DEVELOPMENT OF ETHYLENE BIOSYNTHESIS IN APPLES

by

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ABSTRACT

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The development of the ethylene biosynthesis pathway via 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene was investigated. During the rise of the natural ethylene climacteric, development of ethylene forming enzyme (EFE) in Paulared apples was slow, but ACC levels were high. Law Rome apples had high EFE and low ACC content. Empire apples had moderate EFE activity and moderate levels of ACC. The development of EFE was associated with that of internal ethylene concentration. Preclimacteric Empire and Law Rome apples showed high sensitivity to propylene treatment, but Paulared did not. The rate of ACC conversion to ethylene may play an important role in regulating ACC content in both natural and propylene-induced ethylene climacteric development. Hypobaric storage did not inhibit EFE activity as great as ACC synthesis in Law Rome apples. After hypobaric storage low temperature delayed the development of EFE in preclimacteric fruits, resulting in a massive accumulation of ACC. In postclimacteric fruits EFE activity remained high and there was no accumulation of ACC.

to my grandmother, my uncle, and my parents

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I am especially grateful to Dr. David R. Dilley for his advice and patience throughout the course of this study. I also wish to extend my appreciation to the members of my guidance committee, Dr. Hans Kende, Dr. Robert Herner and Dr. Clarence Suelter.

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TABLE OF CONTENTS

LIST OF FIGURES

PART THREE

INTRODUCTION

Climacteric fruits, such as apples, tomatoes, and pears, are characterized by a surge of ethylene production during ripening (1,30). The biosynthetic pathway of ethylene production has been established as S-adenosylmethionine (SAM)-->1-aminocyclopropane-1-carboxylic acid (ACC)--> ethylene (2,3). The formation of ACC from SAM is generally considered to be the rate limiting step and is catalyzed by ACC synthase (8,41,42). The conversion of ACC to ethylene is the last known step (40,41). The enzyme for this step has been termed ethylene forming enzyme (EFE). Since the establishment of this pathway, many studies have been conducted on the development of ethylene biosynthesis during fruit ripening, as summarized by Yang and Hoffman (41). Preclimacteric fruits have a very limited ability not only to convert SAM to ACC, but also to convert ACC to ethylene (38). The ACC content in these preclimacteric fruits is very low, but increases greatly associated with a concomitant increase in ethylene production. Actually, the dynamic level of ACC is a consequence of the equilibrium between its synthesis and conversion (21). The rapid accumulation of ACC which occurs during the onset of ripening indicates that the rate of ACC formation exceeds the rate of conversion. Conversely, decreased amounts of ACC are indicative that the rate of ACC conversion may be greater than the rate of formation. In tomato fruits, ACC synthase activity and ACC content increase during ripening (23). In avocado fruits, ACC synthase activity, ACC content, and EFE activity increase during the climacteric rise (21,35). As fruits become overripe, EFE becomes impaired and ethylene production declines resulting in a second peak of ACC.

Ethyene production in climacteric fruits is an autocatalytic phenomenon (1,30). Endogenous ethylene triggers the onset of ripening and, as a consequence, a massive increase in ethylene production occurs from the development of both ACC synthase and EFE activities which are low in preclimacteric fruits (38,41). If exogenous ethylene is applied to preclimacteric fruits, autocatalytic ethylene production will be induced if the fruits are sufficiently mature (1). Ethylene analogues, such as propylene, have the same effect as ethylene in inducing the ethylene climacteric (13). Studies with tomato and cantaloupe have brought about an interesting question concerning the order of initiation between the ACC synthesis and ACC conversion. In preclimacteric tomato and cantaloupe fruits EFE is enhanced by short-term ethylene treatment without an increase in ACC synthesis and ethylene production (29). Thus, the initiation of EFE precedes that of ACC synthesis in these ethylene treated fruits. Whether this is also true during the natural ripening in tomato and cantaloupe is unclear. However, studies with avocado suggest that EFE increases only when ethylene increases during natural ripening (35). In apple fruits little is known about the development of EFE activity as related to changes of internal ethylene concentration (IEC) during the development of the ethylene climacteric. To further understand the regulation of ethylene production in ripening apple fruits, information with repect to the changes in ACC level, ACC conversion, and ethylene production is needed.

Although exogenous ethylene hastens the onset of autocatalytic ethylene production, the time between the initial treatment with ethylene and the onset of the ethylene climacteric varies depending on the maturity which conditions the sensitivity of the tissue to ethylene(41). Apples show increased sensitivities to propylene when they are harvested at differet stages of maturity (33). Twenty four hours of ethylene treatment to Golden Delicious apples increased the EFE activity exponentially as a function of maturity (11). It has been suggested that endogenous ripening inhibitors exist in preclimacteric fruit and these inhibitors are destroyed or compartmentalized when fruits are exposed to ethylene and its analogues (41). Two systems have been proposed to explain ethylene action (11,31). System 1 ethylene is related to the low preclimacteric ethylene production and is initiated or controlled by an unknown aging factor. System 1 ethylene then triggers ripening and the formation of vast amounts of system 2 ethylene.

Since the introduction of hypobaric storage by Burg and Burg (12), which allows fruits to be stored under hyponormal ethylene levels, many studies have been devoted to its effect on the ripening process (4,9,15,17,18,19). This has led to a better understanding of the roles of both ethylene and oxygen. Studies by Dilley *et al* (18) and Bangerth (4) show that preclimacteric apple fruits can be kept in the preclimacteric stage without any ripening changes. Moreover, storage under hypobaric conditions was found to uncouple ethylene synthesis from ethylene action. When postclimacteric apples are kept at 0.05 atmosphere at 0°C, softening was retarded and this may indicate that ethylene may play a role in fruit ripening beyond that of initiating the synthesis of ripening enzymes. A continous presence of certain amounts of ethylene and oxygen may be required for autocatalytic ethylene production and ethylene action. This can in turn explain the effect of hypobaric storage which maintains a low oxygen and ultra-low ethylene environment.

The physiological role of environmental factors on ethylene biosynthesis and ripening of fruits has been investigated by many researchers as summarized by Yang and Hoffman (41). These factors can exert their effects on ACC synthesis and the conversion of ACC to ethylene. Temperature is an important environmental factor. Chilling stress induces ethylene production by a number of chilling sensitive tissues. Chilling 'Bosc' pears results in the fruits gaining the capacity to produce ethylene and subsequently ripen (34). Wang and Adam (43,44) found that chilling induces ethylene production in cucumber fruits. They suggested that chilling treatment unmasks or stimulates the production of mRNA coding for ACC synthesis. In Golden Delicious apples, low temperature stimulates ethylene production and the accumulation of high ACC levels during the first month of storage in air (26). ACC content declined after two months, followed by much higher ethylene production. Although these results are not entirely convincing due to the lack of information on the ability of the tissues to convert ACC to ethylene, it is still quite possible that low temperature enhances ACC synthesis but not ACC conversion to ethylene during the initial period of low temperature storage. The ability to convert ACC to ethylene increases after two or three months of storage in cold air(26). The effect of oxygen and low temperature has been studied in 'd'Anjou' pears (5,6) where it was found that low oxygen and low temperature inhibit the development of ACC conversion but not ACC synthesis. This would suggest that the limiting step in ethylene production in treated 'd'Anjou' pears is the conversion of ACC to ethylene.

The objective of this research was to study the ethylene production of apples, focusing on the changes of ethylene levels, ACC content, and the EFE system as estimated by the capacity to convert ACC to ethylene. Three experiments were conducted. The first experiment involved natural ripening, which was based on the log increase in internal ethylene concentration of fruits on the tree. In the second experiment, the effect of propylene on the development of the ethylene climacteric in preclimacteric apples was studied. The third experiment involved the effects of hypobaric storage and temperature on ethylene production of both preclimacteric and postclimacteric Law Rome apples.

PART ONE

DEVELOPMENT OF ETHYLENE BIOSYNTHESIS IN NATURALLY RIPENING APPLES

Introduction

Climacteric fruits, such as apples, tomatoes, avocadoes, and pears, are characterized by a surge of ethylene production during ripening (1,30). The biosynthetic pathway of ethylene production has been established as S-adenosylmethionine (SAM)--> 1-aminocyclopropane-1-carboxylic acid (ACC)--> ethylene (2,3). The formation of ACC from SAM is generally considered to be the rate limiting step and is catalyzed by ACC synthase (8,41,42). The conversion of ACC to ethylene is the last known enzyme catalyzed step (40,41). The enzyme has been termed ethylene forming enzyme (EFE). Since the establishment of this pathway, many studies have been conducted on the development of ethylene biosynthesis capacity during fruit ripening, as summarized by Yang and Hoffman (41). Preclimacteric fruits have a very limited ability not only to convert SAM to ACC, but also to convert ACC to ethylene (38). The ACC content in unripe fruits is very low, but increases greatly associated with a concommitant increase in ethylene production. Actually, the dynamic level of ACC is a consequence of the equilibrium between its synthesis and conversion to ethylene (21). The rapid accumulation of ACC which occurs during the onset of ripening indicates that the rate of ACC formation exceeds the rate of conversion to ethylene. Conversely decreased amounts of ACC are indicative that the rate of ACC conversion to ethylene may exceed the rate of ACC formation or that the amount or activity of ACC synthase has become rate limiting. In tomato fruits, ACC synthase activity and ACC content increase during ripening (23). In avocado fruits, ACC synthase activity, ACC content and EFE activity increase during the climacteric rise (21,35). As fruits become overripe, EFE becomes impaired, and ethylene production declines resulting in a second peak in ACC content.

The order of initiation during ripening between the ACC synthesis and ACC conversion systems is unclear. In preclimacteric tomato and cantaloupe, EFE was enhanced by ethylene treatment without an increase in ACC synthesis and ethylene production (29). Thus, the initiation of EFE precedes that of ACC synthesis in these ethylene treated fruits. The question arises as to whether this reflects natural ripening processes. Studies with avocado suggest that EFE increases only when ethylene increases (35). The ACC synthase is first synthesized, which in turn, produces more ethylene. Once the ethylene threshold level is reached both ACC synthase and EFE are synthesized more rapidly leading to autocatalytic ethylene production.

In apple fruits little is known about the development of EFE activity as related to ethylene production during natural ripening. To futher understand the regulation of ethylene production in ripening apple fruits, information with respect to the changes in ACC level, ACC conversion, and ethylene production is needed.

The criteria to determine the stages of ripening differ in different fruits. Days after harvest and color index are often used because of the difficulties in determining ethylene concentration inside fruits (21). These criteria, however, are often misleading. During the climacteric rise, ethylene levels change rapidly and in fruits with the same color index or harvested at the same time can differ dramatically in internal ethylene (19). To provide a more accurate assessment of physiological maturity and ripening development, internal ethylene concentration (IEC) was used in this experiment. The initiation of the ethylene climacteric is widely regarded as the most definitive measure of physiological developoment prestaging ripening. Moreover, ethylene is obligatory for ripening. In order to understand the ripening phenomenon in different apple fruits three varieties which ripen at different times during growing season were used for comparison.

Materials and Methods

Materials. Paulared and Empire fruits were harvested at the Horticulture Research Center at Michigan State University in 1985. Law Rome fruits were harvested from orchards in the Grand Rapids area of western Michigan. Apples of medium size (125-175 g) were selected. In the study of ethylene biosynthesis during natural ripening on trees, all fruits were collected at a single harvest and grouped according to their internal ethylene concentration. Internal ethylene concentration, ranged from those of the preclimacteric stage up to and including apples at their climacteric peak. Samples of peel and cortex tissue were taken immediately after harvest and lypholyzed for ACC analysis.

Determination of IEC. One ml of internal gas was sampled with a syringe from the core of apples and ethylene was determined by gas chromatography (32).

Determination of ACC. One gram of freeze-dried apple tissue powder was homogenized with cold methanol and the extract was assayed according to the method of Lizada and Yang (28).

Assay of EFE. EFE was estimated *in vivo* by measuring the conversion of exogenous ACC to ethylene. Discs with 2 mm thick and 10 mm in diameter were prepared from individual fruits using a cork borer. One gram of discs were incubated in 25 ml flasks with 2 ml of solution composed of 0.1 mM cycloheximide, 20 mM Mes buffer (pH 6.0), 2% sucrose, with or without of 2 mM ACC. Flasks were incubated at 22°C with constant shaking for 3 hours.

Results and Discussion

Cortical flesh discs were used to measure the natural ethylene production rate when no exogenous ACC was added. Administration of ACC was used to measure the tissue's ability to convert ACC to ethylene. Natural ethylene production was low in preclimacteric Paulared fruits (Figure 1). It increased markedly during the climacteric rise. Addition of exogenous ACC increased ethylene levels in flesh discs. The extent of increase differed at different levels of IEC. Discs from fruits with IEC below 1.0 ul/l had only a slight net gain in ethylene subsequent to addition of ACC. In fruits with IEC above 1.0 ul/l the net gain in ethylene production was one-to-twofold. This indicates that the ability of these fruits to convert exogenous ACC to ethylene remained low in preclimacteric fruits and increased subsequent to development of ACC synthase as with avocado (35).

Once synthesized, ACC has two fates: being converted to ethylene or being conjugated with other compounds (22). Preclimacteric Paulared fruits had very low levels of ACC (Figure 2). ACC content increased rapidly after the initiation of ethylene climacteric reaching approximately 60 nmol per gram dry weight. At the climacteric peak there was more than a 50-fold increase in ACC content while over the same IEC range the increase in ethylene production rate by flesh discs incubated without the addition of ACC added only about 8-fold (Figure 1). Apples harvested at the same time with a wide range of IEC, depicting a succession of physiological developmental stages showed a similar association between ACC content and ethylene production rate.

Although EFE has not been isolate in cell free extracts, the *in vivo* assay using discs is a common way to estimate EFE activity (11,27,29,35). Among the three cultivars of apples studied (Figure 1,3,and 5), Paulared fruits had the lowest EFE activities and this increased slowly with the development of the ethylene climacteric. In Law Rome fruits, on the other hand, EFE activity developed very markedly (Figure 5). The development of EFE activity in Empire fruits (Figure 3) also increased markedly though not as great as in Law Rome fruits.

It is interesting to note that the development of EFE activity in Paulared fruits during the early stages of the ethylene climacteric (<1.0 ul/l) occurred relatively more slowly than the increase in ACC content (Figure 1 and 2). The increase in IEC in Paulared must be due to the increase in ACC content. This agrees with the results obtained from avocado fruits where the increase in EFE activity does not precede the increase in ACC synthesis (35). It may be true that in Paulared apples and in avocado fruits the development of ACC synthesis precedes the development of ACC conversion capacity. Even in Empire and Law Rome fruits EFE activities increased only at the beginning of the ethylene climacteric rise (Figure 3 and 5).

Changes in the levels of ACC are determined by the rate of ACC synthesis relative to its rate of conversion (21). In Paulared fruits, the rapid accumulation of ACC which occurred during the onset of ripening indicates that the rate of conversion. This correlates well with the low activity of EFE found in this cultivar.

