Physiological effects of silver thiosulfate, (aminooxy)acetic acid, and malonate pulse treatments on the inhibition of ethylene biosynthesis and mode of action in carnations

Ruey-Song Lin
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Physiological effects of silver thiosulfate, (aminoxy)acetic acid, and malonate pulse treatments on the inhibition of ethylene biosynthesis and mode of action in carnations

Lin, Ruey-Song, Ph.D.
Iowa State University, 1988
Physiological effects of silver thiosulfate, (aminoxy)acetic acid, and malonate pulse treatments on the inhibition of ethylene biosynthesis and mode of action in carnations

by

Ruey-Song Lin

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

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GENERAL INTRODUCTION

Senescence is the final phase of flower development, and various aspects of it can be influenced or controlled by growth regulators (49). Ethylene ($C_2H_4$) is a gas, and it has been attributed the title of the "plant ripening hormone". Ethylene not only manipulates maturation and senescence of plant tissue (1, 44, 48, 49, 122, 124), but it also controls plant growth and development (32, 76).

High concentrations of $C_2H_4$ within the plant or in the air surrounding the plant tissue produce effects such as "sleepiness" of flower heads and premature wilting, and this causes a shortened vase life, especially in flowers that are sensitive to $C_2H_4$ (1, 108). Cut carnations are one of most sensitive to $C_2H_4$. It is important to reduce the $C_2H_4$ level in the environment in which flowers grow or are packed by use of $C_2H_4$ scrubbers (49). Plant material also contributes to increased $C_2H_4$ levels (95), and the major sources are mechanically damaged leaf and flower tissues (37) and diseased plants, including flowers, such as those infected by Botrytis (38) and other pathogens (92, 110). The manipulation of temperature, sanitation, atmospheric composition, and preservative administration are used commercially. It also is necessary to inhibit $C_2H_4$ biosynthesis by the flower itself.

Numerous studies on the biosynthesis of $C_2H_4$ have been conducted, and methionine now is the generally accepted precursor of $C_2H_4$ in the tissue of higher plants (51, 74, 89, 113, 120, 122, 124). The sequence of the pathway for $C_2H_4$ biosynthesis has been shown to be L-methionine.
(L-MET) » S-adenosylmethionine (SAM) » 1-aminoacyclopropane-1-carboxylic acid (ACC) » C\textsubscript{2}H\textsubscript{4} (4), and C\textsubscript{2}H\textsubscript{4} biosynthesis can be regulated by many factors, including C\textsubscript{2}H\textsubscript{4} itself (124).

Senescence in carnations can be delayed by the application of (aminooxy)acetic acid (AOA) or aminoethoxyvinylglycine (AVG), both of which inhibit the conversion of SAM to ACC (13, 25, 43, 83, 89, 93, 117, 122), and by certain salts of silver, such as silver thiosulfate (STS), which is believed to inhibit the mode of action of C\textsubscript{2}H\textsubscript{4} (115). However, the mechanism by which silver ion (Ag\textsuperscript{+}) inhibits C\textsubscript{2}H\textsubscript{4} biosynthesis and action is still unknown (124).

Longevity of cut flowers can be correlated with carbohydrate level in the flowers (95), and these carbohydrates extend the vase life by reducing the activity of the tricarboxylic acid (TCA) cycle. Malonate (MA) inhibits succinate dehydrogenase, an enzyme exclusively localized in the mitochondria, and it has been shown that this inhibitor blocks the TCA cycle partially (about 50\%) (58). In general, malonate blocks the TCA cycle, and therefore, reduces the respiratory rate (33, 35).

The longevity of cut carnations possibly could be extended through the inhibition of C\textsubscript{2}H\textsubscript{4} biosynthesis, the blockage of the mode of action of C\textsubscript{2}H\textsubscript{4}, or moreover, the preservation of carbohydrates. The physiological effects of pulse treatments that affect the respiratory rate and C\textsubscript{2}H\textsubscript{4} biosynthesis need to be investigated further.

