric rise in respiration. Following induction, active ripening occurs as indicated by softening, changes in protein nitrogen, and by increases in soluble pectin, even though the climacteric fails to develop. This has been demonstrated by the following experiments.

Anjou pears initially treated with ethylene for 24 hours (experiment 1968 A2) developed a sharp increase in respiratory activity which decreased upon discontinuation of treatment and remained at a low steady state thereafter until retreated. The fruit, however, softened to the fully-ripe condition and showed an increase in protein nitrogen and soluble pectin. When again exposed to ethylene for 12 hours, the rate of respiration was stimulated but declined after removal from treatment. Since it has been well documented that no effect on respiration from ethylene can be obtained in the post-climacteric stage, the positive response obtained in this experiment clearly indicated that the fruit was still pre-climacteric, even though full ripe. Similar results were obtained when Anjou pears picked at a later stage of maturity were initially treated for 12 and 18 hours (experiment 1968 A3). Both of these shorter treatments

initiated ripening but rate of respiration declined to a low steady level following the initial stimulation. The fruits also responded similarly to treatment with Ethrel, which releases ethylene when absorbed by the tissues. The Ethrel treated fruits behaved somewhat like 24-hour ethylene treated fruits in all experiments, except a typical climacteric rise in respiration was developed along with the softening and increase in protein nitrogen and soluble pectin in experiment 1968 A4. This was probably due to the effect of the degree of maturity, because the fruits used in experiment 1968 A4 were harvested at the fully mature stage. The mature fruit is physiologically ready to initiate the ripening process and the concentration of ethylene required to stimulate the climacteric rise is not as high as immature fruits.

The length of initial ethylene treatment required for induction of the ripening mechanism decreased with increase in degree of fruit maturity. Thus, 24 hours was required for fruit picked at 61 and 73 percent of full maturity while fruit which had attained 82 percent of maturity ripened after a 12-hour treatment. The typical climacteric rise in respiration failed to develop in all of these experiments but proceeded to the maximum in fruit receiving 48-hour or continuous treatments. These results indicate that the mechanisms involved in ripening and respiration respond differentially to ethylene treatment. The enzyme system, the ability of ethylene production and other factors necessary for the ripening of the fruits apparently are

gradually built up during the maturation process. Hansen (46) found that ethylene does not initiate the ripening process unless the fruit is physiologically mature. The length of treatment required to induce ripening of Bosc pears decreased with increase in fruit maturity.

Separation of the climacteric from ripening, however, cannot always be readily demonstrated. In fully mature Anjou pears treated with Ethrel, the climacteric developed together with ripening. Maturation probably involves an increase in ethylene producing capacity as well as in the development of the ripening mechanism. Also, in cultivars such as Bartlett, with inherently higher rates of respiration and ethylene production than Anjou, development of the climacteric would be difficult to control during ripening. Frenkel, Klein and Dilley (39) however have shown that when ripening was inhibited by cycloheximide, respiration was unaffected. The association of the climacteric with ripening in the past by other investigators probably was due to the fact that mature fruit was used. Also, Biale's separation of various kinds of fruits into climacteric and non-climacteric types, on the basis of these experiments, no longer appears to be valid. Anjou pears, for example, can be classified in either category, depending upon maturity and length of exposure to ethylene.

Ripening of a fruit is always accompanied by softening. This decrease in tissue firmness is considered to be related to the

conversion of protopectin to soluble pectin (34). The chemistry of this substance is not fully elucidated, but it is known that the basic structure is a chain of galacturonic acid units combined through glycosidic linkages.

The results of the pectin studies during ripening in Anjou pears agree with those of Carré (26, 34) who observed that in the early stages there was no soluble pectin in apple fruits but during ripening the content increased, the maximum amount being reached when the fruit was fully ripe. Later there was a steady decline. Biale (11) suggested that the hydrolysis of methyl groups may be brought about by pectase or pectin methyl esterase, and soluble pectin may be broken down to galacturonic acid by pectinase or polygalacturonase. This might account for the decrease in soluble pectin when the fruit is overripe.

Anjou pears treated with ethylene for 24 hours, 48 hours and continuously or with Ethrel showed an increase in protein nitrogen during ripening at 20° C (Figures 5 and 9). This increase in protein actually includes the enzyme required for ripening, according to the evidences provided by Frenkel, Klein, and Dilley (39) and Rhodes and Wooltorton (100). Some of the enzymes which must be present during the ripening of fruit have a hydrolytic function (100). Among these are the enzymes involved in the degradation of chlorophyll, starch and pectic substances.

The changes observed in the chlorophyllase activity in Bartlett pears appear to support the suggestion that chlorophyllase has an essential role in the decomposition of chlorophyll (90). Some workers (29) reported that chlorophyllase has a biosynthetic role for chlorophyll. It is possible, as suggested by Looney (75), that there are actually two enzymes, with similar physical properties present in the chloroplast, one involved with the decomposition of chlorophyll and the other with the synthesis. However, this possibility has not been investigated.

Permeability is one of the currently active theories advanced to explain the climacteric. The concept is based on the consideration that the changes in permeability alter the protoplasmic compartmentalization, resulting in contact between enzymes and substrates (106, 107). This has been demonstrated in banana (7) and avocado (8). However, Hulme et al. (60) measured the uptake of metabolites and found that changes in permeability in apple tissues appeared to be a selective process characteristic of substrates or groups of substrates. Burg (14) also considered that the data on fruit leakage often did not reflect changes in membrane permeability but rather the total solutes available for leakage, especially sugar and malate, which increased during the climacteric. The results of the present studies with pears likewise do not support the permeability theory. While ethylene treatment of both Anjou and Bartlett pears did result in permeability

changes, these were detectable only after ripening had been initiated. Changes in leakage in pears, therefore, appear more likely to be a result rather than the cause of the ripening process.

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