As with Paulared, cortical flesh discs of Empire fruits, without the addition of ACC, produced small amounts of ethylene and the ethylene production rate increased during the climacteric rise (Figure 3). Application of exogenous ACC significantly increased the ethylene production rate by fruit discs. The Net gain in ethylene increased as the climacteric developed. As compared to the flesh discs of Paulared, the capacity of Empire tissue to convert ACC to ethylene was much higher.

ACC content in preclimacteric Empire fruits was low and increased to measurable levels after the IEC in fruits was above 1.0 ul/l (Figure 4). Collectively, the ACC levels in Empire were 2 to 3-fold lower than those in Paulared and this may be related to the observation that Empire fruits ripen more slowly than the Paulared.

The ethylene production rate of Law Rome cortical tissue discs without added ACC was essentially the same as that of Paulared and Empire fruits during the ethylene climacteric (figure 5). However, the ability to convert exogenous ACC to ethylene increased much more rapidly in Law Rome fruits as the IEC increased than with either of the 2 other cultivars. Law Rome tissue was able to convert ACC to ethylene significantly over control tissue without added ACC at IEC levels as low as 0.3 ul/l. After the IEC reached 1.0 ul/l, the ACC conversion rate increased dramatically eventually up to 200 nl per gram per hour.

ACC content was low throughout the development of the ethylene climacteric of Law Rome (Figure 6) while ACC content increased slightly during later stage of ripening. This may indicate that ACC synthesis is the rate limiting step in the Law Rome cultivar and EFE activity is adequate to convert ACC to ethylene as it is produced by ACC synthase.

Law Rome apples had very low ACC content during ripening, indicating that the rate of ACC synthesis was balanced by the rate of ACC conversion. This correlated well with the high EFE activities in Law Rome fruits. In Empire fruits the rate of ACC synthesis was slightly higher than the rate of ACC conversion, resulting in a moderate accumulation of ACC.

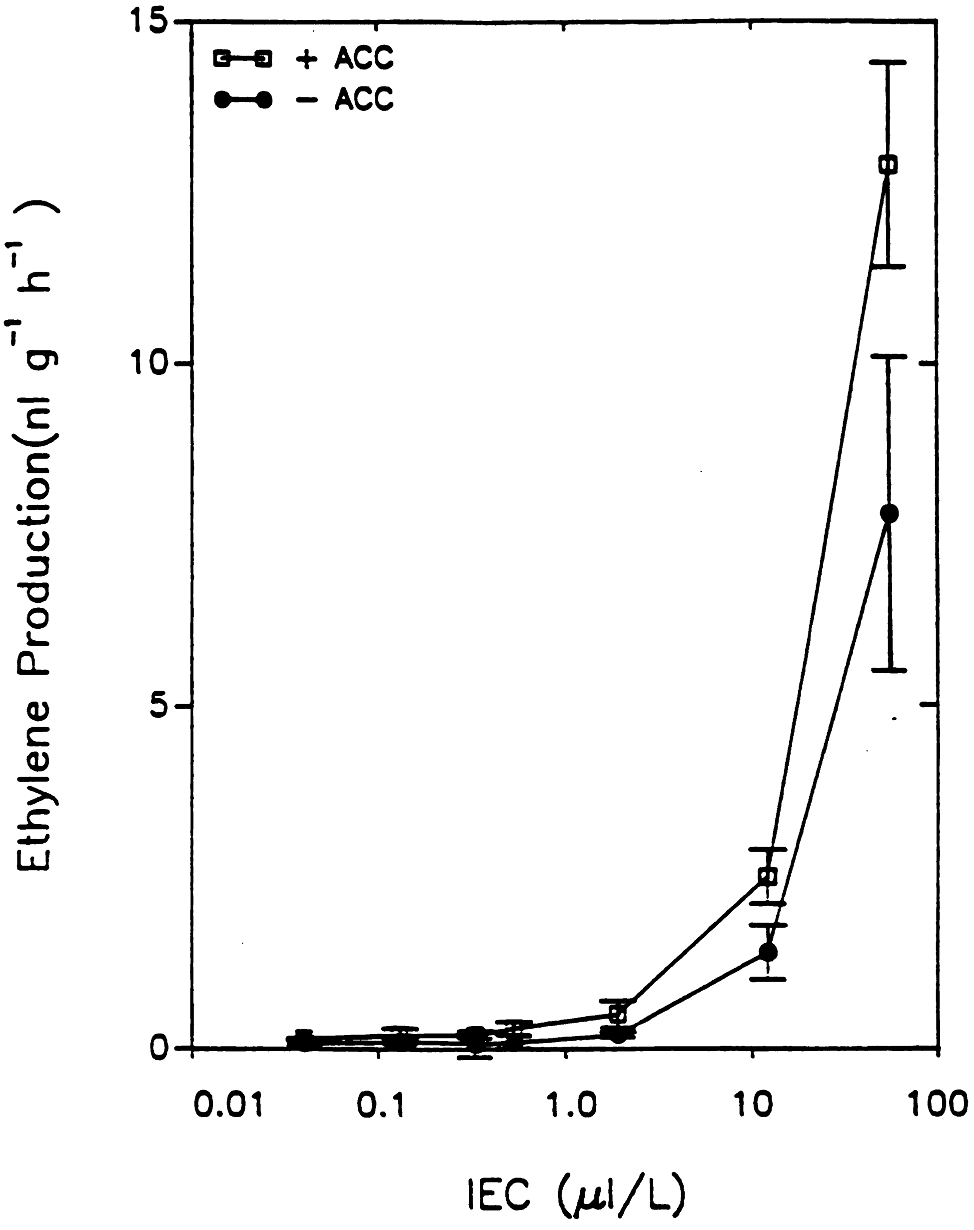

Figure 1. Changes in ethylene production rate in Paulared fruit discs with or without exogenous ACC during the climacteric rise. Ethylene production rate was plotted as a function of IEC. IEC is presented in logarithmic scale to represent the natural log increase in ethylene during ripening.

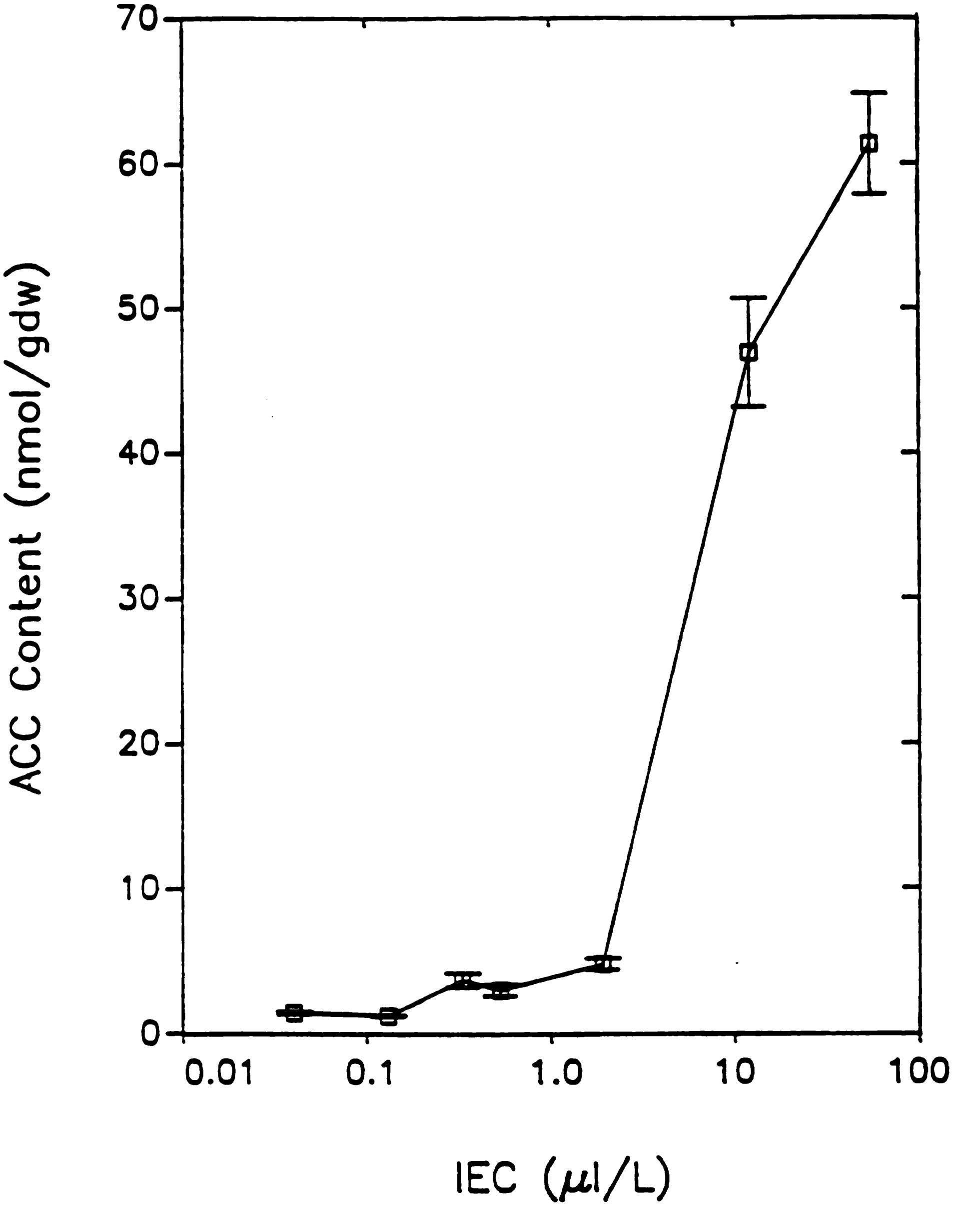

Figure 2. Changes in ACC levels in Paulared fruits during the climacteric rise.

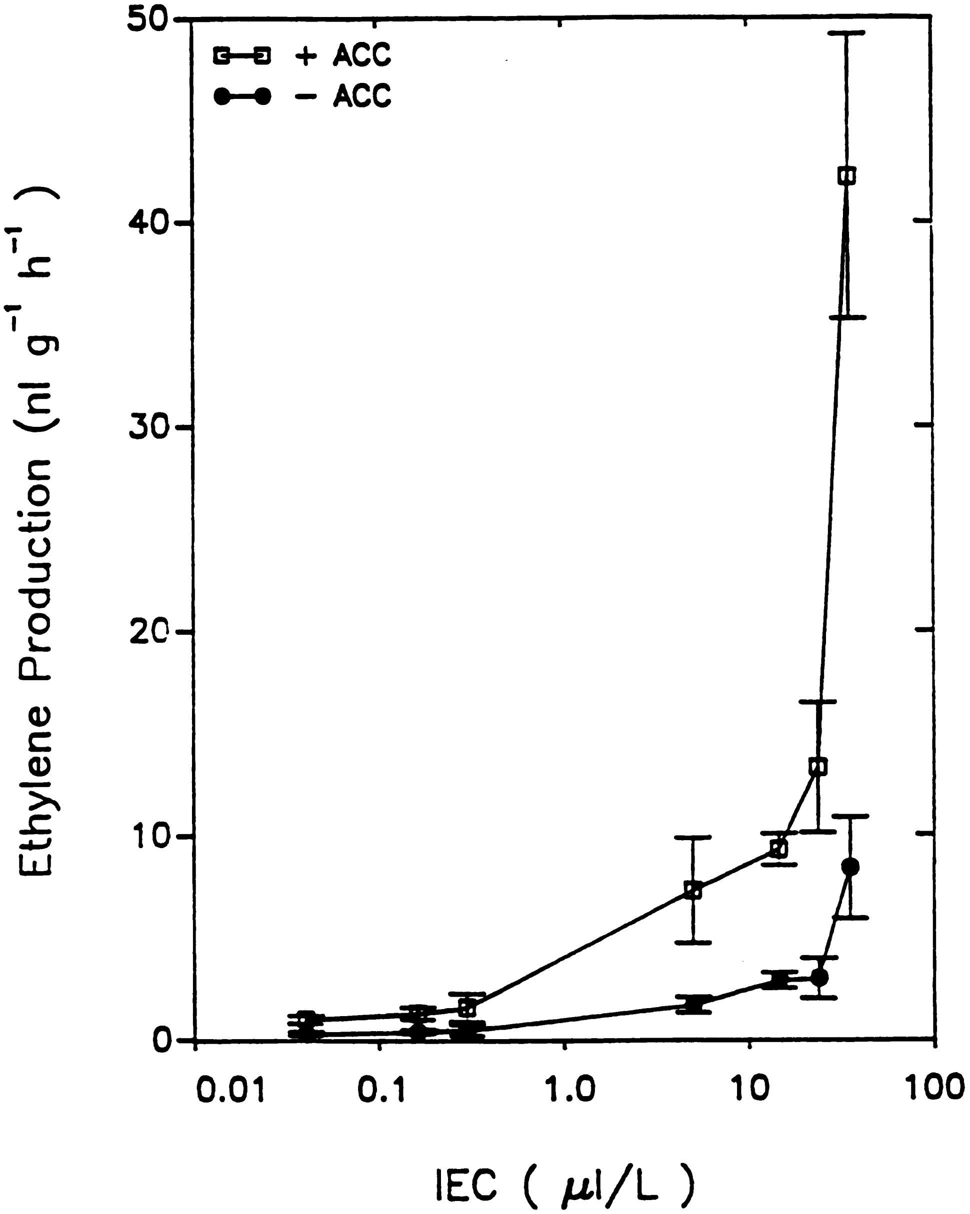

Figure 3. Changes in ethylene production rate in Empire fruit discs with or without added ACC during the climacteric rise.