The overall objective of this research was to study the effects STS, AOA, and MA on C\textsubscript{2}H\textsubscript{4} biosynthesis and CO\textsubscript{2} production in cut carnations. Within this overall objective, the specific objectives
were: 1) To determine the effects of AOA, STS, and MA on the longevity of carnations; 2) To determine the CO\textsubscript{2} and C\textsubscript{2}H\textsubscript{4} production rates of carnations after pulse treatment with AOA, STS, and MA; 3) To evaluate the effect of exogenous MA on 1-(malonylamino)cyclopropane-1-carboxylic acid (MACC) formation and its role in the reduction of C\textsubscript{2}H\textsubscript{4} synthesis; and 4) To determine ACC synthase and ethylene-forming-enzyme (EFE) activities after a pulse treatment with AOA, STS, or MA.

These objectives are presented in separate papers written in journal format.
General recognition of \( \text{C}_2\text{H}_4 \) as a plant hormone has come only in the past 18 years (91). The identity of \( \text{C}_2\text{H}_4 \) as an active agent was not realized until Neljubow, in 1901, discovered that an abnormal growth habit in etiolated pea seedlings was caused by \( \text{C}_2\text{H}_4 \) leaking from illuminating gas lines (86). In 1908, Crocker and Knight found that the leakage of illuminating gas from gas pipes caused greenhouse carnations to become sleepy, and this was the first report of ethylene-induced floral senescence (34). As early as 1935, \( \text{C}_2\text{H}_4 \) was proposed as a natural plant hormone (34); however, this concept met with strong objections (118). Progress in \( \text{C}_2\text{H}_4 \) biology slowed due to difficulties in its bioassay and rising interests in auxin and other plant hormones (1). The application of highly sensitive gas chromatography to \( \text{C}_2\text{H}_4 \) measurement became available around 1960 (27, 57). This stimulated renewed interest in \( \text{C}_2\text{H}_4 \) research, and research papers in \( \text{C}_2\text{H}_4 \) physiology increased dramatically (1). Ethylene, which exists in a gaseous state under physiological conditions of temperature and pressure, was fully established as a natural plant growth hormone by 1969 (91).

Ethylene regulates many aspects of plant growth and development, including breakage of dormancy, seed germination, seedling growth, stress responses, flowering, fruit ripening, leaf abscission, and senescence (1). Ethylene plays a major role in plant senescence through its effects on the regulation of metabolism (63, 122). The
physiological and biochemical effects of $\text{C}_2\text{H}_4$ on harvested horticultural crops includes its effect on respiratory activity. It also increased the activity of enzymes such as polygalacturonase, peroxidase, lipoxidase, alpha-amylase, polyphenol oxidase, and phenylalanine ammonia-lyase (PAL), increased permeability and loss of cell compartmentalization; and altered auxin transport and metabolism (91). This review is especially focused flower senescence and $\text{C}_2\text{H}_4$.

Ethylene and Flower Longevity

Many species of flowers are damaged by $\text{C}_2\text{H}_4$, though differences in sensitivity exist. Several examples include: 1) abscission of flowers and petals (4, 102); 2) fading and in-rolling of the corolla of Ipomoea (65); 3) fading and wilting of sepal tips in orchid (7); and 4) "sleepiness" of carnation and kalanchoe petals (81, 88).

The first report of ethylene-induced floral senescence showed that $\text{C}_2\text{H}_4$ had a number of effects on carnations (34). It prevented the opening of young blossoms, caused opened flowers to close, and discolored and withered the petals. It was reported that 0.5 $\mu$liter $\text{C}_2\text{H}_4$/liter for 12 hours closed open flowers and 1 $\mu$liter/liter for 3 days prevented other flowers from opening (34). This physiological phenomenon was labeled "sleepiness", and it was characterized by a partial closing of the flower due to an incurving, or in-curling, of the distal portions of the petals. The development progressed until the flowers were almost closed (87). Nichols discovered that a surge of $\text{C}_2\text{H}_4$ production and an increase in respiration synchronized the wilting
process of carnations (88). The outer petals were markedly curled and usually appeared drying and dark along the margin. The surge in \( \text{C}_2\text{H}_4 \) accompanying senescence is an autocatalytic phenomenon in which \( \text{C}_2\text{H}_4 \) stimulates its own biosynthesis (122). This phenomenon is common to both flower senescence (65, 88) and the ripening of climacteric fruit (52, 122).

