Precursors of system II ethylene production in senescing carnation petals

by

Pablo Alfredo González Arias

A Thesis Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

MASTER OF SCIENCE

ırticulture
rticulture

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa

1994

DEDICATION

To life, which has given me so much...

TABLE OF CONTENTS

LIST OF TABLES iv

LIST OF FIGURES v

1. GENERAL INTRODUCTION 1

2. GENERAL LITERATURE REVIEW 3

3. PRECURSORS OF SYSTEM II ETHYLENE SYNTHESIS IN SENESCING CARNATION PETALS 8

Abstract 8
Introduction 9
Materials and Methods 12
Results 13
Discussion 15
Literature Cited 18

4. SUMMARY AND CONCLUSIONS 26

GENERAL LITERATURE CITED 28

ACKNOWLEDGEMENTS 33

APPENDIX A. DISTRIBUTION OF RADIOACTIVE δ-AMINOLEVULINIC ACID IN 'WHITE SIM' CARNATION FLOWER PARTS 34

APPENDIX B. pH OF SUBSTRATES USED 35

LIST OF TABLES

Table 1. Cumulative C_2H_4 production ($nl \cdot g^{-1}$) and free ACC ($nmol \cdot g^{-1}$) and conjugated ACC ($nmol \cdot g^{-1}$) content over the 9-day experimental period with constant exposure to 20 mM solutions of each substrate. 22

Table A1. Percentage of total radioactivity in different carnation flower sections. 34

Table B1. pH of 20 mM solutions of each of the different possible C_2H_4 precursors. 35

LIST OF FIGURES

Figure 1. Changes in ethylene production (A) and free ACC (▼) and conjugated ACC (★) content (B) in 'White Sim' carnations treated with ACC. Each data point is the mean of 4 replicates each with 2 observations. 24

Figure 2. Ethylene production (A), free ACC content (B), and conjugated ACC content (C) during senescence of 'White Sim' carnations treated with homocysteine (⊠), MET (▲), D.I. (★), GLU (♦), KG (✣), and ALA (⧖). Each data point is the mean of 4 replicates each with 2 observations. 25

1. GENERAL INTRODUCTION

The carnation flower is unique in that its senescence and death can be attributed almost solely to ethylene (C_2H_4) gas, a natural product of the growth and development of many higher plants. Senescence and death of carnations is accompanied by a massive burst of C_2H_4 production, and methionine (MET) is believed to be the major precursor of this C_2H_4. However, we do not yet understand how so little MET can produce all of the C_2H_4 that a carnation produces during senescence. It has been suggested that another biosynthetic pathway to C_2H_4 may exist during senescence, and this pathway has been labeled System II. Several possible precursors of System II C_2H_4 have been suggested in various species. The overall objective of this research was to explore the utilization of several possible precursors of System II C_2H_4 that is produced during the burst of C_2H_4 synthesis associated with the senescence and death of the carnation. These possible precursors are: 1-aminocyclopropane-1-carboxylic acid (ACC), MET, glutamic acid (GLU), α-ketoglutarate (KG), δ-aminolevulinic acid (ALA), and homocysteine (HOMOC). If one or more of these compounds functions as the precursor of System II C_2H_4, then subsequent research can be focused on control of this pathway of C_2H_4 synthesis. Ultimately, this will lead to a longer-lasting, higher-quality carnation that will reduce postharvest shrinkage and increase consumer satisfaction.

Thesis Organization

This thesis was organized by using journal manuscript format. In addition to the included paper, a general literature review and conclusions were included. A list of

references cited in the general literature review is listed following the summary and conclusions chapter.

2. GENERAL LITERATURE REVIEW

Ethylene (C_2H_4) was the first plant hormone that was shown to be involved with senescence in carnation flowers (Abeles et al., 1992). The C_2H_4 biosynthetic pathway in higher plants has been determined by Adams and Yang (1979) to be: Methionine (MET) → S-adenosylmethionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → C_2H_4. In carnations, this pathway has been shown to operate either when C_2H_4 production has been inhibited by (aminooxy)acetic acid (AOA) (Broun and Mayak, 1981; Mor et al., 1985), which inhibits the conversion of SAM to ACC (Yu et al., 1979), or when C_2H_4 production has been increased and senescence accelerated by the application of exogenous ACC (Hanley and Bramlage, 1987; Mor et al.,1985; Sacalis et al., 1983).

In carnation, MET is present in minute amounts at a young stage of development and increases in concentration with ageing (Van der Westhuizen and De Swardt, 1978). Although the observations of those authors were not related to C_2H_4 production, Cook and Van Staden (1988) suggested that this increase could produce an increase in C_2H_4 production.

A synchronous rise in ACC content and C_2H_4 production has been observed in different carnation organs during the climacteric burst of C_2H_4 production (Hanley and Bramlage, 1987; Hyodo et al., 1990; Peiser, 1986; Whitehead et al., 1984). In addition, ACC synthase activity increased during carnation petal senescence (Hyodo et al. 1990; Peiser, 1986).

Studies that employed petals of 'Arthur' and 'White Sim' carnations have shown that ethylene-forming enzyme (EFE), the enzyme responsible for the conversion of ACC

to C_2H_4 (Yang and Hoffman, 1984), exhibited low activity in preclimacteric petals, and the activity increased with ageing (Serrano et al., 1991; Whitehead et al., 1984; Wu et al., 1989). Accumulation of ACC after C_2H_4 production has decreased has been reported in carnation petals (Bufler et al., 1980; Whitehead et al., 1984). This may have resulted from a rapid fall in EFE activity, and consequently, a more rapid fall in the conversion rate of ACC to C_2H_4 than in the conversion of SAM to ACC (Bufler et al., 1980). Cook and Van Staden (1988) concluded that the conversion of ACC to C_2H_4 also is a rate-limiting step in the C_2H_4 biosynthetic pathway.

Petal tissue produces most of the C_2H_4 during carnation senescence (Hyodo et al., 1990; Nichols, 1977). Mor et al. (1985) found that C_2H_4 production was greater in the proximal portion of the petals than in the distal region and that greater ACC synthase and EFE activities were found in the proximal portion of the petal.

The conjugated form of ACC in carnation petals has been identified as a malonyl conjugate (Borochov and Woodson, 1989). In several studies, a more active conjugation system has been found in young petals than in senescing petals (Hanley et al., 1989; Peiser, 1986; Serrano et al., 1991). Borochov and Woodson (1989) have suggested that this step could limit the availability of free ACC for conversion to C_2H_4 by active EFE. In carnations, it has been suggested that conjugated ACC is hydrolyzed to free ACC, which then could be oxidized to C_2H_4 (Hanley et al., 1989). Cook and Van Staden (1988) suggested that the concentration of conjugates could be important for the regulation of ACC content by reversible conversion reactions. It has been shown that conjugated ACC content rises coincidentally with the C_2H_4 climacteric senescence burst during carnation

senescence (Hanley et al., 1989; Peiser, 1986; Serrano et al., 1991; Whitehead et al., 1984). However, in some of these studies, a greater conjugated ACC content than free ACC content has been observed (Hanley et al., 1989; Peiser, 1986; Serrano et al., 1991).