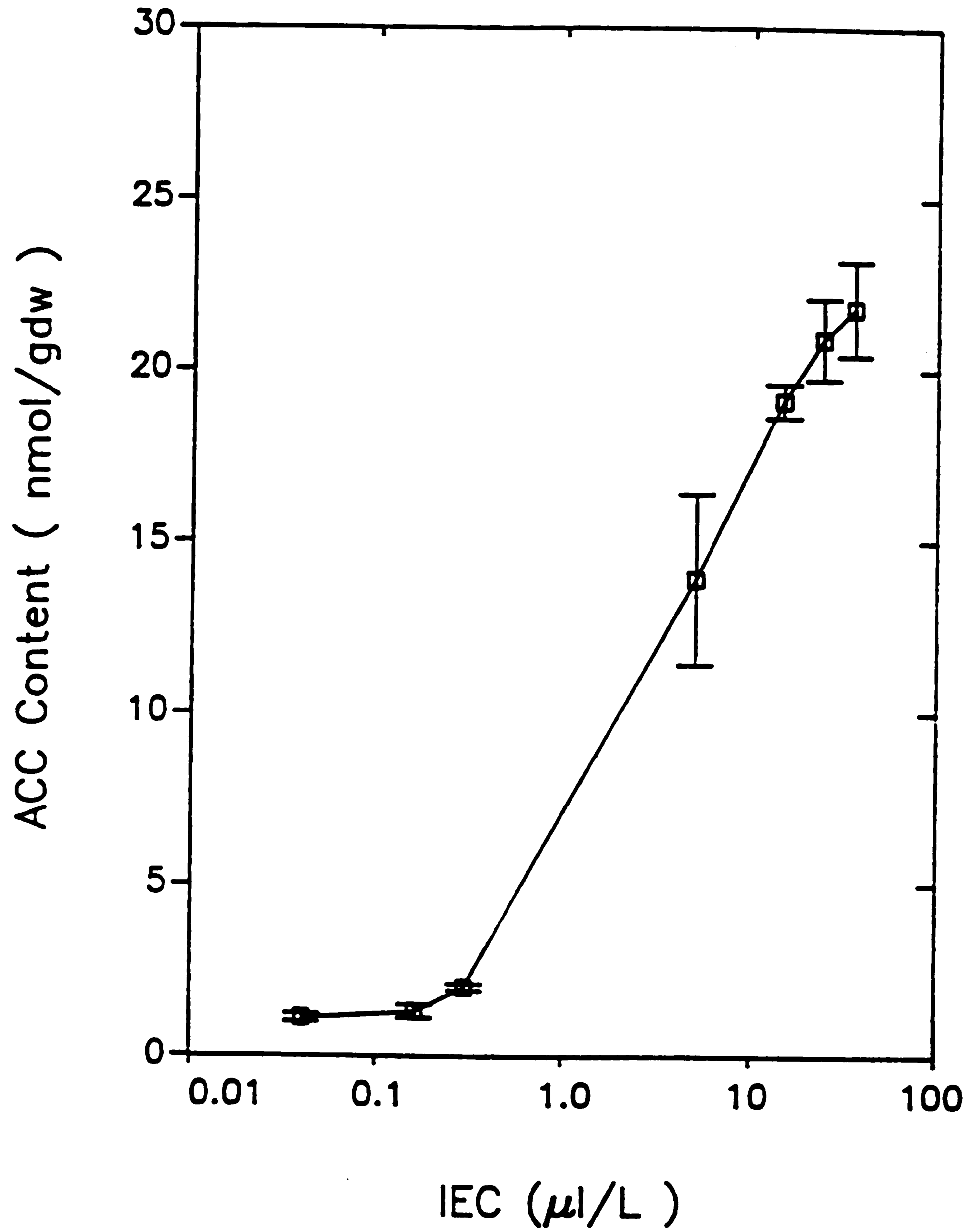

Figure 4. Changes in ACC levels in Empire fruits during the climacteric rise.

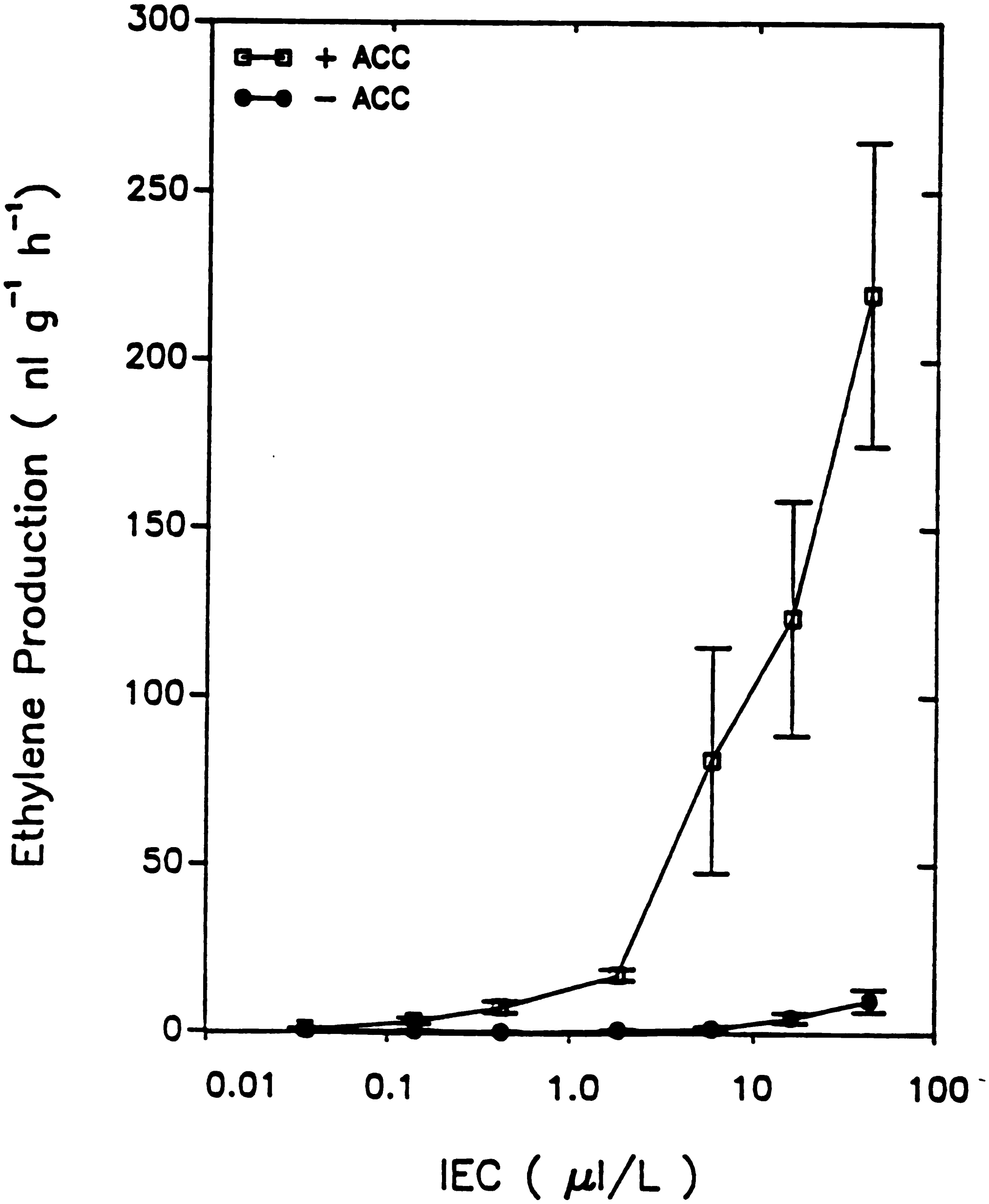

Figure 5. Changes in ethylene production rate in Law Rome fruit discs with or without ACC addition during the climacteric rise.

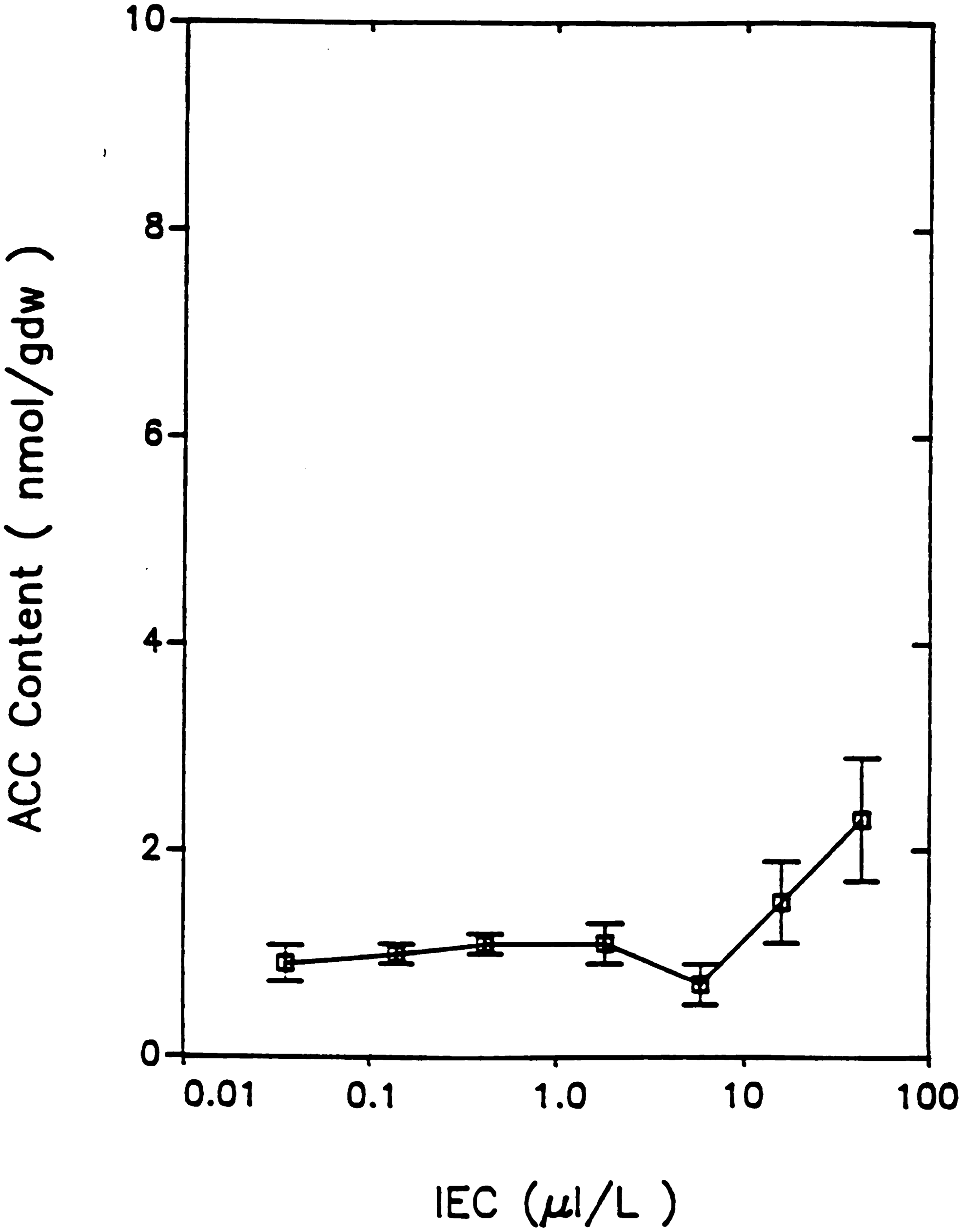

Figure 6. Changes in ACC levels in Law Rome fruits during the climacteric rise.

PART TWO

EFFECT OF PROPYLENE ON ETHYLENE BIOSYNTHESIS

Introduction

Ethylene production in climacteric fruits is an autocatalytic phenomenon (1,30). Endogenous ethylene triggers the onset of ripening and, as a consequence, a massive increase in ethylene production occurs from the development of both ACC synthase and EFE activities which are low in preclimacteric fruits (41). If exogenous ethylene is applied to preclimacteric fruits autocatalytic ethylene production is induced (1). Ethylene analogues such as propylene have the same effect as ethylene in inducing the ethylene climacteric (13). Studies in tomato, cantaloupe, avocado, and Golden Delicous apples show that short term ethylene treatment stimulates development of EFE without enhancing ethylene production (11,27,35). Ethylene production, extractable ACC synthase activity, and ACC level are increased by long term ethylene or propylene treatment (9,10).

Although exogenous ethylene hastens the onset of autocatalytic ethylene production, the time between the initial treatment with ethylene and the onset of the ethylene climacteric varies depending on the maturity which conditions the sensitivity of the tissue to ethylene (41). Apples show increased sensitivities to propylene when they are harvested at different stages of maturity (33). Twenty-four hours of ethylene treatment to Golden Delicious apples increased the EFE activity exponentially as a function of maturity (11). It has been suggested that endogenous ripening inhibitors exist in preclimacteric fruits, and these inhibitors are destoyed or compartmentalized when fruits are exposed to ethylene and its analogues (31,41). Alternatively, increased sensitivity to exogenous or endogenous ethylene with development may reflect a decline in a substance derived from the tree that conditions the fruits sensitivity to ethylene. Calcium is a likely candidate for this putative substance because its translocation to the fruit slows relative to fruit growth in late development. This decrease by dilution coupled with compartmentalization of calcium pre- or postharvest may explain the decrease in sensitivity of fruits to ethylene. Fruit calcium level has been positively related to a decrease in sensitivity of avocado fruit to ripen and supplementation of fruit with calcium is known to delay the onset of the ethylene climacteric (36). The sequence of ethylene action is postulated to be comprised of two systems (11,31). System 1 ethylene is related to the low preclimacteric ethylene production and is initiated or controlled by an unknown aging factor. System 1 ethylene then triggers ripening and the formation of vast amounts of system 2 ethylene.

The objective of this experiment was to study the effect of propylene on the development of the ethylene climacteric in preclimacteric apples. The focus was on the changes in ethylene levels, EFE, and ACC content by short-term propylene treatment at different stages of maturity and by long-term propylene treatment.

The development of the ethylene climacteric induced by propylene treatment was compared to that in apples ripened naturally on the tree.

Materials and Methods

Materials. Three varieties of apples, Paulared, Empire, and Law Rome were harvested in 1985 growing season from the same sources as described in Part One. Fruits were harvested at different stages of maturity ranging from very immature to mature based on growth development and internal ethylene levels.

Propylene Treatment. Apples were placed in 21 liter plastic buckets and ventilated with a constant flow of moist air at 22°C containing 100 ul/l propylene. Apples in buckets ventilated with ethylene or propylene-free air were used as controls.

Determination of Ripening. The time when approximately 30% of apples harvested had IEC above 1.0 ul/l was considered as the time initiated.

Determination of IEC, EFE, and ACC content were determined as described in Part One.

Results

Sensitivity of Propylene Treatment in Preclimacteric Apples during Maturation. Apples harvested at different stages of maturation were treated with propylene at 100 ul/l for two days to test their sensitivity. In Paulared apples, IEC was stimulated by propylene and the increase in IEC progressed as maturation advanced (Figure 1, A). There was a slight increase in IEC in apples without propylene treatment but the levels remained below 0.08 ul/l. The capacity of fruit to convert exogenous ACC to ethylene was also stimulated slightly but did not show any changes in sensitivity to propylene treatment at different stages of maturity (Figure 1, B). In Empire fruits, propylene stimulated both IEC and EFE and showed a trend to increase during maturation (Figure 2). However, IEC and EFE of the control fruits also increased over the same period in similar but parallel manner. When Law Rome fruits were harvested at early stages of maturation, propylene had no effect on IEC (Figure 3, A). At later stages of maturation IEC was increased markedly by propylene treatment, indicating a great increase in sensitivity with maturation. The capacity to convert exogenous ACC to ethylene was induced only slightly through the early stages of maturity and dramatically at later stages (Figure 3, B). ACC content in Paulared, Empire and Law Rome fruits showed no significant change by two-day propylene treatment (Figure 4).