The Biosynthetic Pathway of Ethylene in Higher Plants

Adams and Yang elucidated the sequence for the pathway of \( \text{C}_2\text{H}_4 \) biosynthesis in ripening apples (3), and this pathway has since been shown to be operative in all other plant tissues tested (122, 124). The pathway of \( \text{C}_2\text{H}_4 \) synthesis in higher plants has been established as \( \text{L-MET} \rightarrow \text{SAM} \rightarrow \text{ACC} \rightarrow \text{C}_2\text{H}_4 \). It has been shown that ACC synthase, which converts SAM to ACC, is the control site of \( \text{C}_2\text{H}_4 \) biosynthesis (122). The conversion of ACC to \( \text{C}_2\text{H}_4 \) by the EFE system has been shown to be an important step in the control of \( \text{C}_2\text{H}_4 \) production (122).

In the carnation, both the ACC content and \( \text{C}_2\text{H}_4 \) formation are low before the start of flower senescence (26). It was suggested that the rate of formation of ACC and its rate of conversion to \( \text{C}_2\text{H}_4 \) may both determine the overall rate of \( \text{C}_2\text{H}_4 \) production (26). With the onset of senescence and the synchronizing autocatalytic rise in \( \text{C}_2\text{H}_4 \) production, ACC content increased rapidly. In cut carnations, as senescence progressed \( \text{C}_2\text{H}_4 \) production fell, but the ACC content of the tissue remained high (124). The narration of \( \text{C}_2\text{H}_4 \) biosynthesis is described
Methionine as the precursor in vivo

Direct evidence of the role of methionine as an \( \text{C}_2\text{H}_4 \) precursor in vivo was reported first by Lieberman et al. (73). They found that labeled methionine itself can act as a substrate and \( \text{C}_2\text{H}_4 \) was derived from carbons 3 and 4 of the methionine molecule (72). Subsequent studies showed that methionine could enhance \( \text{C}_2\text{H}_4 \) production in vivo and was efficiently converted to \( \text{C}_2\text{H}_4 \) in apple (73), and in many other tissues (74).

S-adenosylmethionine as an intermediate

Ethylene production from plant tissues was known to be oxygen dependent (28, 50) and was inhibited by 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation in apple tissue (29). Burg, Murr, and Yang suggested that SAM was an intermediate in \( \text{C}_2\text{H}_4 \) biosynthesis (31, 85). In the proposed scheme, methionine was activated first by ATP to form SAM, which is then fragmented into \( \text{C}_2\text{H}_4 \), \( \text{CO}_2 \), formic acid, \( \text{NH}_3 \) and 5'-methylthioadenosine (MTA). Adams and Yang first provided conclusive evidence that SAM was an intermediate in \( \text{C}_2\text{H}_4 \) biosynthesis (2). Their experimental evidence included: (a) MTA, which is the expected nucleoside fragment from SAM, and methylthioribose (MTR), which is the hydrolyzed product of MTA, were formed from methyl- and sulfur-labeled methionine in climacteric apple tissue. Both MTA and MTR are known metabolites of SAM, (b) When apple plugs were fed with labeled methionine, radioactive SAM was produced, (c) The formation of
MTA and MTR were observed only in climacteric apple but not in preclimacteric apple, and (d) Aminoethoxyvinylglycine (AVG) inhibited MTA and \( \text{C}_2\text{H}_4 \) production but the concentration of SAM was not affected (2).

1-amino-cyclopropane-1-carboxylic acid (ACC) as the immediate precursor of ethylene

Adams and Yang compared the metabolism of methionine in air and in a nitrogen atmosphere. In air containing \( \text{O}_2 \), methionine was efficiently converted to \( \text{C}_2\text{H}_4 \). But in nitrogen without \( \text{O}_2 \), it was metabolized to MTR and ACC. 1-Aminocyclopropane-1-carboxylic acid was converted rapidly to \( \text{C}_2\text{H}_4 \) in the presence of oxygen, indicating that ACC was an intermediate. Moreover, this observation indicated that the conversion of ACC to \( \text{C}_2\text{H}_4 \) was oxygen-dependent (3).