McMurchie et al. (1972) proposed that two systems control C_2H_4 biosynthesis. Mattoo and White (1991) mentioned that System I controls basal C_2H_4 production, and responds to C_2H_4 by increasing the activity of EFE and the malonylation of ACC. It has been determined that System II controls autostimulation of C_2H_4 production, responds temporally to C_2H_4, and increases ACC synthase activity.

MET now is accepted as the major precursor of C_2H_4 in higher plants (Yang and Hoffman, 1984). However, the existence of alternate routes for C_2H_4 biosynthesis in some aquatic and lower plant species and certain higher plants under stress has been shown (Mattoo and White, 1991). In young soybean leaves, high concentrations of MET stimulated C_2H_4 synthesis slightly, whereas ACC greatly enhanced it (Lürsen et al., 1979). Also, MET was ineffective in increasing C_2H_4 production compared with ACC in several plant species (Cameron et al., 1979). These authors mentioned that this response occurred because MET was distant from C_2H_4 in the biosynthetic pathway, and its conversion to C_2H_4 may be restricted by one or more rate-limiting steps.

Baker et al. (1978) found in tomato that aminoethoxyvinylglycine (AVG), which inhibits the conversion of SAM to ACC, blocked the formation of C_2H_4 in green tomato fruit tissue, but sensitivity of C_2H_4 production to AVG declined considerably during ripening. These results suggest that the ability of tomato fruits to convert MET into C_2H_4 does not parallel their ability to produce C_2H_4 naturally. Baker et al. (1976) suggested that,

during ripening, tomato fruit tissue switches from MET to an unknown compound as the major precursor of C_2H_4, but it retains its ability to utilize MET as a precursor of C_2H_4. Thus, two pathways may be involved in C_2H_4 production, as McMurchie et al. (1972) have postulated.

Woodson et al. (1992) found an increase in the abundance of transcripts for both ACC synthase and EFE in senescent carnations petals. In contrast, the level of SAM syntethase mRNA decreased during senescence.

In higher plants, δ-aminolevulinic acid (ALA) is the precursor of chlorophyll and heme (Castelfranco and Beale, 1983). However, there is evidence that ALA can be metabolized via nonporphyrin pathways in some species (Beale, 1978; Duggan et al., 1982). El-Rayes (1987) found that the application of (2,3-^{3}H)ALA caused the accumulation of radioactivity in both ACC and C_2H_4, but not MET, in tomato fruit tissue that was at its maximum rate of C_2H_4 production. These data suggest that during ripening of tomato fruits, C_2H_4 may be formed from ALA via ACC.

Glutamic acid (GLU) and α-ketoglutarate (KG) serve as possible ALA precursors (Castelfranco and Beale, 1993), and it has been shown that they participate in the C_2H_4 biosynthetic pathway in *Penicillium digitatum* (Yang, 1973). In this system, C_2H_4 was derived from carbons 3 and 4 of GLU and KG (Chou and Yang, 1973).

In apple tissue, it has been shown that homocysteine (HOMOC) serves as a precursor of C_2H_4 through its conversion into MET (Baur and Yang, 1972). In *Ipomoea tricolor* floral buds, MET had little effect on C_2H_4 production (Konze et al., 1978). However, in the presence of HOMOC thiolactone, C_2H_4 synthesis was enhanced and

occurred prematurely (Hanson and Kende, 1976). Schilling and Kende (1979) subsequently found, in pea stem sections, that MET slightly promoted C_2H_4 synthesis (5 to 10%), whereas HOMOC thiolactone enhanced C_2H_4 production by 20 to 25%. These authors also found that when unlabelled MET or HOMOC was supplied to incubating stem sections of pea with L-[U-^{14}C]MET, MET was most effective in reducing the specific radioactivity of C_2H_4, but HOMOC was almost as effective. These results suggest that HOMOC either serves as a direct precursor of C_2H_4 or as an indirect precursor through its conversion to MET.

3. PRECURSORS OF SYSTEM II ETHYLENE SYNTHESIS IN SENESCING CARNATION PETALS

A paper to be submitted to HortScience

Pablo Gonzalez and Richard J. Gladon[1]

Abstract. Methionine is considered the first committed precursor of C_2H_4, and the C_2H_4 biosynthetic pathway has been established as methionine → S-adenosylmethionine → ACC → C_2H_4. It has been suggested that another pathway to C_2H_4 may exist, and this pathway has been labeled System II. Our objective was to evaluate several compounds as possible precursors of System II C_2H_4 in senescing carnation petals. Cut 'White Sim' carnations were held continuously in 20 mM solutions of water, methionine, ACC, δ-aminolevulinic acid, glutamic acid, α-ketoglutarate, or homocysteine. C_2H_4 production from entire flowers was measured, and free ACC and conjugated ACC in the proximal portion of the petal was quantified. Flowers treated with ACC exhibited the greatest C_2H_4 production and accumulation of free ACC and conjugated ACC. These results confirm the role of ACC as the immediate precursor of C_2H_4 in senescing carnation petals. Homocysteine caused premature production of C_2H_4 and accumulation of more free ACC than methionine and the other possible precursors. Thus, homocysteine may be involved in System II C_2H_4 production in senescing carnation petals. However, whether homocysteine is converted to C_2H_4 via methionine or via a different pathway can not yet be proposed.

---

[1]Associate Professor

Methionine increased C_2H_4 production at the same time as the control, but it caused accumulation of more free ACC. These results suggest that methionine may be a precursor of System II C_2H_4 production. A possible toxicity was observed with δ-aminolevulinic acid and α-ketoglutarate, thus evidence of their participation in the synthesis of C_2H_4 during senescence is not conclusive. Chemical name used: ethylene (C_2H_4), 1-aminocyclopropane-1-carboxylic acid (ACC).

Introduction

The ethylene (C_2H_4) biosynthetic pathway in higher plants has been determined by Adams and Yang (1979) to be: Methionine (MET) → S-adenosylmethionine (SAM)→1-aminocyclopropane-1-carboxylic acid (ACC) → C_2H_4. In carnations, this pathway has been shown to operate either when ACC synthase has been inhibited by (aminooxy)acetic acid (AOA), or when C_2H_4 production has been increased and senescence accelerated by the aplication of exogenous ACC (Mor et al.,1985). In carnations, MET is present in minute amounts at a young stage of development and increases in concentration with ageing (Van der Westhuizen and De Swardt, 1978). These results, however, were not related to the role of MET in C_2H_4 production.