Ethylene Climacteric Development of Propylene Treated Apples. In this study the preclimacteric apples were treated with continuous propylene until the ethylene climacteric peak was reached. All data presented here were obtained on apples which were quite immature being harvested approximately one month before the fruit remaining on the tree ultimately reached physiological maturity (approximately 30% of apples had IEC above 1.0 ul/l). In Paulared apples, propylene stimulated the development of the ethylene climacteric (Figure 5 A). EFE activity was slow to develop (Figure 5 B), requiring 5 days for initiation. ACC slightly stimulated ethylene production over that of fruits without added ACC. Fruits treated with air showed no increase in IEC (Figure 5 A), EFE (Figure 5 C), and ACC levels (Figure 6). ACC content increased as the ethylene climacteric developed as a result of propylene treatment (Figure 6).

In Empire fruits, propylene greatly enhanced both IEC and EFE (Figure 7 A,B and C). ACC levels, on the other hand, were low and only showed a slight increase beginning after 4 days of continuous propylene treatment (Figure 8).

In Law Rome fruits, IEC was low during the first two days of propylene treatment (Figure 9 A), after which it increased markedly. The capacity to convert ACC to ethylene was also increased significantly (figure 9 B and C). ACC content in Law Rome, similar to that of Empire fruits, was low for the first 7 days of continuous propylene treatment. And also like Empire apples, the time of the increase in ACC content coincided with the increase in IEC and EFE activity as measured by adding ACC to the tissue.

Preclimacteric fruits of all cultivars without propylene treatment did not show an increase in IEC and in EFE as measured by incubating fruit discs with ACC.

Discussion

Studies with these apple cultivars showed that the effects of propylene treatment in apple fruits depends on the stage of maturity and cultivar. Propylene induced ethylene production increased as a function of maturity (Figures 1-3). The propylene effect on ACC synthesis and ACC conversion may vary. In tomato, cantaloup, avocado, and Golden Delicious apples, short-term ethylene treatment only stimulated the EFE system (11,29,35). In this study, however, two days of propylene treatment to Paulared apples stimulated ethylene production and EFE activities at all the maturity stages of studied (Figure 1, A and B). The increase in EFE did not show any changes at the different stages of maturity. This indicates that the increase in IEC is due to the increase in ACC availability which accounts for the increase in sensitivity to propylene treatment. In Law Rome fruits harvested 20 or more days before the fruits were physiologically mature, short-term propylene treatment stimulated EFE without increasing the IEC (Figure 3). This may suggest that propylene and supposedly, ethylene, preferentially induces the EFE system at early stage of maturity in Law Rome fruits.

As compared to the development of the ethylene climacteric in naturally ripened fruits described earlier in Part One, propylene treated apples show a similar trend in the development of EFE as related to IEC. The increase in EFE is slow in Paulared fruits but in Law Rome fruits it is dramatically fast. It is noteworthy that in

Empire fruits that both IEC and EFE were greatly enhanced by propylene (Figure 7) while the ACC content was low (Figure 8). This suggests that propylene stimulates the development of EFE to a greater extent than it does ACC synthesis, resulting in low levels of ACC in the tissue while ethylene production is high. This is different compared to naturally ripened Empire fruits (Part One), which had high ACC accumulation due to greater ACC synthesis.

Since the sensitivity to ethylene or propylene is a very reliable paramater, it can be used as a way to predict the ripening of apple fruits. Propylene is advantageous because the ethylene produced by fruits can be measured easily and apples produce much more uniform ethylene levels in response to propylene treatment. The sensitivity curve expressed by the increase in ethylene production with propylene treatment can be established depending on the variety of apples as showed in Figures 1,2,and 3. It will be worthwhile to approach this problem with computer modeling. Other ripening parameters such as decrease in chlorophyll and flesh firmness softening after propylene treatment might also be considered.

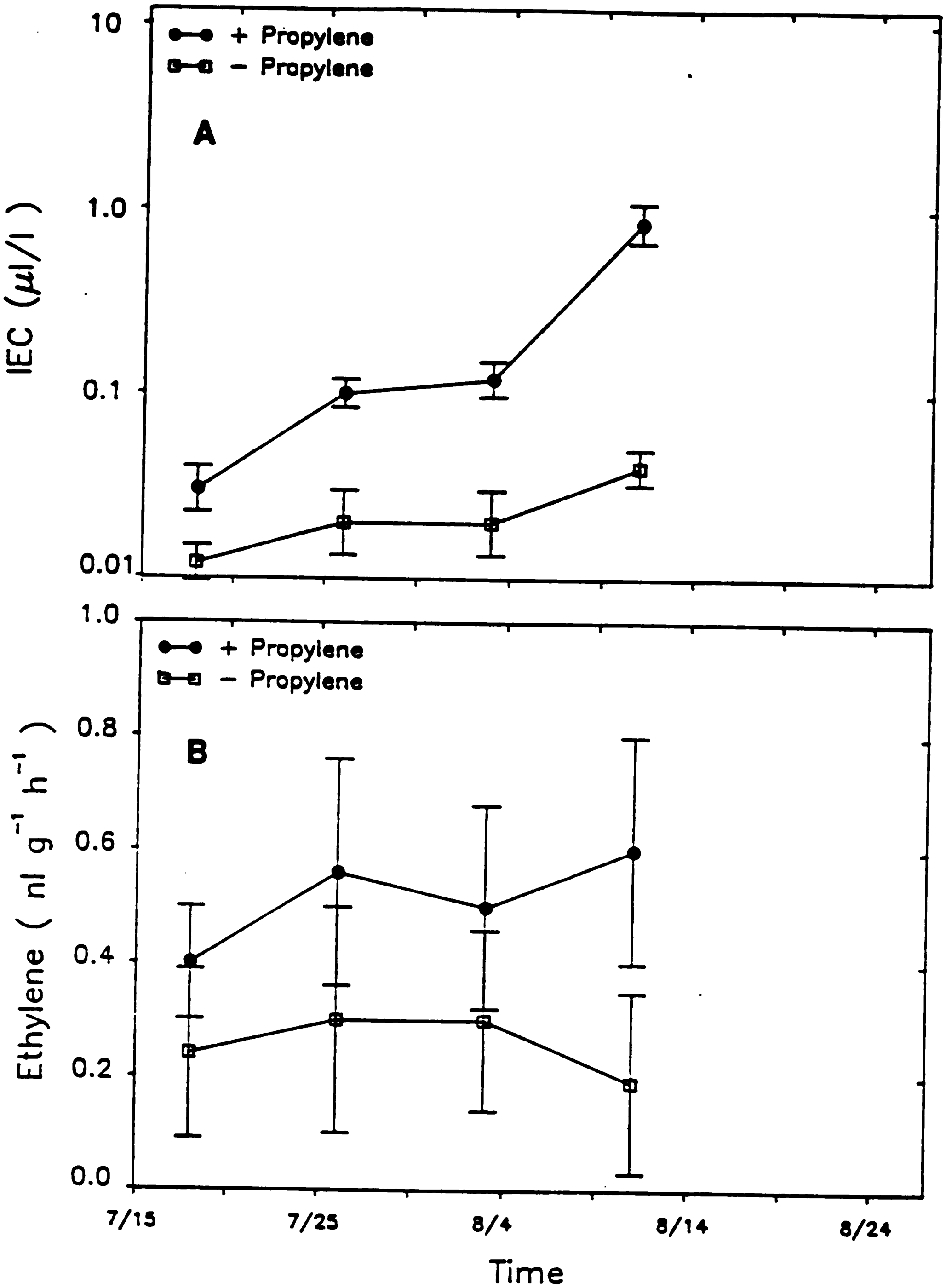

Figure 1. Changes in IEC (A) and in the ability to convert exogenous ACC to ethylene (B) in preclimacteric Paulared fruits with or without propylene treatment (24 hours at 100 ul/l) at different stages of maturity at harvest. August 24 was the date when 30 % of apples had IEC above 1.0 ul/l.

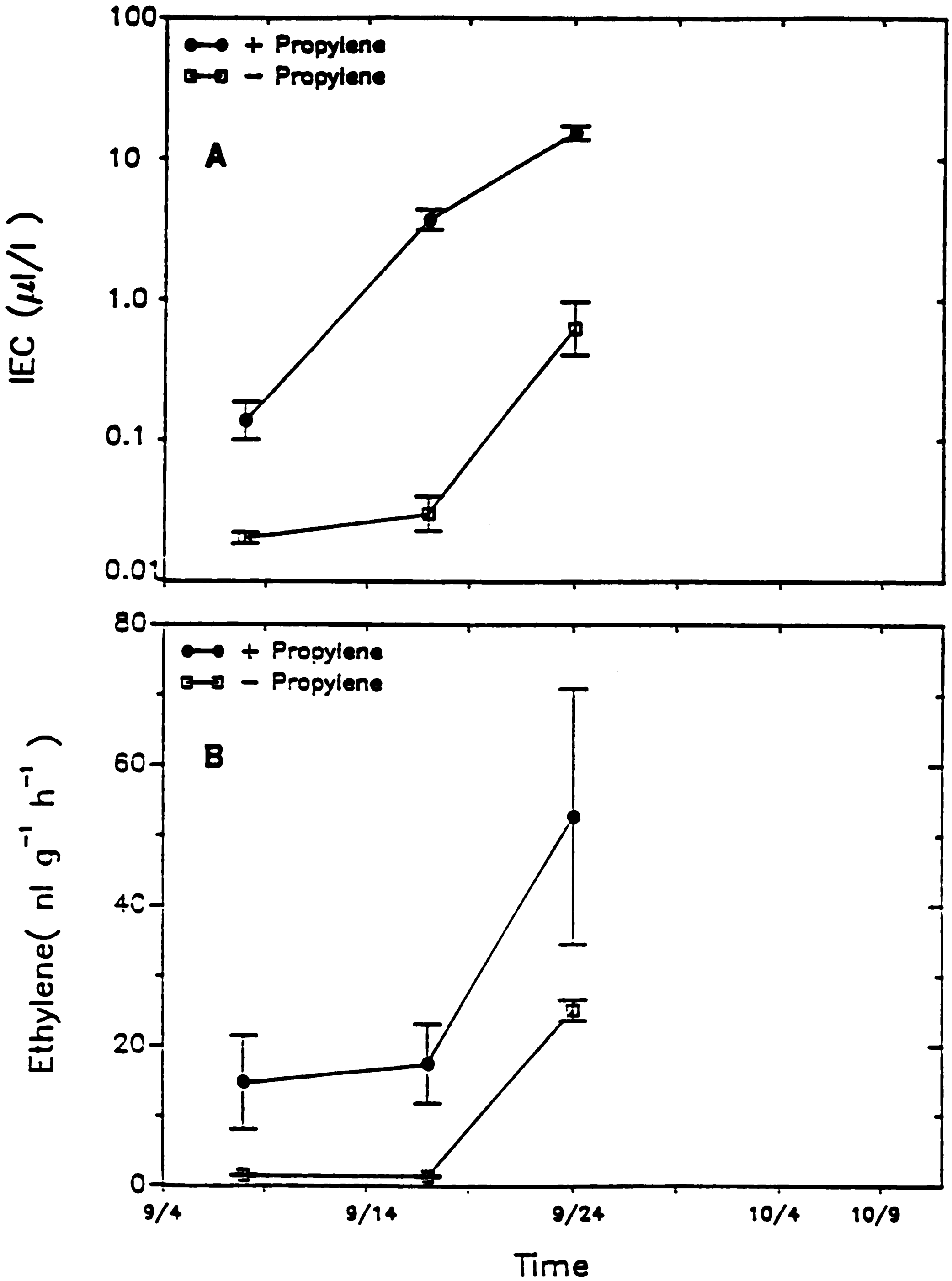

Figure 2. Changes in IEC (A) and in the ability to convert exogenous ACC to ethylene (B) in preclimacteric Empire fruits with or without propyplene treatment (24 hours at 100 ul/l) at different stages of maturity. October 9 was the date when 30 % of apples had IEC above 1.0 ul/l.

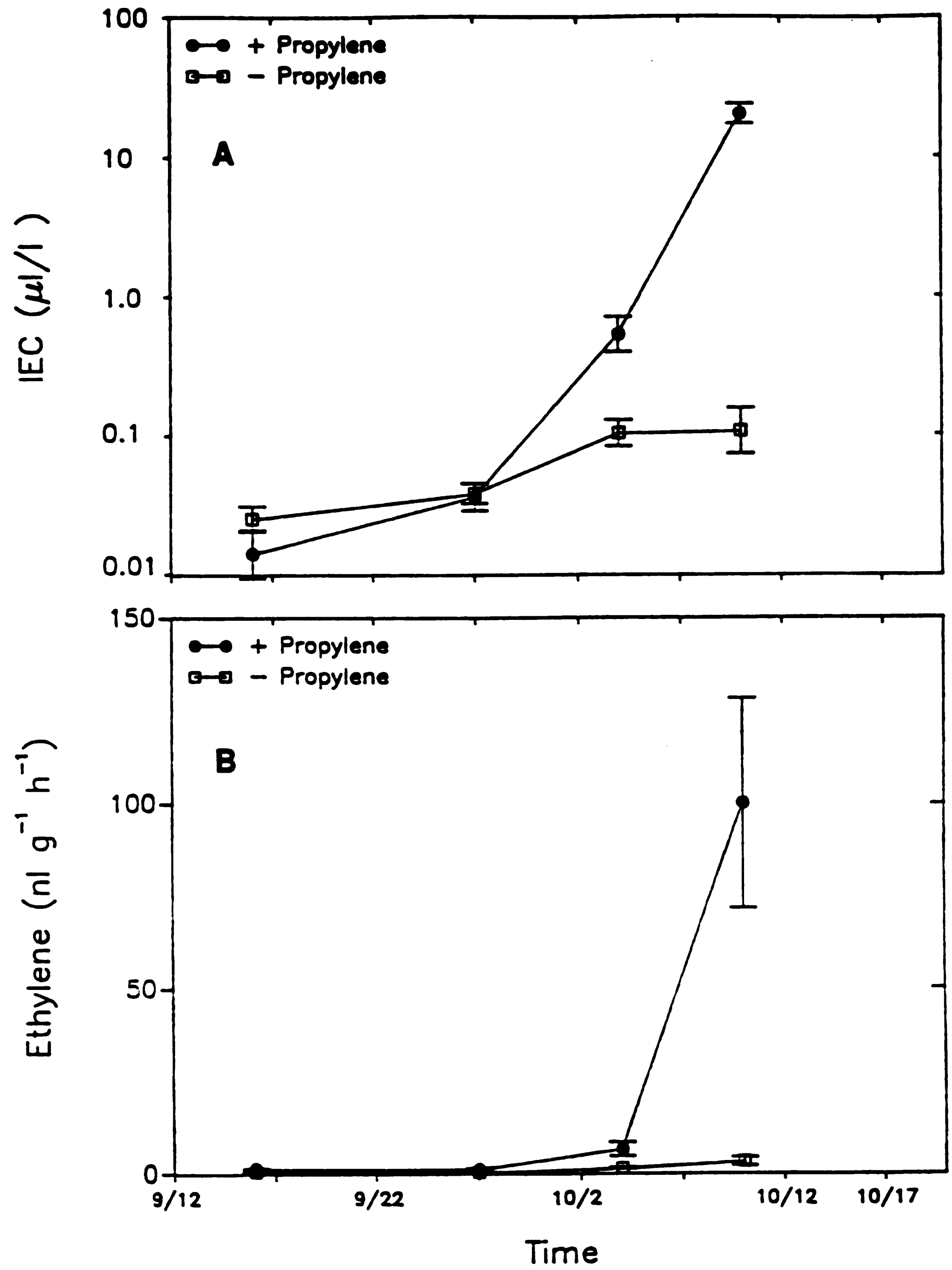

Figure 3. Changes in IEC (A) and in the ability to convert exogenous ACC to ethylene (B) in preclimacteric Law Rome fruits with or without propylene treatment (24 hours at 100 ul/l) at different stages of maturity. October 17 was the date when 30 % of apples had IEC above 1.0 ul/l.