Hence, the pathway of ethylene biosynthesis was indicated by the following sequence: L-MET \( \rightarrow \) SAM \( \rightarrow \) ACC \( \rightarrow \) \( \text{C}_2\text{H}_4 \).

Regulation of Ethylene Biosynthesis

Regulation of the rate-limiting steps

Ethylene production in vivo is known to be regulated by various physiological and environmental factors. The SAM concentration remains substantially constant whether the conversion of SAM to ACC was stimulated or inhibited (36, 130). It was suggested that the formation of ACC was the rate-limiting step in \( \text{C}_2\text{H}_4 \) biosynthesis. Application of exogenous ACC to various plant organs from a number of plants resulted in a marked increased in \( \text{C}_2\text{H}_4 \). The conversion of ACC to \( \text{C}_2\text{H}_4 \) was done
by EFE, which is largely constitutive (32). However, ACC applied to preclimacteric fruits or young petals of carnation flowers resulted in only a slight increase in \( C_2H_4 \) production rates, much lower than those from ripe fruit or senescing flowers (52, 83). In general, this evidence indicated that the preclimacteric or presenescing tissues have a very limited ability not only for the conversion of SAM to ACC but also for the conversion of ACC to \( C_2H_4 \). The restriction for these two steps is released at the onset of ripening or senescence and results in a burst of \( C_2H_4 \) production. Consequently, not only ACC synthase but also EFE can be influenced during certain developmental stages.

**Regulatory factors of ethylene biosynthesis**

The regulation of \( C_2H_4 \) production has been reviewed previously (1, 74, 124). There are several factors that regulate \( C_2H_4 \) biosynthesis, and these factors are described as follows.

**Hormones** Ethylene is a plant hormone that regulates many aspects of plant growth and development, ranging from seed germination to organ senescence (1). The other plant hormones may or may not affect \( C_2H_4 \) production (1). The interaction relationships between \( C_2H_4 \) and other plant hormones can be described as follows.

**Auxin** Auxin increases both ACC synthase activity and ACC concentration (127, 128, 130). Auxin promotion of \( C_2H_4 \) production was discovered initially by Zimmerman and Willooxon in 1935 (132). The research indicated that IAA stimulated \( C_2H_4 \) production by inducing the synthesis of ACC from SAM, and this step is the rate-limiting reaction in the pathway (128).
Abscisic acid Abscisic acid inhibits the increase of ACC in response to stress or IAA (84, 126).

Cytokinin Cytokinin has no or only little stimulatory effect on $C_2H_4$ production. But, cytokinin plus calcium ion ($Ca^{2+}$) or auxin has a synergistic effect on $C_2H_4$ production (69, 70, 71). Indeed, it has been suggested that cytokinin plays a role in the suppression of the formation of IAA conjugates, and it increases the concentration of free IAA, which stimulates $C_2H_4$ production (59, 69, 70). Moreover, Hoffman suggested that it may increase the lifetime of ACC synthase mRNA and accordingly increase ACC synthase activity (53).

Ethylene Ethylene can play two roles, that of an autocatalyst and that of an autoinhibitor (124). Autocatalysis of $C_2H_4$ production is caused by both ACC synthesis and the conversion of ACC to $C_2H_4$ (52, 96). On the contrary, it has been suggested that autoinhibition of $C_2H_4$ production is caused by either inhibition of ACC synthase activity (97) or suppression of ACC synthase synthesis (127).

Gibberellic acid Gibberellic acid has only a slight effect on $C_2H_4$ production (74, 119).

Plant physiological development The restraints of ACC synthesis and the conversion of ACC to $C_2H_4$ will be released by the plant physiological development to senescence resulting in a mass production of $C_2H_4$. The production of $C_2H_4$ is caused by flowers or fruit at certain developmental stages such as the climacteric stage (124).
Carbon dioxide  Carbon dioxide inhibits \( \text{C}_2\text{H}_4 \) action by competing for binding sites with \( \text{C}_2\text{H}_4 \). However, it also increases EFE activity and accordingly enhances the conversion of ACC to \( \text{C}_2\text{H}_4 \) (42, 45, 46, 47, 64, 123).