A synchronous rise in free ACC and conjugated ACC content with the rise of C_2H_4 production has been observed in different carnation organs during the climacteric burst of C_2H_4 production (Peiser, 1986). In addition, ACC synthase and ethylene-forming enzyme (EFE) activities increased during carnation petal senescence (Whitehead et al., 1984). Petal tissue produces most of the C_2H_4 during carnation senescence (Hyodo et al., 1990). Mor et

al. (1985) found that C_2H_4 production was greater in the proximal portion of the petals than in the distal region. In addition, greater ACC synthase and EFE activities were found in the proximal portion of the petals. McMurchie et al. (1972) proposed that two systems control C_2H_4 biosynthesis. System I controls basal C_2H_4 production, and System II controls autostimulation of C_2H_4.

MET now is accepted as the major precursor of C_2H_4 in higher plants (see Yang and Hoffman, 1984). However, contrary to the findings in some plant tissues (Burg and Clagett, 1967), it has been shown in other species that MET stimulates C_2H_4 synthesis either slightly or not at all, whereas ACC had a definite stimulatory effect on C_2H_4 production (Cameron et al., 1974).

Baker et al. (1978) found in tomato that inhibiting the conversion of SAM to ACC by aminoethoxyvinylglycine (AVG) blocked the formation of C_2H_4 in green tomato fruit tissue, but sensitivity of C_2H_4 production to AVG declined considerably during ripening. These results showed that the ability of tomato fruits to convert MET into C_2H_4 did not parallel their ability to produce C_2H_4 naturally. Baker et al. (1976) suggested that during ripening, tomato fruit tissue switches from MET to an unknown compound as the major precursor of System II C_2H_4, but it retains its ability to utilize MET as a precursor of C_2H_4. Thus, two pathways may be involved in C_2H_4 production, as McMurchie et al. (1972) have postulated.

Woodson et al. (1992) found an increase in the abundance of transcripts for both ACC synthase and EFE in senescent carnation petals. In contrast, the level of SAM synthetase mRNA decreased during senescence.

El-Rayes (1987) found that the application of (2,3-^{3}H) δ-aminolevulinic acid (ALA) caused the accumulation of radioactivity in both ACC and C_2H_4, but not MET, in tomato fruit tissue that was at its maximum rate of C_2H_4 production. These data suggest that during ripening of tomato fruits, C_2H_4 may be formed from ALA via ACC.

Glutamic acid (GLU) and α-ketoglutarate (KG) serve as possible ALA precursors (Castelfranco and Beale, 1983), and it has been shown that they participate in C_2H_4 synthesis in *Penicillium digitatum* (Yang, 1973). In apple tissue, it has been shown that homocysteine (HOMOC) serves as a precursor of C_2H_4 through its conversion into MET (Baur and Yang, 1972). In *Ipomoea tricolor* floral buds, MET had little effect on C_2H_4 production (Konze et al., 1978). However, in the presence of HOMOC thiolactone, C_2H_4 synthesis was enhanced and occurred prematurely (Hanson and Kende, 1976). Schilling and Kende (1979) found similar results in pea stem sections. These authors also found that when either unlabelled MET or HOMOC was supplied to incubating pea stem sections with L-[U-^{14}C]MET, MET was most effective in reducing the specific radioactivity of C_2H_4, but HOMOC was almost as effective. These results suggest that HOMOC either serves as a direct precursor of C_2H_4 or as an indirect precursor through its conversion to MET.

The overall objective of this research was to evaluate the effect of several of these possible precursors of System II C_2H_4 synthesis on C_2H_4 production and free ACC and conjugated ACC synthesis in senescing carnation petals.

Materials and Methods

Handling flowers and chemical treatments. *Dianthus caryophyllus* L. 'White Sim' flowers were grown in Colorado, and they were harvested when the outer whorl of petals was perpendicular to the flower axis. Flowers were handled and shipped dry by air to Iowa. All leaves were removed and flower stems were recut to 7.5 cm. Flowers were placed in a 50 ml erlenmeyer flask that contained 20 ml of a 20 mM solution of: one of deionized water (D.I., control), ACC, L-MET, ALA, KG, L-GLU and L,D-HOMOC (Sigma Chemical Co., St. Louis, MO). Flowers in flasks were held for 9 days on the laboratory bench at 20C under continuous, low-level irradiance from fluorescent lamps.

Measurement of ethylene production. C_2H_4 was measured every 24 h by placing the entire flower into a sealed 1-liter jar for 30 min. A 1-ml sample was drawn, and gas composition was determined by using a Carle AGC-211M gas chromatograph fitted with a flame ionization detector (EG & G-Chandler Engineering, Broken Arrow, OK).

Free ACC and total ACC assays. Entire petals were removed from the flowers after C_2H_4 production was measure. The proximal 2 cm of each petal (green portion) were excised and used to measure free ACC and total ACC contents. Petal tissue was ground in liquid N_2 and homogenized in 10 ml of 80% (v/v) ethanol by using a Polytron homogenizer at 4C. The homogenate was centrifuged at 10,000x*g* for 10 min, the pellet was resuspended in 10 ml of ethanol, and the mixture was centrifuged again. The supernatants were combined and evaporated to dryness under an air stream at 22C. The dry extract was dissolved in 2 ml water, and 0.5-ml samples of this solution were used to determine free ACC and total ACC. Free ACC was quantified by the modified method of

Lizada and Yang (Wang and Woodson, 1989), and total ACC was quantified by the method of Hoffman et al. (1983). Efficiency of conversion of ACC to C_2H_4 was uniformly above 87%. Conjugated ACC was calculated by subtracting free ACC from total ACC.

Statistical analysis. The experiment was replicated four times independently, and each replicate consisted of two, single-flower observations. The data were pooled for statistical analysis. Data from ACC treatments were not included in the statistical analysis because the amounts of C_2H_4, free ACC, and conjugated ACC were extraordinarily large compared with the other treatments. The amounts of accumulated C_2H_4, free ACC, and conjugated ACC for each treatment were estimated by integrating the areas under the curves of daily C_2H_4 production, free ACC content, and conjugated ACC content, respectively, over the 9 days of the experiment. When statistical significance ($P<0.05$) was found, LSD values were calculated from the error mean square.

Results

ACC as a substrate. ACC increased C_2H_4 production (Fig. 1A) and free ACC content (Fig. 1B) within 24 h of exposure to the substrate. Cumulative C_2H_4 production and free ACC and conjugated ACC also were so large that application of ACC was deemed a separate treatment (Table 1). The increase in C_2H_4 production was accompanied by visual signs of premature petal inrolling. The content of conjugated ACC rose at 120 h (Fig. 1B). The amount of conjugated ACC that accumulated was only about 30% of the amount of free ACC (Table 1).