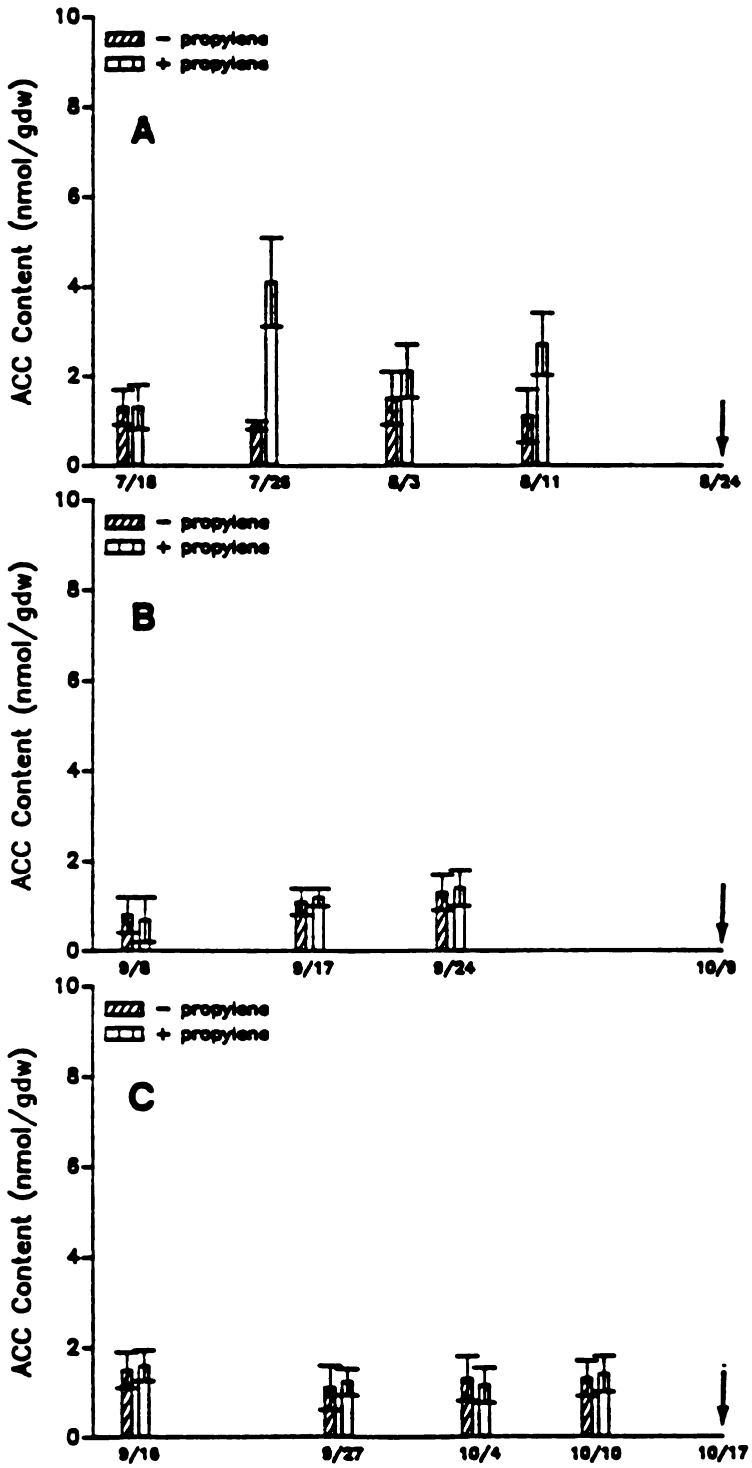

Figure 4. Changes in ACC content in apples with or without propylene treatment (24 hours at 100 ul/l) at different stages of maturity. A: Paulared. B: Empire. C: Law Rome. Arrows indicate the dates when 30 % of apples had IEC above 1.0 ul/l.

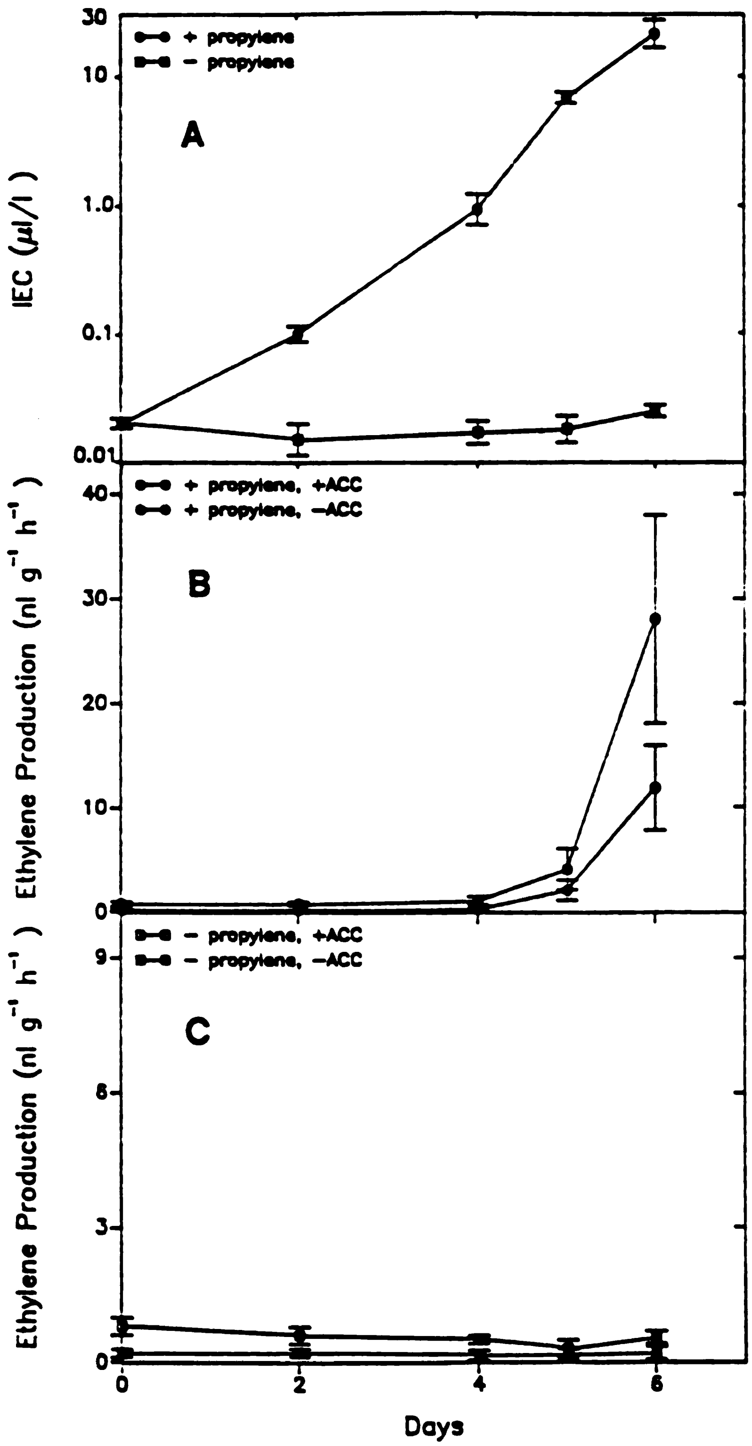

Figure 5. Changes in IEC in intact fruits (A) and in ethylene production rate in fruit discs from Paulared apples with (B) or without (C) continuous propylene treatment at 100 ul/l at 20°C.

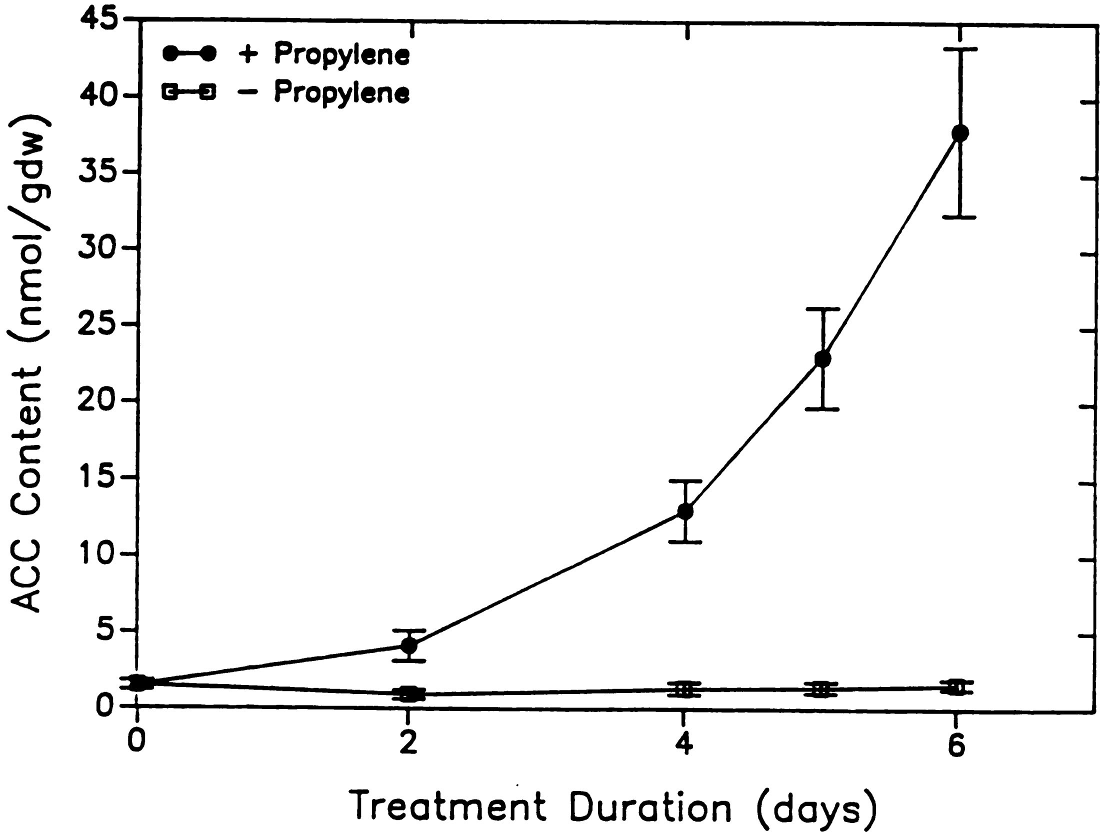

Figure 6. Changes in ACC content in Paulared apples with or without continuous propylene treatment at 100 ul/l at 25°C.

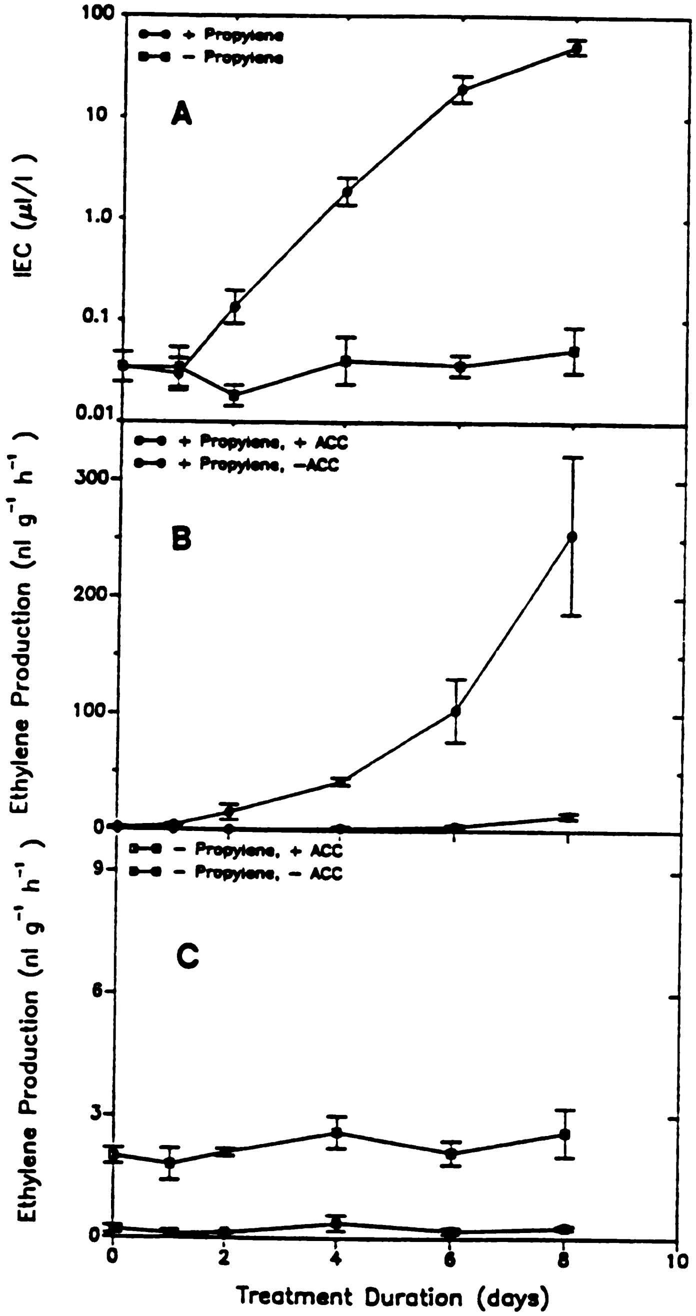

Figure 7. Changes in IEC in intact fruits (A) and in ethylene production rate in fruit discs from Empire apples with (B) or without (C) continuous propylene treatment at 100 ul/l at 25°C.