Light  In the presence of sufficient \( \text{CO}_2 \), light stimulates \( \text{C}_2\text{H}_4 \) production in many green tissues (14, 46, 47, 64). However, the role of light in the stimulation of \( \text{C}_2\text{H}_4 \) production still is unknown.

Stresses  Stress-induced \( \text{C}_2\text{H}_4 \) can be caused by mechanical wounding such as cutting, bruising, radiation and insect infestation, by temperature, including freezing, chilling, and occasionally high temperature, and by water stress including drought, flood, and salts. All these factors induce de novo synthesis of ACC synthase, which, in turn, increase the ACC level and enhance \( \text{C}_2\text{H}_4 \) production (12, 121 124).

ACC conjugate formation  An ACC conjugate, which was identified as MACC, may play a role in regulating \( \text{C}_2\text{H}_4 \) production by decreasing the ACC concentration (9).

Inhibitors  Many compounds inhibit \( \text{C}_2\text{H}_4 \) biosynthesis. These compounds include calcium compounds, sulfhydryl reagents, pyridoxal enzyme inhibitors, uncouplers, free-radical scavengers, polyamines, and ACC analogs. Most of these inhibitors affect either the conversion of SAM to ACC or the conversion of ACC to \( \text{C}_2\text{H}_4 \).

Calcium ions  Depending on the concentration, calcium ions (\( \text{Ca}^{2+} \)) play one of two roles. Indirect prevention of membrane structure deterioration in senescing tissues and inhibition of \( \text{C}_2\text{H}_4 \) biosynthesis may occur when the \( \text{Ca}^{2+} \) concentration is greater than 0.01 M (74). On
the contrary, Ca\(^{2+}\) treatment (1 to 100 mM) induced a higher concentration of C\(_2\)H\(_4\) production. It has been suggested that Ca\(^{2+}\) stabilizes the cellular membrane, which is a requirement for C\(_2\)H\(_4\) biosynthesis (41, 75). It is well known that Ca\(^{2+}\) plus cytokinin has a synergistic effect on C\(_2\)H\(_4\) production as described previously (124).

**Sulfhydryl reagents** Several sulfhydryl reagents, including cobaltic ion (Co\(^{2+}\)), nickle ion (Ni\(^{2+}\)), p-chloromercuribenzoate, and N-ethylmaleimide, react with the sulfhydryl group of EFE and block C\(_2\)H\(_4\) production (130). Cobaltic ion is the most effective inhibitor among the above sulfhydryl reagents. For 50% inhibition of C\(_2\)H\(_4\) production, it has been reported that Co\(^{2+}\) should about 20 \(\mu\)M in mungbean hypocotyls (130).

**Pyridoxal-enzyme inhibitors** There are two types pyridoxal enzyme inhibitors that have been shown to be inhibitors of ACC synthase *in vivo* as well as *in vitro* (23, 124, 129). 1-Aminocyclopropane-1-carboxylic acid synthase is known to be a pyridoxal enzyme. The first inhibitors of this enzyme that were discovered included vinylglycine analogs such as rhizobitoxine (89), aminooxyvinylglycine (AVG) (129, 130), and hydroxylamine analogs, such as (aminooxy)acetic acid (AOA) (128, 129). Aminooxyvinylglycine is a very influential inhibitor of ACC synthase with a \(K_i\) of 0.2 \(\mu\)M (23, 130), whereas the \(K_i\) of AOA is 0.8 \(\mu\)M (130).
Uncouplers, anaerobiosis, and respiratory poisons  The uncouplers of oxidative phosphorylation are potent inhibitors of C$_2$H$_4$ production (29, 71, 85, 107). Low concentrations of 2,4-dinitrophenol (DNP) inhibited C$_2$H$_4$ synthesis by blocking the conversion of ACC to C$_2$H$_4$ without affecting the conversion of methionine to SAM (131). On the other hand, DNP may disrupt membrane structure, and the integrity of membrane structure may be essential for EFE activity (61, 74, 131). Anaerobiosis primarily inhibits the conversion of ACC to C$_2$H$_4$ (128). Several respiratory poisons such as KCN, azide (67), amytal, rotenone, antimycin A, and oleomycin also inhibit the conversion of ACC to C$_2$H$_4$ (11).