C_2H_4 production. Flowers treated with HOMOC exhibited an increase in C_2H_4 production within 48 h (Fig. 2A). Application of MET increased C_2H_4 production at 96 h (Fig. 2A). With the other substrates, C_2H_4 production began to increase at either 96 or 120 h (Fig. 2A). Flowers treated with ALA exhibited no detectable C_2H_4 production rate (Fig. 2A). KG caused low accumulation of C_2H_4 (Table 1). For all substrates except ALA, the increase in C_2H_4 production was accompanied by visual petal inrolling. Application of KG caused brown discoloration of the stem, and flowers treated with ALA exhibited a green petal discoloration and "drying" of the calyx 24 h after the start of the treatment.

Free ACC content and accumulation. HOMOC caused an increase in free ACC content within 48 h (Fig. 2B). MET increased free ACC content by 96 h (Fig. 2B). With other substrates, free ACC content began to increase at 144 h (Fig. 2A). Flowers treated with ALA exhibited a constant low free ACC content (Fig. 2B). All substrates except ALA caused an increased free ACC content at the final stages of senescence, although C_2H_4 production had decreased (Fig. 1A, 1B, 2A and 2B). Flowers treated with HOMOC accumulated more free ACC than any of the other substrates (Table 1). Those treated with ALA and KG exhibited lesser accumulation of free ACC than the control (Table 1).

Conjugated ACC content and accumulation. All substrates began to increase conjugated ACC content between 96 and 144 h after the start of exposure to the substrates (Fig. 2C). Flowers treated with MET showed greatest accumulation of conjugates (Table 1). Those in the control, HOMOC and GLU treatments accumulated nearly equal amounts of free ACC and conjugated ACC (Table 1). Flowers treated with MET accumulated almost twice as much conjugated ACC as free ACC (Table 1).

Discussion

When exogenous ACC was applied to whole carnation flowers, we observed premature senescence and a dramatic increase in C_2H_4 production (Fig. 1A). Mor et al. (1985) observed these same responses, and Yang and Hoffman (1984) concluded that this response indicated that EFE, which converts ACC to C_2H_4, is present constitutively and at a relatively high activity.

In flowers treated with HOMOC, C_2H_4 production and free ACC content increased sooner than those treated with any of the other substrates (Figs.2A and 2B), and flowers that received HOMOC accumulated more free ACC than those in other treatments (Table 1). In addition, HOMOC induced a maximum peak of C_2H_4 production at 96 h, whereas the other substrates induced peak C_2H_4 production between 120 and 168 h (Fig. 2A). Premature C_2H_4 production induced by HOMOC thiolactone has been observed previously (Hanson and Kende, 1976). Additionally, enhancement of C_2H_4 production by HOMOC compared with MET has been observed (Hanson and Kende, 1976; Schilling and Kende, 1979). Our results did not show a significant difference in cumulative C_2H_4 production (Table 1). However, in preliminary experiments, when C_2H_4 production was measured on the same flowers rather than different flowers due to destruction during free ACC and conjugated ACC analysis, HOMOC induced a cumulative C_2H_4 production greater than MET and the control (data not presented). Daily measurements of C_2H_4 production on different flowers may have introduced excessive variability, resulting in the lack of significant differences between HOMOC and the rest of the treatments. Cameron et al. (1979) suggested that the ineffectiveness of MET in increasing C_2H_4 synthesis compared

with ACC in several plant tissues is due to the fact that MET is at a distant point from C_2H_4 in the biosynthetic pathway, and its conversion to C_2H_4 is restricted by one or more rate-limiting steps. In addition, Baur and Yang (1972) have shown that HOMOC can serve as an C_2H_4 precursor in apple tissue, as long as it is converted to MET first. Our results, however, showed that HOMOC itself increased C_2H_4 production and free ACC content before MET did (Figs. 2A and 2B). In view of these observations, these data suggest that HOMOC may be involved in the control of autostimulation of C_2H_4 (System II) during senescence. However, because the rate of absorption was not considered and may differ among the different substrates, it is possible that these results may be due in part to a faster rate of absorption of HOMOC compared with the other substrates. Thus, conversion of HOMOC into C_2H_4 through MET may still have to be considered.

MET caused greater free ACC content than the control (Table 1), but the increase in C_2H_4 occurred at the same time as the control (Fig. 2). This greater free ACC content may be explained by the observation that in mungbean hypocotyls the L-form of MET inhibited conversion of ACC to C_2H_4 because ACC is converted stereospecifically to C_2H_4 (Kionka and Amrhein, 1984). Because we used the L-form of MET in this experiment, this could be the reason for the greater free ACC accumulation with MET.

ALA and KG induced lower total C_2H_4 and cumulative free ACC than the control (Table 1), and we observed symptoms of a possible toxicity caused by these treatments. In preliminary experiments, ALA was used in lower concentrations (data not presented), but similar results were obtained. Evidence of the participation of these substrates in System II C_2H_4 synthesis is inconclusive because of the toxic effect that we have observed.

All substrates caused a greater content of free ACC when C_2H_4 production decreased in the terminal stages of senescence (Figs. 1A, 1B, 2A, and 2B), as compared with a less mature stage. This response could be the result of a more rapid fall in the rate of ACC conversion to C_2H_4 rather than a change in the rate of ACC synthesis. This might be expected because EFE is associated with membranes, whereas ACC synthase is located in the cytosol. Therefore, the membrane disintegration that accompanies senescence also may affect the conversion of ACC to C_2H_4 (see Yang and Hoffman, 1984).

Application of exogenous ACC also promoted the accumulation of conjugated ACC (Table 1). However, the amount of conjugated ACC that accumulated represented only about 30% of the amount of free ACC (Table 1). Whitehead et al. (1984) found similar results when ACC was applied to carnation petals. They concluded that the low rate at which applied ACC was conjugated suggested that this alternative use of ACC is unlikely to control C_2H_4 production in this tissue. The control, HOMOC, and GLU caused the accumulation of nearly equal quantities of free ACC and conjugated ACC (Table 1). These results suggest that HOMOC and GLU induced a normal rate of conjugation. In contrast, flowers treated with MET showed a relatively high rate of conjugation, as this represented twice the amount of free ACC accumulated. If the conversion of ACC to C_2H_4 was inhibited because of the use of the L-form of the amino acid, as was mentioned above, conjugation of ACC could be an alternate way to metabolize the excess free ACC that was formed through the application of L-MET.