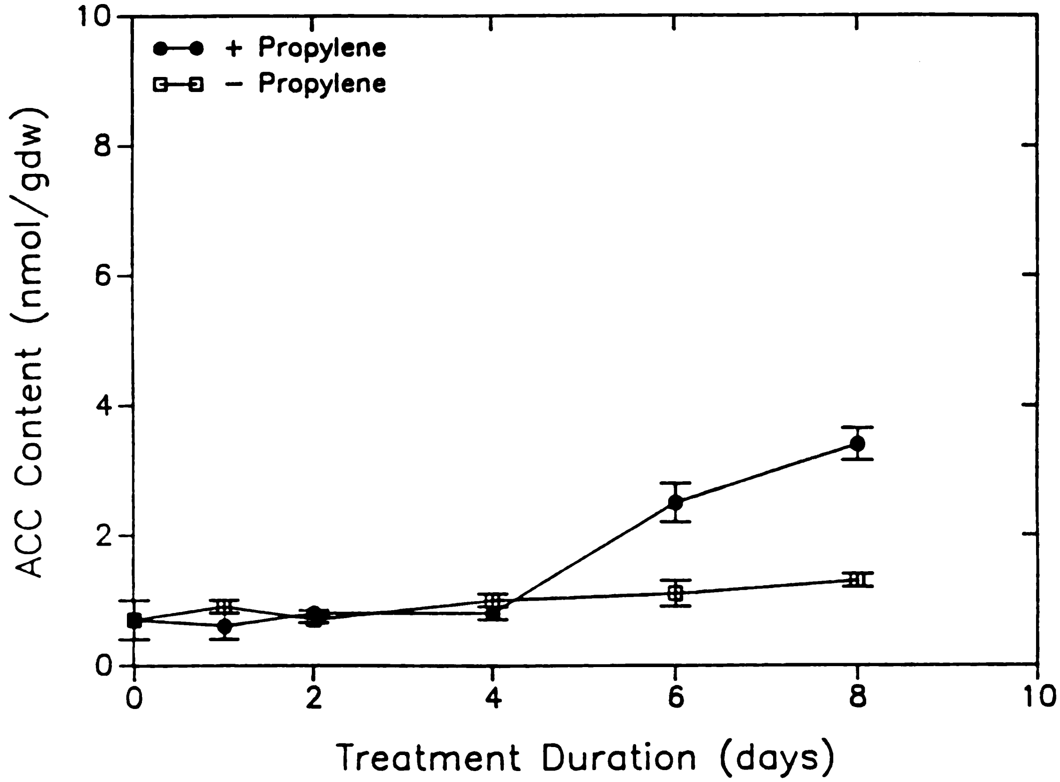

Figure 8. Changes in ACC content in Empire apples with or without continuous propylene treatment at 100 ul/l at 25°C.

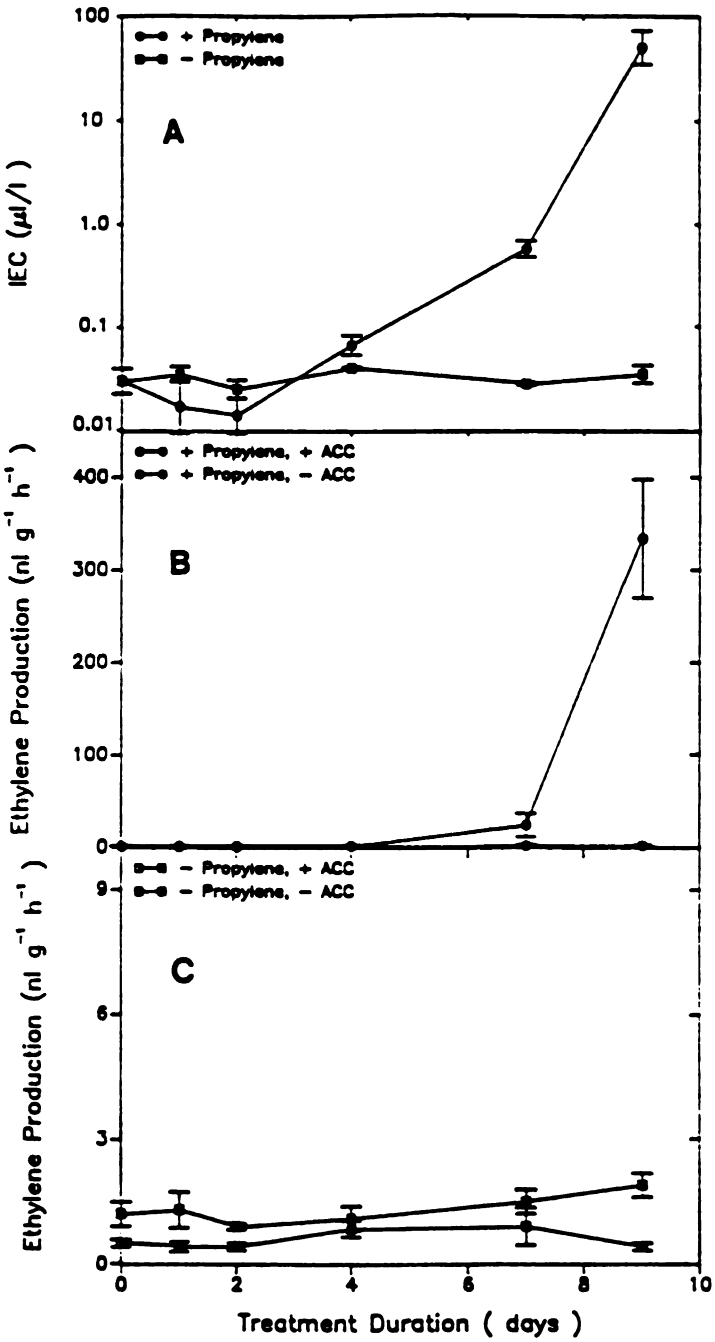

Figure 9. Changes in IEC in intact fruits (A) and in ethylene production rate in fruit discs from Law Rome apples with (B) or without (C) continuous propylene treatment at 100 ul/l at 25°C.

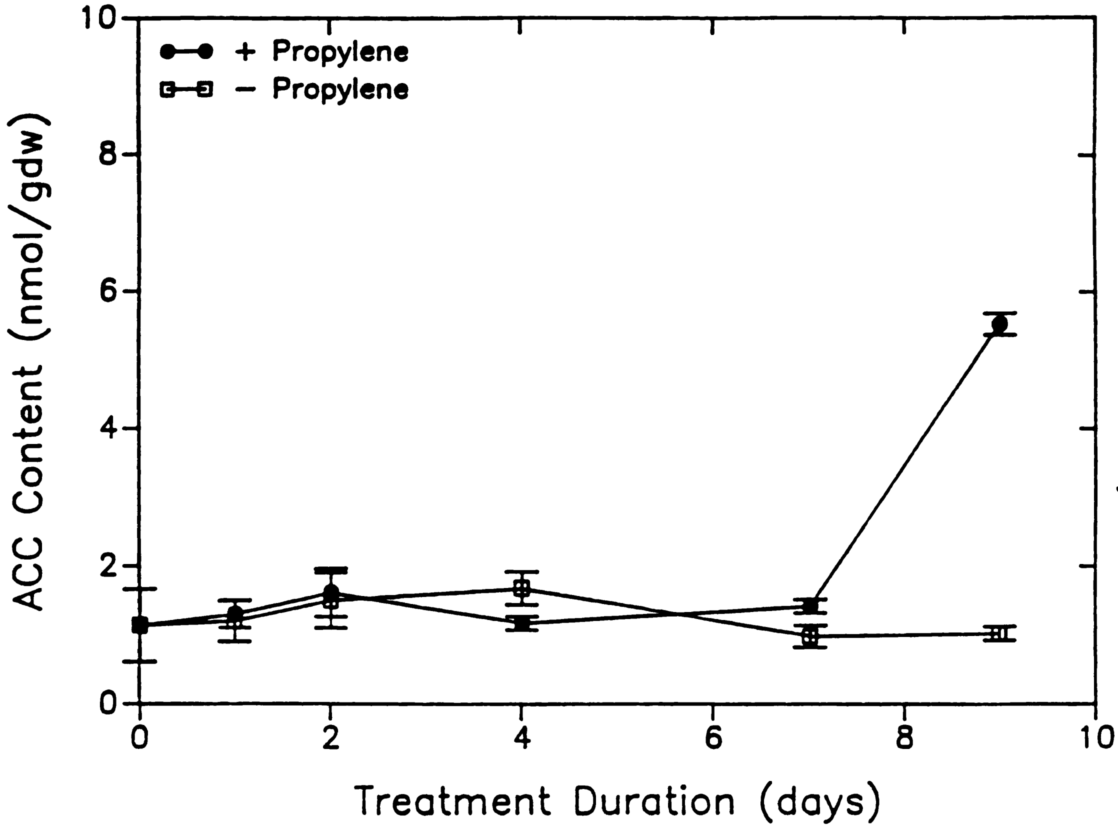

Figure 10. Changes in ACC content in Law Rome apples with or without continuous propylene treatment at 100 ul/l at 25°C.

PART THREE

EFFECT OF HYPOBARIC STORAGE AND TEMPERATURE ON ETHYLENE BIOSYNTHESIS IN PRECLIMACTERIC AND POSTCLIMACTERIC LAW ROME APPLES

Introduction

Since the introduction of hypobaric storage by Burg and Burg (12), which allows fruits to be stored under hyponormal ethylene levels, many studies have been devoted to its effect on the ripening process (4,9,15,17,18,19). This has led to a better understanding of the roles of both ethylene and oxygen. Studies by Dilley *et al* (18) and Bangerth (4) show that preclimacteric apple fruits can be kept in the preclimacteric stage without any ripening changes. Moreover, storage under hypobaric conditions was found to uncouple ethylene synthesis from ethylene action. When post-climacteric apples are kept at 0.05 atmosphere at 0°C, softening was retarded and this may indicate that ethylene plays a role in fruit ripening beyond that of initiating the synthesis of ripeing enzymes. A continuous presence of certain amounts of ethylene and oxygen may be required for autocatalytic ethylene production and ethylene action. This can in return explain the effect of hyobaric storage which maintains a low oxygen and ultra-low ethylene environment.

The physiological role of environmental factors on ethylene biosynthesis and ripening of fruits has been investigated by many researchers as summarized by Yang and Hoffman (41). These factors can exert their effects on ACC synthesis, the key step in the biosynthesis pathway for ethylene (8,42), and on the last known step, the conversion of ACC to ethylene (40,41). Temperature is an important environmental factor. Chilling stress induces ethylene production in a number of chilling sensitive tissues. Chilling 'Bosc' pears results in the fruits gaining the capacity to produce ethylene and susequently ripen (34). Wang and Adam (42,43) found that chilling induces ethylene production in cucumber fruits. They suggested that chilling treatment unmasks or stimulates the production of mRNA coding for ACC synthesis. In Golden Delicious apples, low temperature stimulates ethylene production and accumulation of high ACC levels during the first month of storage in air (26). ACC content declined after two months, followed by much higher ethylene production. Although these results are not entirely convincing due to the lack of information on the ability to convert ACC to ethylene it is still quite possible that low temperature enhances ACC synthesis but not ACC conversion during the initial period of low temperature storage. The ability to convert ACC to ethylene increases after the second or third month of storage (26). The effect of oxygen and low temperature has been studied in 'd'Anjou' pears (5,6), where it was found that low oxygen and low temperature inhibited the development of ACC conversion ability but not ACC synthesis. This suggests that the limiting step in ethylene production in these treated 'd'Anjou' pear fruits is the conversion of ACC to ethylene.

The objective of this experiment was to determine the effect of hypobaric storage and temperature on ethylene biosynthesis in apple fruits.

Materials and Methods

Materials. Only Law Rome apples were used in this experiment. Fruits were harvested from the Grand Rapids area in western Michigan in 1985.

Hypobaric Storage. After cooling to 0°C, preclimacteric fruits were stored in a hypobaric chamber at 40 mm Hg. The chamber was ventilated with moisture air brought to near saturation after pressure reduction. Post-climacteric fruits stored at 0°C in air for three months were also placed under hypobaric conditions. After at least four months of hypobaric storage, fruits were taken from the chamber for analysis.

Temperature Treatment. After hypobaric storage, fruits were placed at normal atmospheric pressure conditions at 0°C or 25°C continuously and samples were taken periodically.

Measurement of Flesh Firmness. Apple flesh firmness was obtained as described by Blanpied *et al* (7).

The measurements for ethylene, ACC and EFE were the same as described in Part One of this thesis.

Results

Effect of Hypobaric Storage on Ethylene Production of Preclimacteric and Postclimacteric Fruits. Preclimacteric fruits placed in hypobaric storage remained in the preclimacteric stage without any ripening changes. Ethylene production rate in fruit discs without added ACC was low (Figure 1), and adding ACC increased ethylene production 5 to 12-fold, respectively, for fruits before or after hypobaric storage. ACC levels (Figure 2) were low before and after storage. High flesh firmness was maintained by hypobaric storage (Figure 3).

Postclimacteric fruits stored at 0°C in air had a high ethylene production rate (Figure 4, -ACC) and very high capacity to convert ACC to ethylene (Figure 4, +ACC), but the level of ACC was low (Figure 2). After 5 months of hypobaric storage, these fruits showed a great reduction in ethylene production rate (Figure 4, - ACC). The capacity to convert ACC to ethylene was reduced appreciably, but it was still high. A reduction in ACC was also found (Figure 2). No further reduction of flesh firmness was observed (Figure 3).

Ripening of Preclimacteric Fruits After Hypobaric Storage. After hypobaric storage, preclimacteric fruits were placed at 0°C and 25°C to evaluate ethylene production characteristics. At 25°C fruits maintained at preclimacteric ethylene levels (0.1-0.2 ul/l) for 6 to 9 days (Figure 5 A), and then IEC increased abruptly, reaching about

150 -200 ul/l in a further one to two days. The capacity to convert ACC to ethylene showed a similar trend of development (Figure 5, B). ACC content increased slightly but generally remained low during the entire period of development of ethylene production capacity (Figure 5 C).

Preclimacteric fruits held at 0°C in air following hypobaric storage produced very little ethylene for the first 5 days (Figure 6 A). Then IEC increased in a log-linear manner eventually reaching about 25 ul/l after 14 days. As compared to those ripened at 25°C, increase in IEC in these apples at 0°C was more uniform and slow. Elevated levels of ACC were detected to coincide with the increase in IEC. This was followed by rapid accumulation of ACC in the fruit tissue (Figure 6 C). The increase in ethylene production by fruit discs without added ACC paralelled the increase in IEC and ACC (Figure 6 B). The stimulating effect on ethylene production by adding ACC was small after 8 days of cold treatment, indicating the ability to convert exogenous ACC to ethylene was not changed significantly.

Ethylene Production of Post-climacteric Fruits. When post-climacteric apples were removed from hypobaric conditions, a high ethylene production rate was achieved in one day at 25°C, followed by a burst of higher ethylene production as seen in the IEC (Figure 7 A). The capacity to convert ACC to ethylene showed a similar trend (Figure 7 B), while there was no increase in ACC content which remained at a level of about 2 nmol/gdw (Figure 7 C).