Free radical scavengers  In fruit slices, flowers, and vegetative tissue, the conversion of ACC to C$_2$H$_4$ is sensitive to the inhibition of free radical scavengers such as n-propylgallate (0.1 to 10 mM) and sodium benzoate (0.1 to 1 mM) (11, 101).

Polyamines  Polyamines such as spermidine and spermine have been shown to inhibit C$_2$H$_4$ production at 1 mM in Tradescantia petals and in etiolated soybeans (109). Ben-Arie et al. confirmed similar results in apple discs (15). Because the biosynthesis of C$_2$H$_4$ and polyamines share a common precursor, SAM, it is relevant to inquire whether the flux of SAM into polyamines is enhanced when C$_2$H$_4$ biosynthesis rates are inhibited (124).

ACC analogs  α-aminoisobutyric acid significantly and competitively inhibited C$_2$H$_4$ production, and it is oxidatively metabolized by EFE (79).
Ethylene Action

Ethylene binding site and metabolism

Several researchers have reported that C₂H₄ binding sites are associated with membranes (16, 17, 104). Evans et al. showed that the endoplasmic reticulum and protein-body membranes were C₂H₄ binding sites (39, 40). Recently, Thomas et al. suggested that the binding site is a hydrophobic, integral, membrane protein that requires a hydrophobic environment to retain its activity. The C₂H₄ binding protein has not been purified. Further, it is yet to be shown whether the binding site also is associated with subcellular organelles other than the endoplasmic reticulum and protein bodies (111).

There are two hypotheses suggested to describe the interaction of C₂H₄ with its receptors after C₂H₄ binds to the binding site. The first hypothesis suggests that C₂H₄ combines with its receptor and forms a dissociable ethylene-receptor complex that acts as a switch and turns on a cascade of reactions. This complex is believed to be subjected to rapid dissociation after a conformational change on the receptor, resulting in the release of C₂H₄ from the ethylene-receptor complex. Upon this set of circumstances, the switch will be turned off (1, 106). According to this hypothesis, C₂H₄ incorporation and metabolism are not associated with C₂H₄ action.

The second hypothesis suggests that the receptor reacts with C₂H₄ molecules, resulting in physiological actions. Ethylene must be metabolized in order to trigger physiological events. Beyer reported that ¹⁴C₂H₄ was metabolized to ¹⁴CO₂ and also incorporated into tissues.
and showed physiological responses to \( \text{C}_2\text{H}_4 \) under aseptic conditions (18, 19, 20, 21). In many respects, \( \text{C}_2\text{H}_4 \) metabolism resembles \( \text{C}_2\text{H}_4 \) biosynthesis because both are dependent on a high degree of tissue integrity. However, further evidence is necessary to confirm the metabolism of \( \text{C}_2\text{H}_4 \) to its metabolites in a variety of tissues and species (22).

**Antagonists of ethylene action**

There are 3 known types of \( \text{C}_2\text{H}_4 \) antagonists that can inhibit \( \text{C}_2\text{H}_4 \) action when applied exogenously to tissues.