The effect of several possible precursors of System II C_2H_4 on C_2H_4 production, free ACC, and conjugated ACC synthesis in senescing carnation petals was evaluated. The

role of ACC as the immediate precursor of C_2H_4 during senescence in carnation petals, in agreement with previous studies, was confirmed by the observation that application of ACC to carnation flowers induced the greatest C_2H_4 production and accumulation of free ACC in the petals. MET may be a precursor of System II C_2H_4 production because it accumulated more free ACC than did the control, but the increase in C_2H_4 production for both occurred at the same time. HOMOC may be a precursor of System II C_2H_4 production in carnation petals because it induced premature C_2H_4 production and accumulated more free ACC than MET and the other substrates. However, whether HOMOC is converted to C_2H_4 through MET or through another pathway can not yet be proposed. Evidence of involvement of ALA and KG in System II C_2H_4 synthesis was not conclusive because a possible toxicity was present with these substrates. Future research that employs radioactive MET and HOMOC should be conducted to confirm the results obtained in this research and to have a better understanding of the role of these possible precursors in System II C_2H_4 production in carnation petals.

Literature Cited

Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Nat. Acad. Sci. (USA) 76:170-174.

Baker, J.E., M. Lieberman, and A.T. Kunishi. 1976. Inhibition of ethylene production in tomato and avocado fruit slices by a rhizobitoxine analog and free radical scavengers. Plant Physiol. 57:S-97.

Baker, J.E., M. Lieberman, and L.D. Anderson. 1978. Inhibition of ethylene production in fruit slices by a rhizobitoxine analog and free radical scavengers. Plant Physiol. 61:886-888.

Baur, A.H. and S.F. Yang. 1972. Methionine metabolism in apple tissue in relation to ethylene biosynthesis. Phytochemistry 11:3207-3214.

Burg, S.P. and C.O. Clagett. 1967. Conversion of methionine to ethylene in vegetative tissue and fruits. Biochem. Biophys. Res. Commun. 27:125-130.

Cameron, A.C., C.A.L. Fenton, Y. Yu, D.O. Adams, and S.F. Yang. 1979. Increased production of ethylene by plant tissues treated with 1-aminocyclopropane-1-carboxylic acid. HortScience 14:178-180.

Castelfranco, P.A. and S.I. Beale. 1983. Chorophyll biosynthesis: recent advances and areas of current interest. Annu. Rev. Plant Physiol. 34:241-278.

El-Rayes, D.E. 1987. Biosynthesis of 1-aminocyclopropane-1-carboxylic acid and ethylene from δ-aminolevulinic acid in ripening tomato fruits. PhD Diss., Iowa State University, Ames.

Hanson, A.D. and H. Kende. 1976. Methionine metabolism and ethylene biosynthesis in senescent flower tissues of morning glory. Plant Physiol. 57:528-537.

Hoffman, N.E., Y. Liu, and S.F. Yang. 1983. Changes in 1-(malonylamino) cyclopropane-1-carboxylic acid content in wilted wheat leaves in relation to their ethylene production rates and 1-aminocyclopropane-1-carboxylic acid content. Planta 157:518-523.

Hyodo, H., Y. Terada, and S. Noda. 1990. Effects of 2,5-norbornadiene and ethylene on the induction of activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase, and on increases in the ACC content and the rate of ethylene production in petals of cut carnation flowers during senescence. J. Japan. Soc. Hort. Sci. 59:151-156.

Kionka, C. and N. Amrhein. 1984. The enzymatic malonylation of 1-aminocyclopropane-1-carboxylic acid in homogenates of mung-bean hypocotyls. Planta 162:226-235.

Konze, J.R., N. Schilling, and H. Kende. 1978. Enhancement of ethylene formation by selenoamino acids. Plant Physiol. 62:397-401.

McMurchie, E.J., W.B. McGlasson, and I.L. Eaks. 1972. Treatment of fruit with propylene gives information about the biosynthesis of ethylene. Nature 237:235-236.

Mor, Y., A.H. Halevy, H. Spiegelstein, and S. Mayak. 1985. The site of 1-aminocyclopropane-1-carboxylic acid synthesis in senescing carnation petals. Physiol. Plant. 65:196-202.

Peiser, G. 1986. Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity, ACC and ACC-conjugate in cut carnation flowers during senescence. Acta Hortic. 181:99-104.

Schilling, N. and H. Kende. 1979. Methionine metabolism and ethylene formation in etiolated pea stem sections. Plant Physiol. 63:639-642.

Van Der Westhuizen, A.J. and G.H. De Swardt. 1978. Changes in the individual concentrations in the petals of carnations during the vase life of the flowers. Z. Pflanzenphysiol. 86:125-134.

Wang, H. and W.R. Woodson. 1989. Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by norbornadiene. Plant Physiol. 89:434-438.

Whitehead, C.S., A.H. Halevy, and M.S. Reid. 1984. Control of ethylene synthesis during development and senescence of carnation petals. J. Amer. Soc. Hort. Sci. 109:473-475.

Woodson, W.R., K.Y. Park, P. Larson, and H. Wang. 1992. Expression of ethylene biosynthetic pathway mRNAs during carnation senescence. HortScience 27:580. (Abstr.)

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. 35:155-189.

Yang, S.F. 1973. Biochemistry of ethylene: biogenesis and metabolism. Recent Advan. Phytochemistry 7:131-164.

Table 1. Cumulative C_2H_4 production ($nl \cdot g^{-1}$) and free ACC ($nmol \cdot^{-1}$) and conjugated ACC ($nmol \cdot^{-1}$) content over the 9-day experimental period with constant exposure to 20 mM solutions of each substrate[z].

		Substrate					
Metabolite	ACC[y]	Control	HOMOC	MET	GLU	KG	ALA
C_2H_4	26529	1896a	2347a	1679ab	1599ab	929b	0[x]
Free ACC	37891	248c	699a	531b	194dc	139de	112e
Conj. ACC	12088	322c	634b	934a	249c	237c	345c

[z]Means in rows followed by different letters are statistically different at an LSD of 0.05. Each mean represents the average of 4 replications each with 2 observations.

[y]ACC treatment was not included in statistical analysis.

[x]ALA treatment was not included in statistical analysis for C_2H_4.

Fig.1. Changes in ethylene production (A) and free ACC (▼) and conjugated ACC (★) content (B) in 'White Sim' carnations treated with ACC. Each data point is the mean of 4 replicates each with 2 observations.

Fig. 2. Ethylene production (A), free ACC content (B), and conjugated ACC content (C) during senescence of 'White Sim' carnation treated with homocysteine (⊠), MET (▲), D.I. (★), GLU (♦), KG (✣), and ALA (⧗). Each data point is the mean of 4 replicates each with 2 observations.