Postclimacteric fruits placed in 0°C recovered the ability to produce ethylene slowly (Figure 8 A). During the first three days, IEC remained at about 2 to 5 ul/l. After 13 days, most of the fruits had IEC of about 10 to 20 ul/l. The capacity to convert exogenous ACC to ethylene remained as high as that measured immediately after removal from hypobaric storage and did not change significantly from 1 to 12 days (Figure 8 B). There was a slight increase in ACC content but the level was low (Figure 8 C), as was true of fruits held at 25°C following hypobaric storage (Figure 7 C)..

Discussion

Immediately after hypobaric storage, cortical tissue discs from preclimacteric fruits produced very little ethylene (Figure 1, -ACC). In post-climacteric fruits ethylene production was reduced greatly (Figure 4, -ACC). However, the capacity to convert ACC to ethylene was slightly higher in preclimacteric fruits and remained very high in postclimacteric fruits. This may indicate that hypobaric storage has a greater effect on ACC synthesis than it does on the EFE system. ACC content accumulated during the development of the ethylene climacteric of hypobarically stored preclimacteric fruits after returning fruits to normal atmospheric pressure in air at 0°C (Figure 6 C). The development of the capacity to convert exogenous ACC to ethylene in fruit discs was slow (Figure 6 B). This provides some evidence that the development of EFE system is inhibited at low temperature, resulting in an increase in ACC levels (26). Increase in the ethylene production rate in fruit discs without exogenous ACC may result from the increase in endogenous ACC to near saturation levels. This may explain the phenomenon that the addition of ACC only increased ethylene production slightly. The other possibility is that the transport of exogenous ACC to the location of EFE is also limited. Diffusion of ACC into the cell should not have been a problem in this experiment. Discs used were thin (2mm) and the EFE assay was carried out for three hours at 25°C. In fruits with similar IEC and similar flesh firmness, such as those treated with propylene (Part One) and those maintained at 25°C in air for about 8 to 10 days after hypobaric storage, addition of ACC greatly enhanced the ethylene production rate. EFE is considered to be membrane bound and localized in vacuoles in *Pisum sativum* L. (20). Utilization of endogenous ACC by EFE can be favored. Nevertheless, it is certain that at 0°C in air the rate of ACC synthesis is greater than ACC conversion making the conversion of ACC to ethylene the limiting factor for higher ethylene production. Unlike preclimacteric fruits, postclimacteric fruits did not accumulate ACC when they were held at 0°C in air after hypobaric storage (Figure 8 C). This is not surprising because the ability to convert ACC to ethylene is already high in this fruit tissue. ACC synthesis is the limiting factor for ethylene production in this case.

Many fruits and vegetables show a burst of ethylene production and a coinciding increase in respiration rate subsequent to being transferred from refrigerated storage in air or under controlled atmosphere storage conditions (14). This phenomenon can now be explained in terms of the accumulation of ACC in postclimacteric tissues leading to an increase in ethylene production upon returning the fruits to warmer temperature (Figure 7).

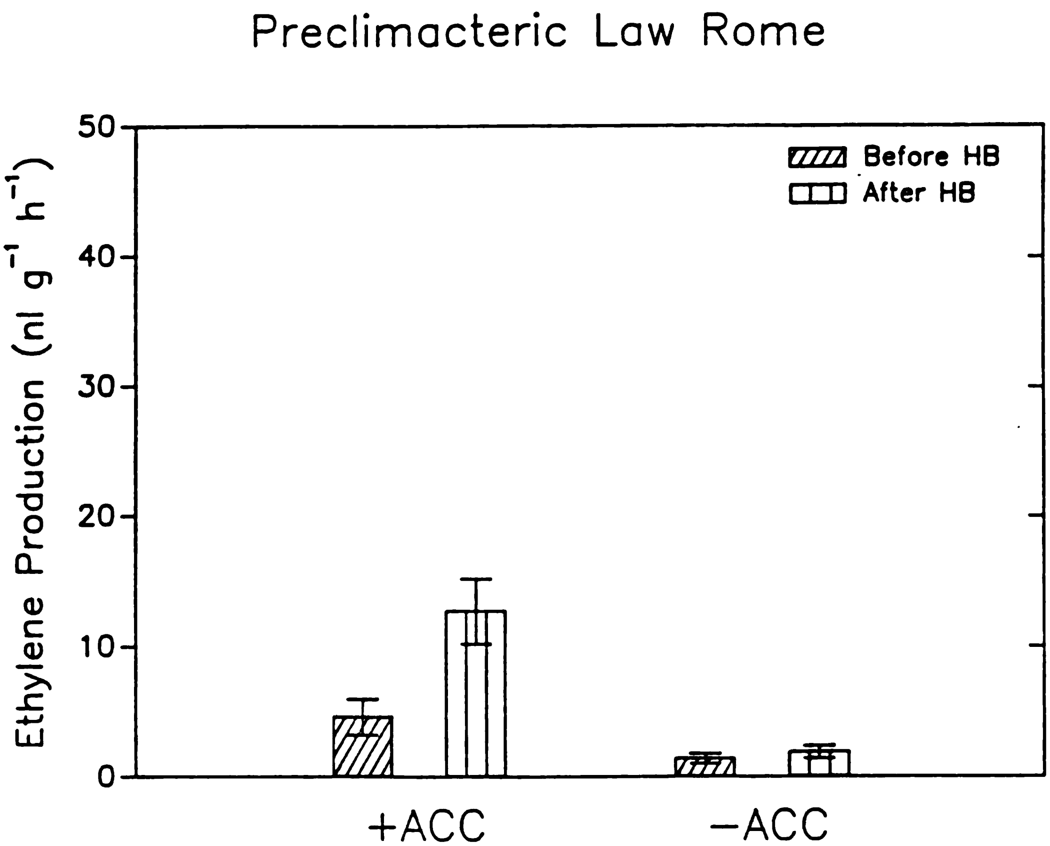

Figure 1. Ethylene production rate of preclimacteric Law Rome fruit discs before and after hypobaric storage.

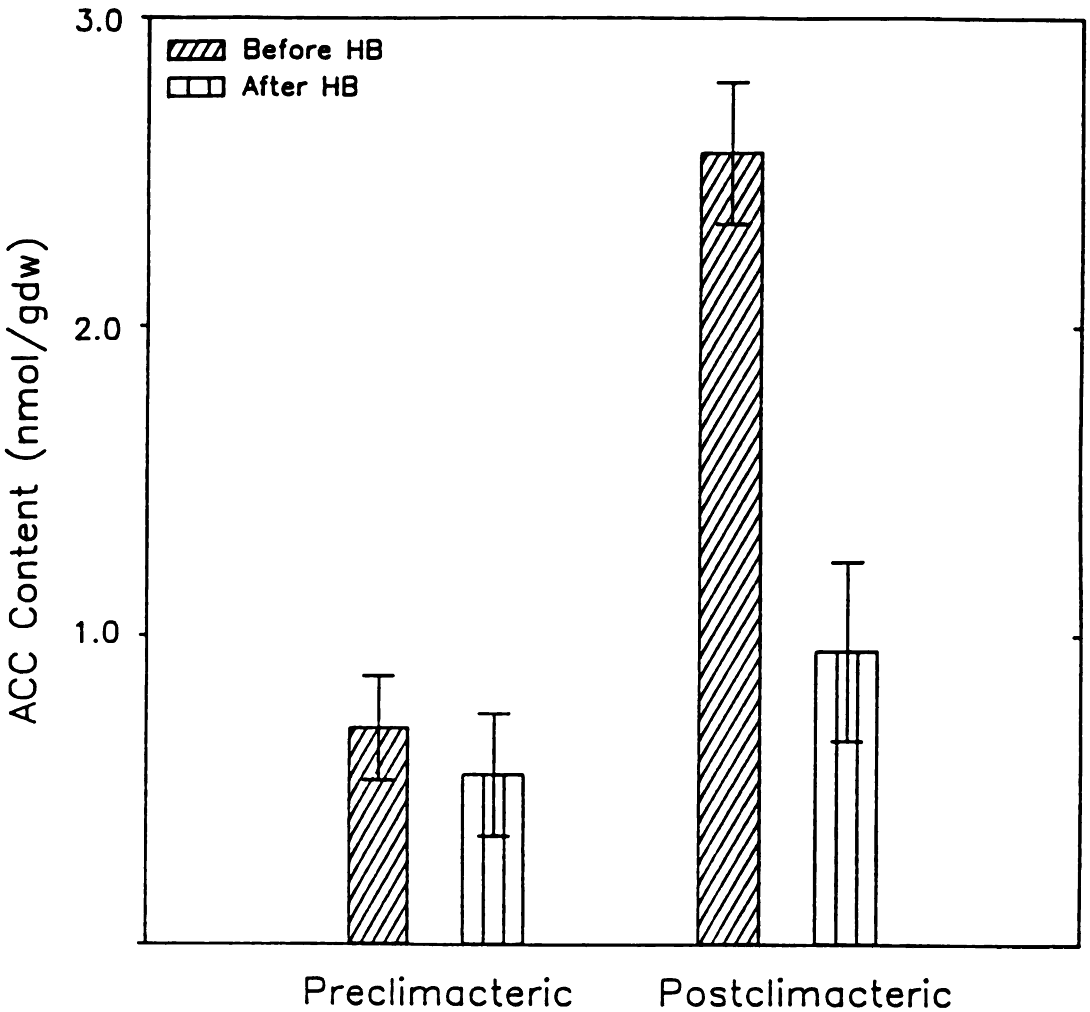

Figure 2. ACC content in Law Rome fruits before and after hypobaric storage.

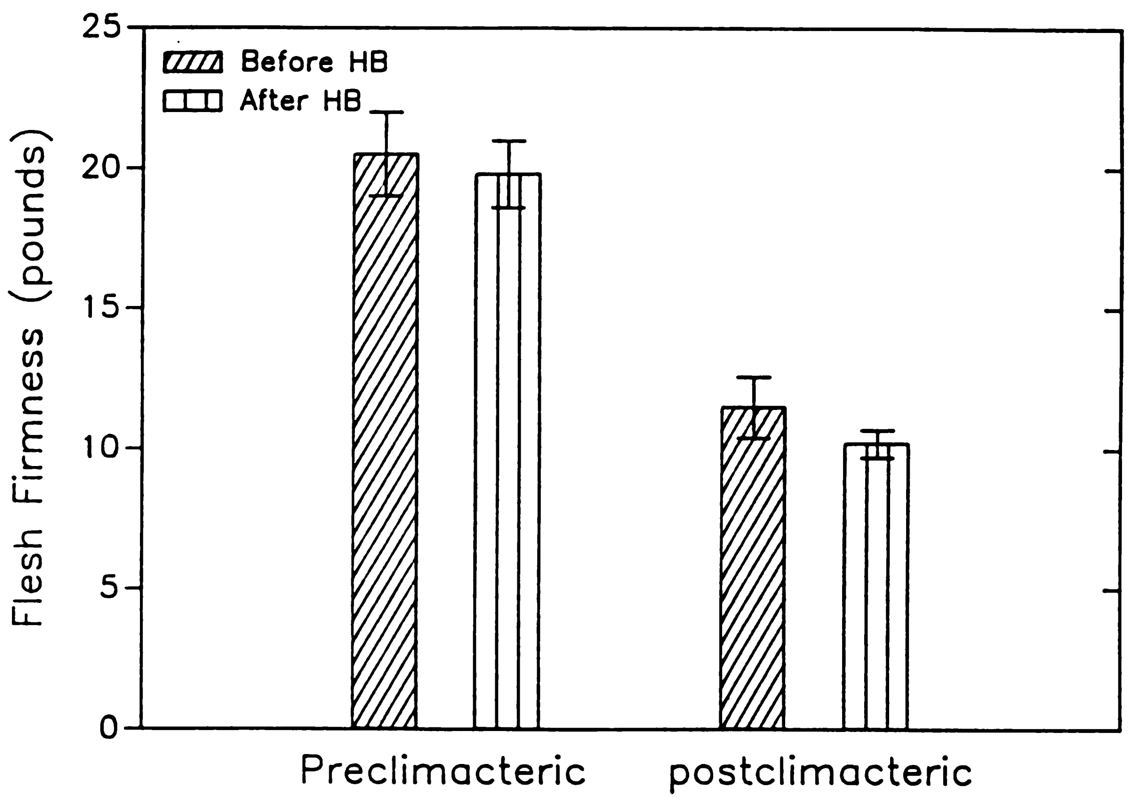

Figure 3. Flesh firmness in Law Rome fruits before and after hypobaric storage.

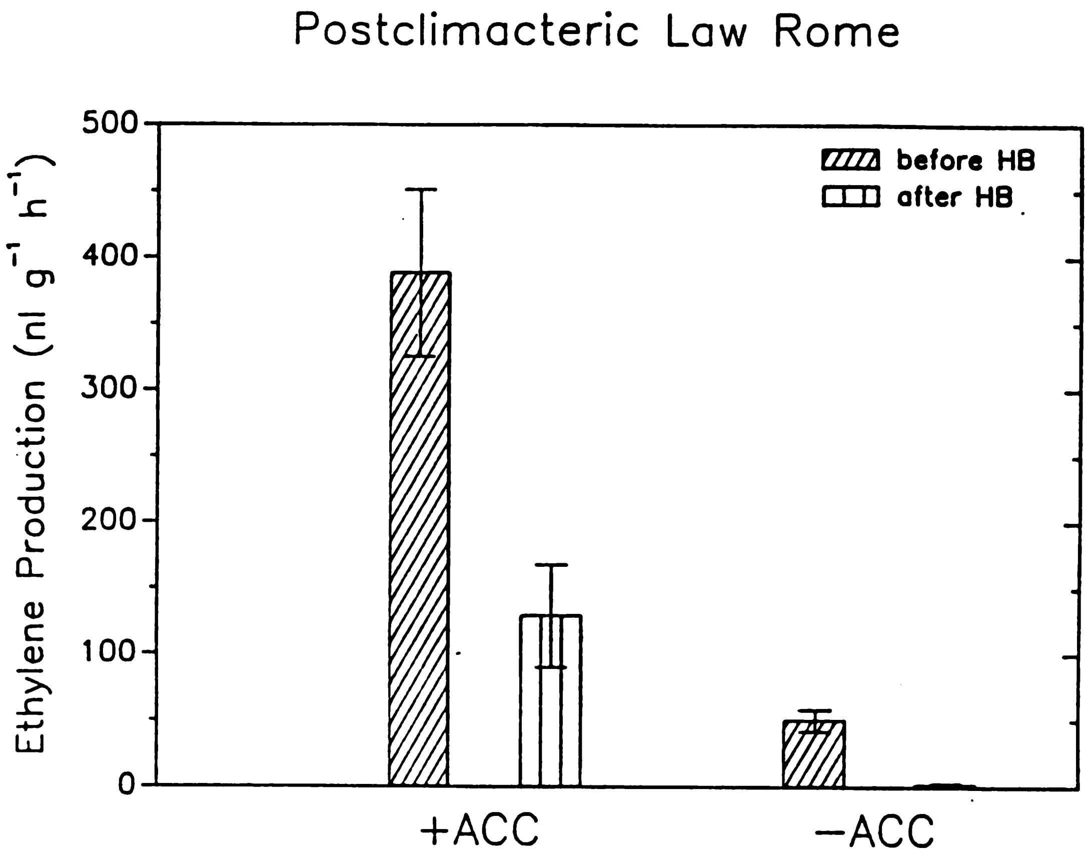

Figure 4. Ethylene production rate of postclimacteric Law Rome fruit discs before and after hypobaric storage.

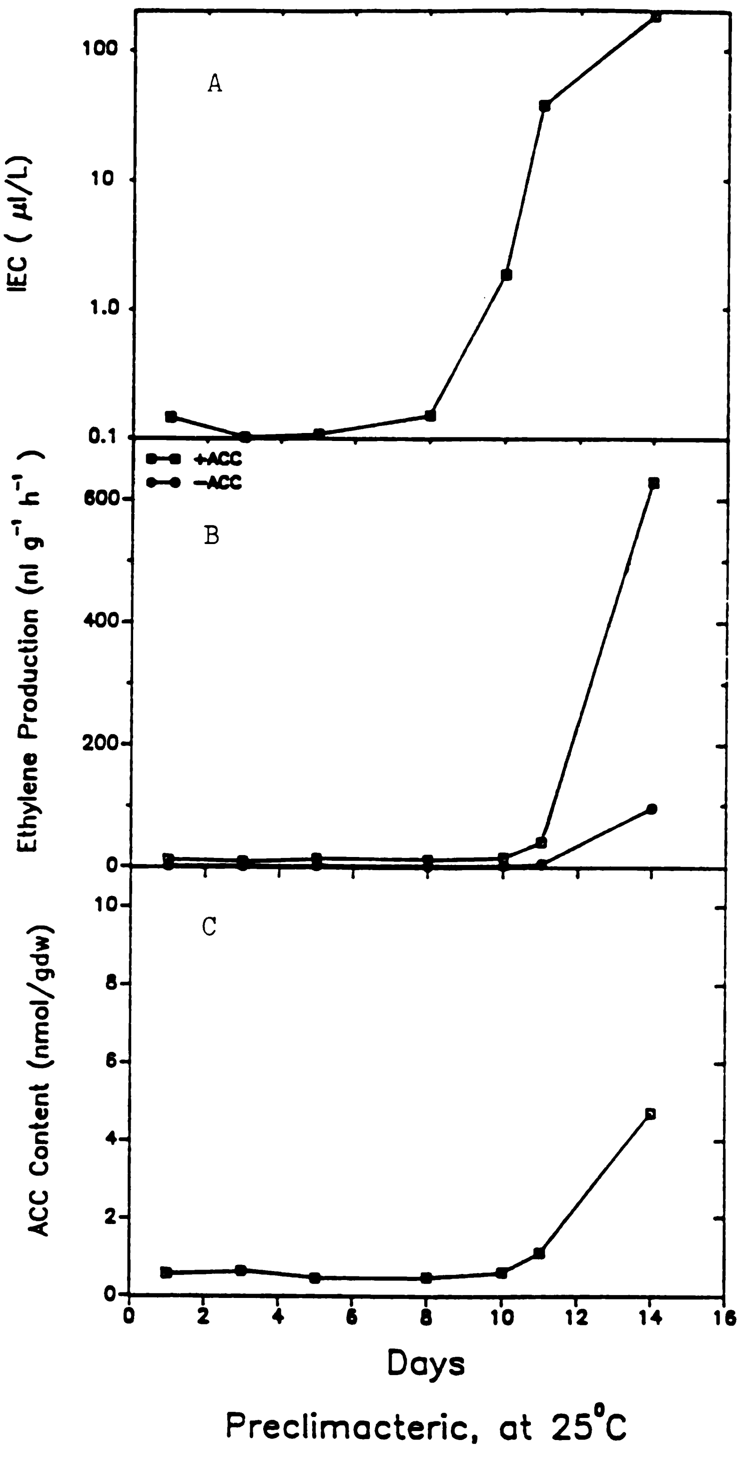

Figure 5. Development of ethylene biosynthesis of preclimacteric Law Rome fruits at 25°C after hypobaric storage.

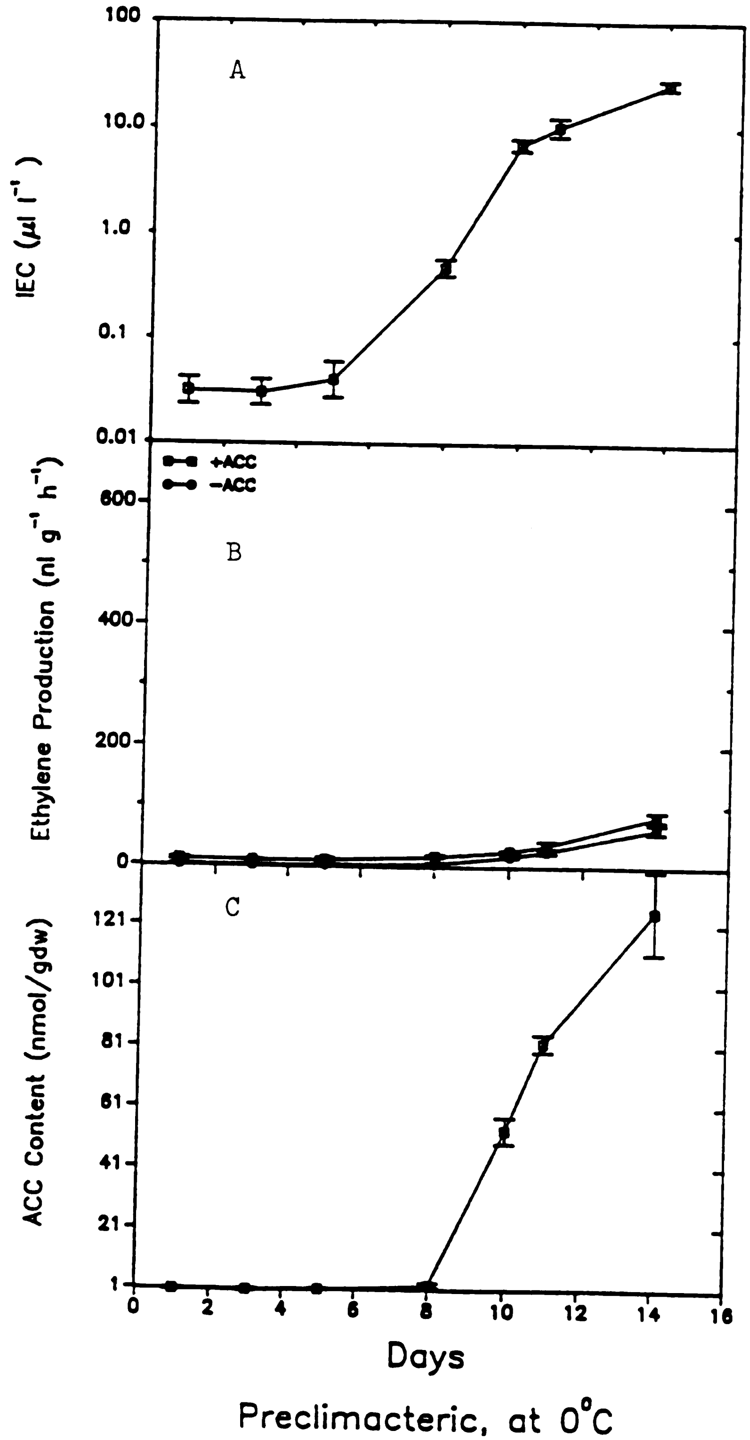

Figure 6. Development of ethylene biosynthesis of preclimacteric Law Rome fruits at 0°C after hypobaric storage.

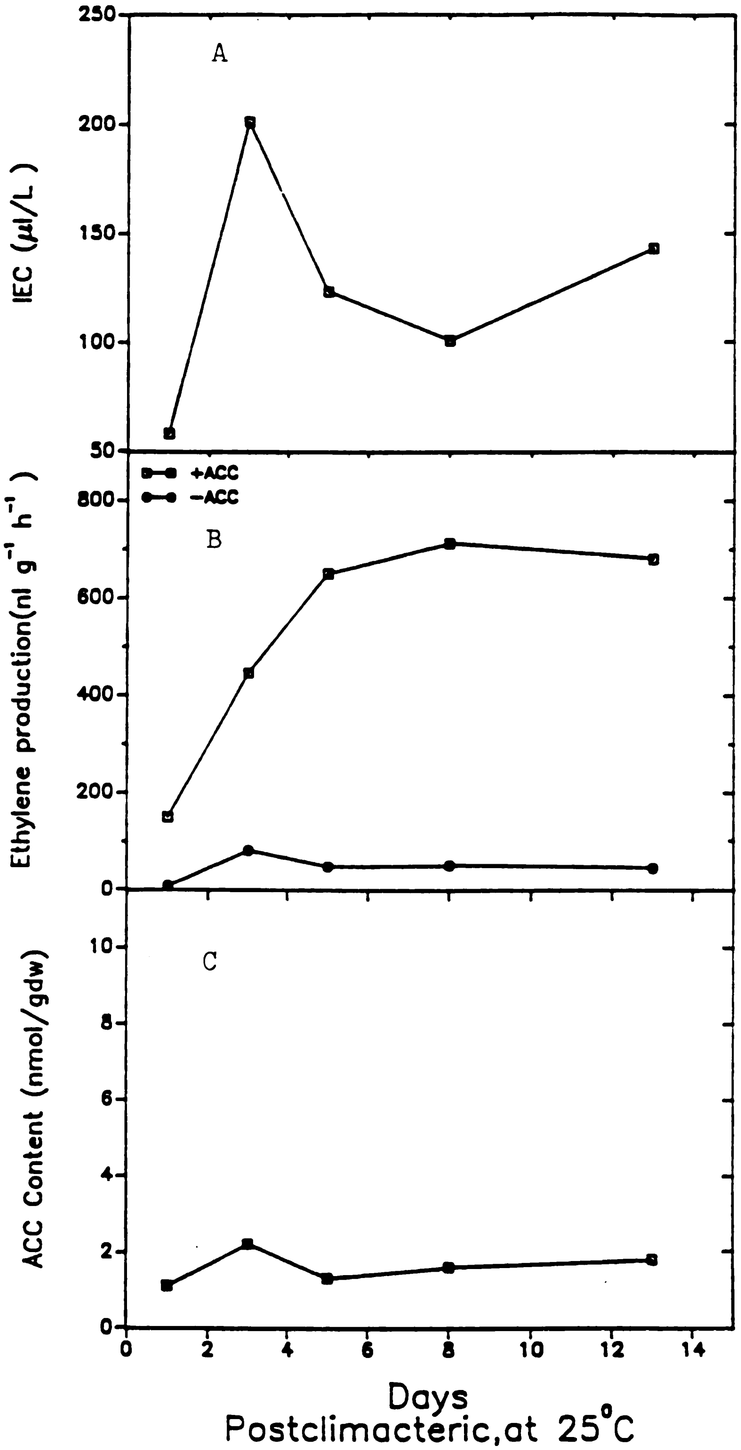

Figure 7. Development of ethylene biosynthesis in postclimacteric Law Rome fruits at 25°C after hypobaric storage.

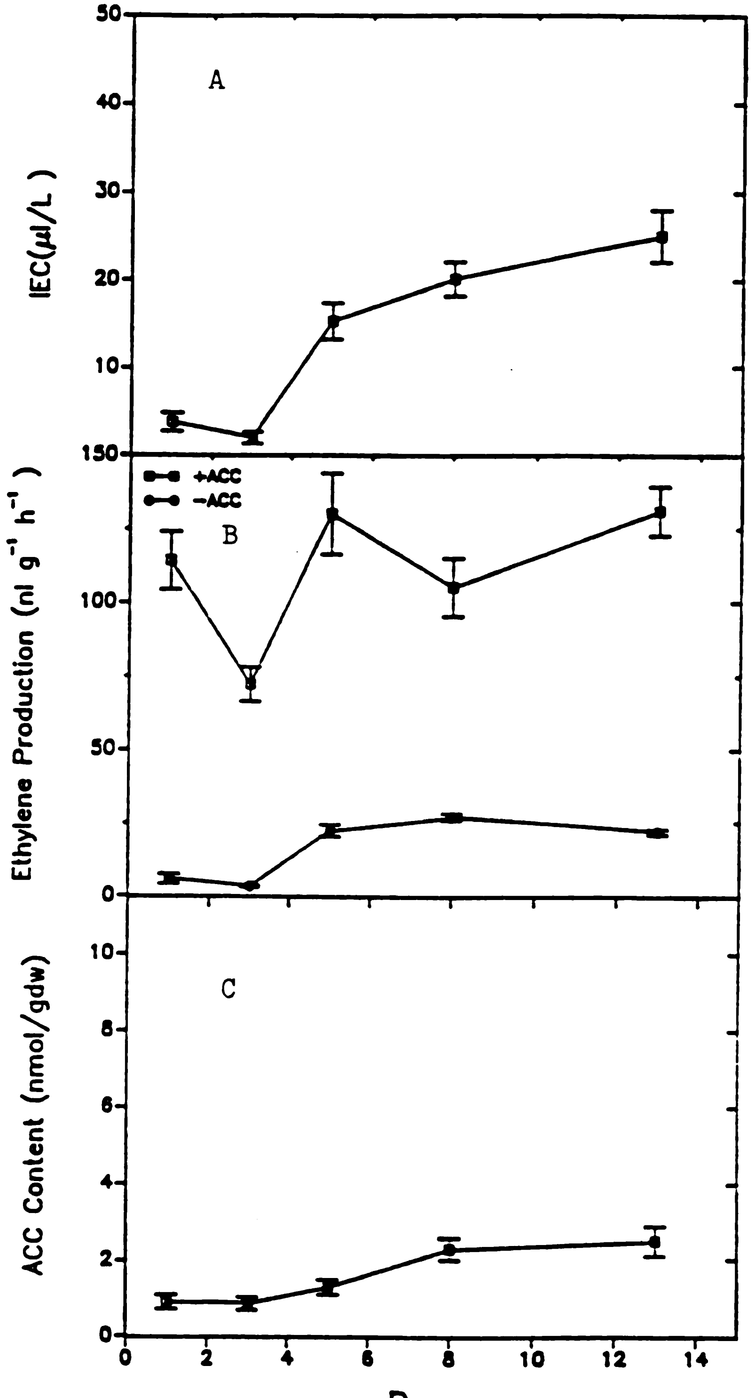

Figure 8. Development of ethylene biosynthesis in post-climacteric fruits at 0°C after hypobaric storage.

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