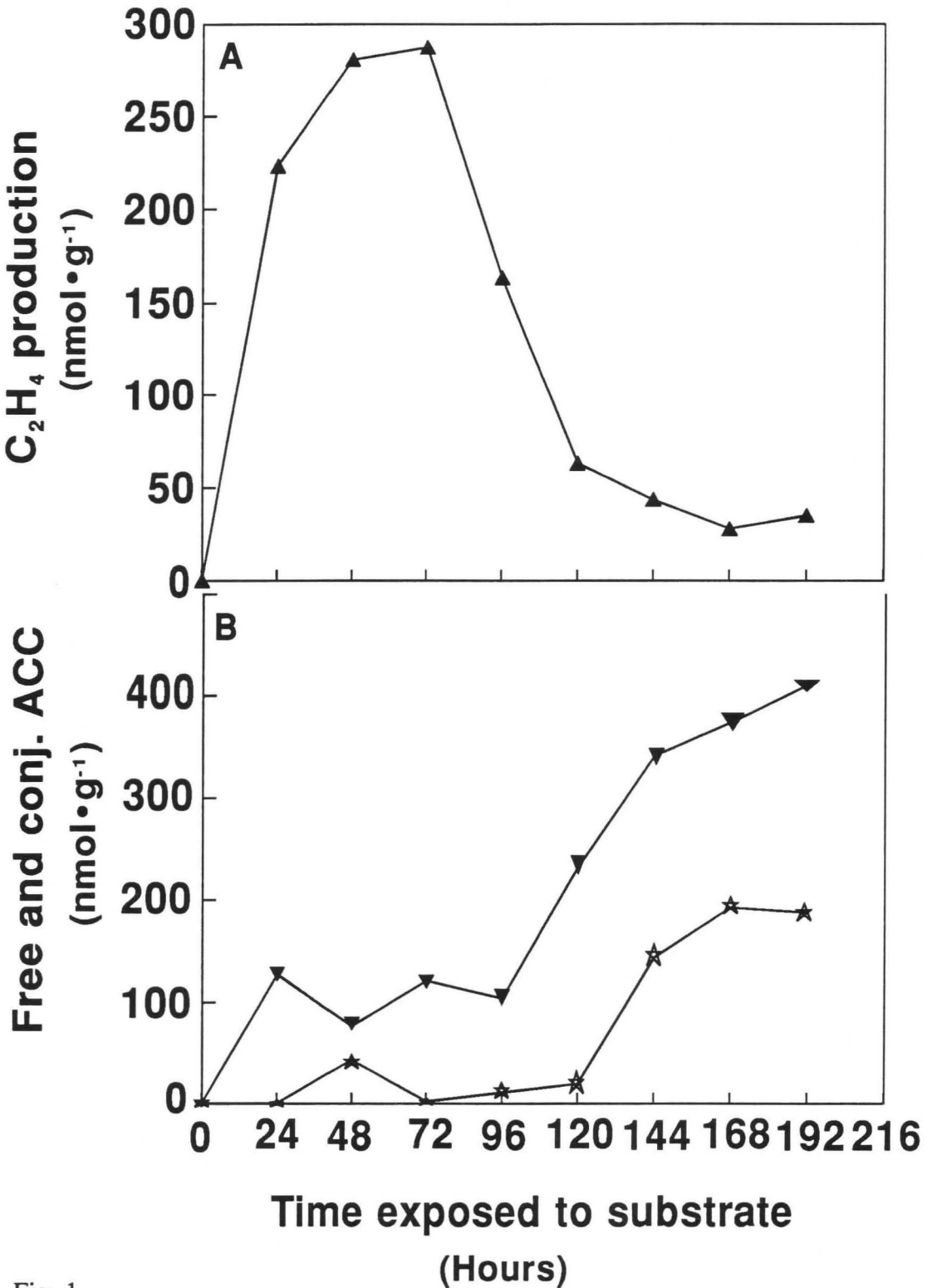
A
B
C_2H_4 production
(nmol•g^{-1})
Free and conj. ACC
(nmol•g^{-1})
0
50
100
150
200
250
300
100
200
300
400
0
24
48
72
96
120
144
168
192
216
Time exposed to substrate
(Hours)

Fig. 1

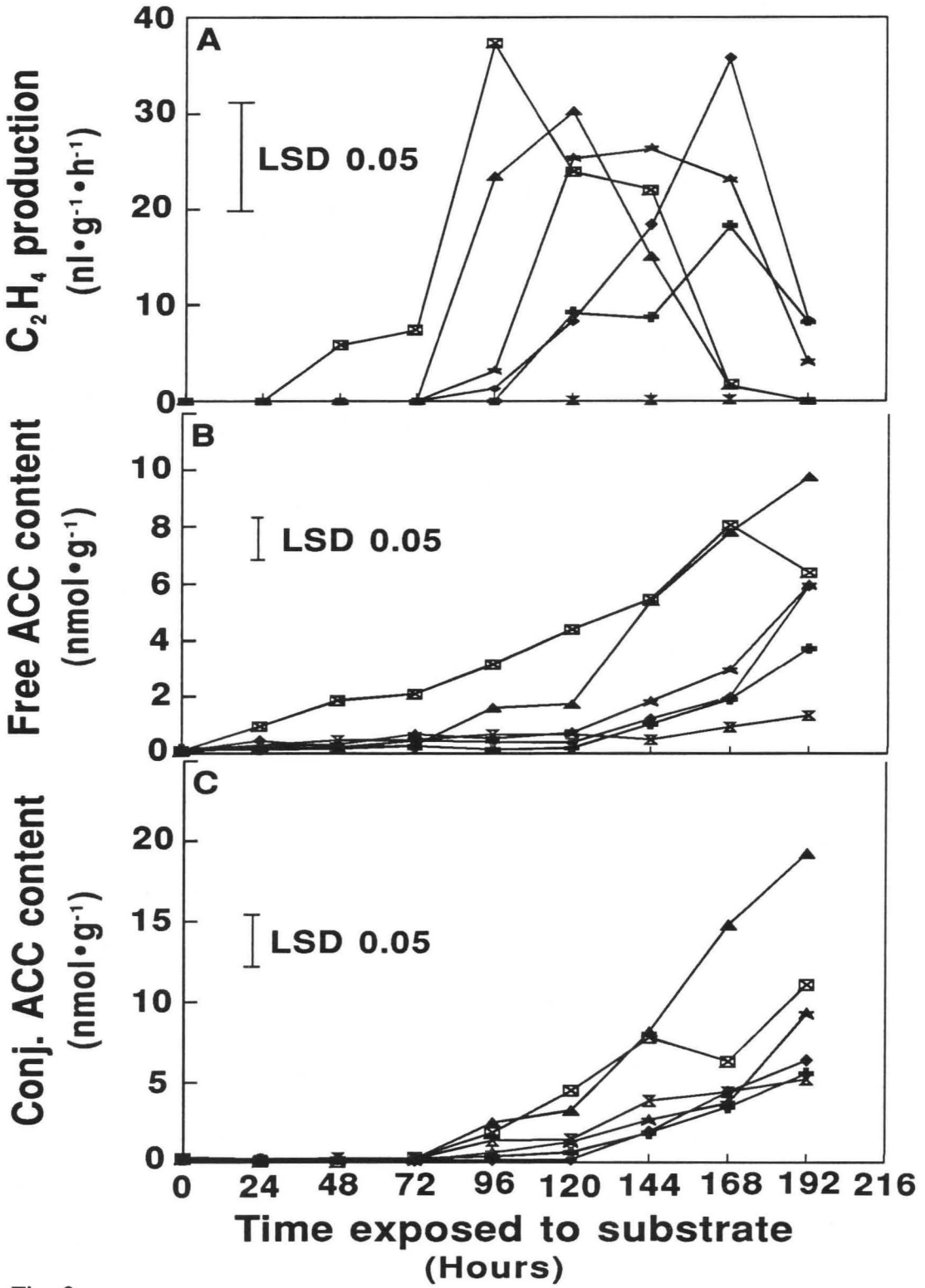
A
B
C
LSD 0.05
LSD 0.05
LSD 0.05
C_2H_4 production
(nl•g^{-1}•h^{-1})
Free ACC content
(nmol•g^{-1})
Conj. ACC content
(nmol•g^{-1})
0
10
20
30
40
0
2
4
6
8
10
0
5
10
15
20
0
24
48
72
96
120
144
168
192
216
Time exposed to substrate
(Hours)

Fig. 2

4. SUMMARY AND CONCLUSIONS

The effect of several possible System II ethylene (C_2H_4) precursors on C_2H_4 production and free ACC and conjugated ACC synthesis was studied. The possible precursors 1-aminocyclopropane-1-carboxylic acid (ACC), methionine (MET), α-ketoglutarate (KG), glutamic acid (GLU), δ-aminolevulinic acid (ALA), and homocysteine (HOMOC) were evaluated. 'White Sim' carnations were placed continuously in 20 ml of 20 mM solutions of the different substrates for 9 days. C_2H_4 production was measured from entire flowers, and free ACC and conjugated ACC content were quantified in the basal portion of the petals. All substrates except ALA induced inrolling of petals accompanying the climacteric C_2H_4 burst and the increase in free ACC content. KG caused a brown discoloration of the stem, and ALA showed a green discoloration of the petals and "drying" of the calyx. These additional symptoms were associated with possible toxic effects of these substrates (see appendixes A and B).

Flowers treated with ACC showed a dramatic and premature increase in C_2H_4 production. This substrate also induced the greatest free ACC and conjugated ACC content, which began to increase at 24 and 144 h respectively, after the substrate was supplied to the flowers. These results confirm the role of ACC as an immediate precursor of C_2H_4 during senescence in carnation flowers.

HOMOC caused premature C_2H_4 production compared with the other substrates. C_2H_4 production started to increase within 48 h, and free ACC and conjugated ACC content began to increase at 48 and 96 h, respectively. HOMOC induced greater accumulation of free ACC than MET and the other treatments. These results suggest that

HOMOC may be involved in System II C_2H_4 production during senescence of carnation petals. However, because the rate of absorption was not considered and may differ among the different substrates, it is possible that these results may be due in part to a faster rate of absorption of HOMOC compared with the other substrates. Thus, a conversion of HOMOC into C_2H_4 through MET may still have to be considered.

MET caused an increase in C_2H_4 production, free ACC content, and conjugated ACC content at 96 h. Flowers treated with MET exhibited greater accumulation of free ACC than the control, and a greater accumulation of conjugated ACC than HOMOC and the other substrates. However, C_2H_4 production in flowers treated with MET began to increase at the same time as the control. These results suggest that the L-form of MET may inhibit the conversion of MET into C_2H_4, stimulating the conjugation system. In addition, MET may be involved in C_2H_4 production during senescence in carnations.

With GLU, ALA, and KG, C_2H_4 production started to increase at either 96 or 120 h, whereas free ACC content started to increase at 144 h. Conjugated ACC content began to increase between 96 and 144 h. GLU and the control induced accumulation of similar amounts of free ACC. With ALA and KG a possible toxicity was observed. Therefore, their participation on C_2H_4 synthesis during senescence is not conclusive.

Further research should be done with radioactive MET and HOMOC to confirm the results obtained with the present research, and to develop a better understanding of the role of these possible precursors in C_2H_4 production during senescence of carnation flowers.

GENERAL LITERATURE CITED

Abeles, F.B., P.W. Morgan, and M.E. Saltveit, Jr. 1992. Ethylene in plant biology. 2nd ed. Academic Press, Inc., San Diego, CA.

Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Nat. Acad. Sci. (USA) 76:170-174.

Baker, J.E., M. Lieberman, and A.T. Kunishi. 1976. Inhibition of ethylene production in tomato and avocado fruit slices by a rhizobitoxine analog and free radical scavengers. Plant Physiol. 57:S-97.

Baker, J.E., M. Lieberman, and L.D. Anderson. 1978. Inhibition of ethylene production in fruit slices by a rhizobitoxine analog and free radical scavengers. Plant Physiol. 61:886-888.

Baur, A.H. and S.F. Yang. 1972. Methionine metabolism in apple tissue in relation to ethylene biosynthesis. Phytochemistry 11:3207-3214.

Beale, S.I. 1978. δ-aminolevulinic acid in plants: its biosynthesis, regulation, and role in plastid development. Annu. Rev. Plant Physiol. 29:95-120.

Borochov, A. and W.R. Woodson. 1989. Physiology and biochemistry of flower petal senescence. Hort. Rev. 11:15-43.

Broun, R. and S. Mayak. 1981. Aminooxyacetic acid as an inhibitor of ethylene synthesis and senescence in carnation flowers. Sci. Hort. 15:277-282.

Bufler, G., Y. Mor, M.S. Reid, and S.F. Yang. 1980. Changes in 1-aminocyclopropane-1-carboxylic acid content of cut carnation flowers in relation to their senescence. Planta 150:439-442.

Cameron, A.C., C.A.L. Fenton, Y. Yu, D.O. Adams, and S.F. Yang. 1979. Increased production of ethylene by plant tissues treated with 1-aminocyclopropane-1-carboxylic acid. HortScience 14:178-180.

Castelfranco, P.A. and S.I. Beale. 1983. Chorophyll biosynthesis: recent advances and areas of current interest. Annu. Rev. Plant Physiol. 34:241-278.

Chou, T.W. and S.F. Yang. 1973. The biogenesis of ethylene in *Penicillium digitatum.* Arch. Biochem. Biophys. 157:73-82.

Cook, E.L. and J. Van Staden. 1988. The carnation as a model for hormonal studies in lower senescence. Plant Physiol. Biochem. 26:793-807.

Duggan, J.X., E. Meller, and M.L. Gassman. 1982. Catabolism of 5-aminolevulinic acid to CO_2 by etiolated barley leaves. Plant Physiol. 69:19-22.

El-Rayes, D.E. 1987. Biosynthesis of 1-aminocyclopropane-1-carboxylic acid and ethylene from δ-aminolevulinic acid in ripening tomato fruits. PhD Diss., Iowa State University, Ames.

Hanley, K.M. and W. Bramlage. 1987. Synchronous production of ACC, MACC, ethylene, and CO_2 in senescing carnation flower parts. HortScience 22:126. (Abstr.)

Hanley, K.M., S. Meir, and W.J. Bramlage. 1989. Activity of ageing carnation flower parts and the effects of 1-(malonylamino)cyclopropane-1-carboxylic acid- inducing ethylene. Plant Physiol. 91:1126-1130.

Hanson, A.D. and H. Kende. 1976. Methionine metabolism and ethylene biosynthesis in senescent flower tissues of morning glory. Plant Physiol. 57:528-537.

Hyodo, H., Y. Terada, and S. Noda. 1990. Effects of 2,5-norbornadiene and ethylene on the induction of activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase, and on increases in the ACC content and the rate of ethylene production in petals of cut carnation flowers during senescence. J. Japan. Soc. Hort. Sci. 59:151-156.

Konze, J.R., N. Schilling, and H. Kende. 1978. Enhancement of ethylene formation by selenoamino acids. Plant Physiol. 62:397-401.

Lürsen, K., K. Naumann, and R. Schröder. 1979. 1-aminocyclopropane-1-carboxylic acid-An intermediate of the ethylene biosynthesis in higher plants. Z. Pflanzenphysiol. 92:285-294.

Mattoo, A.K. and W.B. White. 1991. Regulation of ethylene biosynthesis. pp.21-42. *In* A.K. Mattoo and J.C. Suttle (eds.). The plant hormone ethylene. CRC Press, Inc, Boca Raton, FL.

McMurchie, E.J., W.B. McGlasson, and I.L. Eaks. 1972. Treatment of fruit with propylene gives information about the biosynthesis of ethylene. Nature 237:235-236.

Mor, Y., A.H. Halevy, H. Spiegelstein, and S. Mayak. 1985. The site of 1-aminocyclopropane-1-carboxylic acid synthesis in senescing carnation petals. Physiol. Plant. 65:196-202.

Nichols, R. 1977. Sites of ethylene production in the pollinated and unpollinated senescing carnation (*Dianthus caryophyllus*) inflorescence. Planta 135:155-159.

Peiser, G. 1986. Levels of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase activity, ACC and ACC-conjugate in cut carnation flowers during senescence. Acta Hortic. 181:99-104.

Schilling, N. and H. Kende. 1979. Methionine metabolism and ethylene formation in etiolated pea stem sections. Plant Physiol. 63:639-642.

Serrano, M., F. Romojaro, J.L. Casas, and M. Acosta. 1991. Ethylene and polyamine metabolism in climacteric and nonclimacteric carnation flowers. HortScience 26:894-895.

Van Der Westhuizen, A.J. and G.H. De Swardt. 1978. Changes in the individual concentrations in the petals of carnations during the vase life of the flowers. Z. Pflanzenphysiol. 86:125-134.

Whitehead, C.S., A.H. Halevy, and M.S. Reid. 1984. Control of ethylene synthesis during development and senescence of carnation petals. J. Amer. Soc. Hort. Sci. 109:473-475.

Woodson, W.R., K.Y. Park, P. Larson, and H. Wang. 1992. Expression of ethylene biosynthetic pathway mRNAs during carnation senescence. HortScience 27:580. (Abstr.)

Wu, M.J., W. Van Doorn, S. Mayak, and M.S. Reid. 1989. Senescence of 'Sandra' carnation. Acta Hort. 261:221-225.

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. 35:155-189.

Yang, S.F. 1973. Biochemistry of ethylene: biogenesis and metabolism. Recent Advan. Phytochemistry 7:131-164.

Yu, Y., D.O. Adams, and S.F. Yang. 1979. 1-aminocyclopropane carboxylate synthase, a key enzyme in ethylene biosynthesis. Arch. Biochem. Biophys. 198:280-286.

ACKNOWLEDGEMENTS

I want to express my gratitude to Dr. Richard J. Gladon, my major professor, for so kindly taking me on as a graduate student on such short notice and for his guidance during my graduate career (and for helping me with my pronunciation of important words in English). Sincere thanks to the other members of my committee, Dr. Clinton Hodges and Dr. Martin Spalding for their guidance and thoughtful ideas.

I am also very grateful to Gary Polking for his constant support and helpful ideas, and to Marios, Tony, and Giridhar for all their encouragement and friendship, which have been vital to me in this endeavor. My sincerest thanks to Carlos Echandi, for the hours of time and effort he devoted to helping me with this thesis, and most of all, for his friendship, which has sustained me during my time here.

I also owe a debt of gratitude to the U. S. Agency for International Development for their very generous scholarship which provided me with this opportunity to study at Iowa State, and to Dr. George Karas, Associate Dean of the Graduate College, for all his help in obtaining financial assistance.

Thanks to my mother, my family and my people--your words of encouragement and advice were most welcome. Special thanks to you, Susana...you make all my efforts make sense.

APPENDIX A. DISTRIBUTION OF RADIOACTIVE δ-AMINOLEVULINIC ACID IN 'WHITE SIM' CARNATION FLOWER PARTS

To determine the distribution of absorbed, labelled δ-aminolevulinic acid (ALA) in carnations, flowers were placed for 72 h in 12 x 75 mm tubes that contained 2.0 ml of a solution of ALA at a concentration of 20 mM containing 1 μCi of [4-^{14}C]ALA. The flowers were kept in this solution, in a desiccator, until 1.0 ml was absorbed. After the pulse treatment, the different parts of the flowers were excised and burned in a Packard sample oxidizer. Radioactivity was measured by using a scintillation counter (Table A1).

Table A1. Percentage of total radioactivity in different carnation flower sections

Flower section	Percentages of radioactivity
Lower stem portion	13.8
Upper stem portion	8.5
Receptacle	5.0
Calyx	38.0
Ovary	1.0
Style	0.5
Petal distal portion	20.0
Petal proximal portion	13.0

APPENDIX B. pH OF SUBSTRATES USED

We determined the pH of the substrate solutions (Table B1) for two reasons. First, because phytotoxicity occurred, we wanted to determine whether or not this phytotoxicity was induced by the pH or by the chemical itself. Secondly, solution pH, in some flower species, affects the rate of absorption, and this possible differential rate may have affected our results due to variable amounts of the substrate arriving in the proximal portion of the petals.

Table B1. pH of 20 mM solutions of each of the different posible C_2H_4 precursors*

Substrate	pH
ACC	5.84
MET	5.94
HOMOC	6.02
ALA	2.83
KG	2.15
GLU	3.38

*D.I. water (control) pH was 5.53