**Carbon dioxide**

In commercial use, a controlled-atmosphere storage technique, which combines increased \( \text{CO}_2 \) concentration with low \( \text{O}_2 \) levels, has been used successfully to prolong the life of many commodities (62, 82). Carbon dioxide prevents or delays many \( \text{C}_2\text{H}_4 \) responses, and it often has been used as a diagnostic test for \( \text{C}_2\text{H}_4 \) action. However, only under the conditions of low \( \text{C}_2\text{H}_4 \) concentrations is it an effective inhibitor of \( \text{C}_2\text{H}_4 \) action. When the \( \text{C}_2\text{H}_4 \) concentration exceeds 1 \( \mu \)liter/liter, the inhibitory capabilities are lost. In certain fruits, \( \text{CO}_2 \) accumulates in the intercellular space and functions as a natural \( \text{C}_2\text{H}_4 \) antagonist (30). The mode of action of \( \text{CO}_2 \) inhibition is not known. However, it has been suggested that \( \text{CO}_2 \) competes with \( \text{C}_2\text{H}_4 \) for the same binding site with a \( K_i \) of 15 ml/liter (30).
Silver ions have been used commercially in cut carnations to extend their vase life (93, 94, 114). Silver ions inhibit $C_2H_4$ action in a wide variety of plant responses including growth inhibition, abscission, and change in sex expression of cucurbit flowers (20). Silver nitrate and STS complexes are the two forms of $Ag^+$ reported in experimental use. The silver thiosulfate complex is less phytotoxic and is mobile in the plant transport system. Accordingly, it has been used commercially, especially in the cut flower industry (116). Silver ion reacts with $C_2H_4$ to form a complex, but such a simple scavenging effect of $Ag^+$ has been excluded as a possible mechanism of action (125), and the mechanism by which it inhibits $C_2H_4$ action is not understood fully (125). The effectiveness of $Ag^+$ in reducing $C_2H_4$ action declines as the $C_2H_4$ concentration is increased. The anti-ethylene effect of $Ag^+$ is more conspicuous than that of $CO_2$ at increased $C_2H_4$ concentrations. It has been suggested that the inhibiting action of $Ag^+$ is not simply competitive with $C_2H_4$ (124). Several investigators reported that $Ag^+$ not only inhibits $C_2H_4$ binding but also $C_2H_4$ biosynthesis (77, 98, 112, 115). It was assumed that $Ag^+$ inhibits the autocatalytic increase in $C_2H_4$ by blocking the $C_2H_4$ receptor site (52). On the contrary, some experiments have shown that $Ag^+$ increased $C_2H_4$ production (5, 6), supposedly by inhibiting the autoinhibition of $C_2H_4$ production (116). Yang presented a simple model accounting for the antiethylene effect of $Ag^+$. In this model, it is implied that one or more of the coordinating ligands in the receptor site facilitates the binding of $C_2H_4$ to the receptor, forming a biologically active complex.
Once Ag⁺ interacts with these ligands when it is exogenously administered, it results in the receptor having little capability to bind C₂H₄, or in an C₂H₄ receptor complex that is inactive or less active (125).

Norbornadiene  Sisler and Pian first reported that some cyclic olefins counteracted ethylene-induced increases in the respiratory rate of tobacco leaves (103). By comparing the structure-activity relationships of a number of olefins that possess antiethylene activity, Sisler and Yang found, by using the pea seedling bioassay, that 2,5-norbornadiene was the most active compound and that it inhibited C₂H₄ action in a competitive fashion with a Kᵢ of 170 μliter/liter. It is assumed that norbornadiene resembles C₂H₄ structurally, competes with C₂H₄ for the same binding site, and induces the norbornadiene receptor complex that makes it biologically inactive (105). Peacock showed that the low endogenous levels of C₂H₄ existing in green fruit exert their ripening influence by shortening the green life of the fruit (90). While the effect of low endogenous levels of C₂H₄ existing in green fruit was counteracted by norbornadiene, the onset of ripening was inhibited. When C₂H₄ was applied to those norbornadiene-treated green fruit, the ripening processes were accelerated. Thus, C₂H₄ was capable of counteracting norbornadiene action, as expected for competitive inhibition (125). Norbornadiene is a gas, and it can be applied and removed reversibly. In this way, it has proved to be a useful tool for the study of C₂H₄ action.
Metabolism of ACC to MACC

Honma and Shimomura demonstrated that ACC was degraded to \( \alpha \)-ketobutyrate and ammonia in microorganisms, a Pseudomonas species, and yeast. Their discovery indicated an alternative metabolic pathway for ACC in forming \( C_2H_4 \) (56). However, Amrhein et al. found no such fragmentation of ACC in buckwheat, but instead detected \( \alpha \)-ketobutyrate. The labeled ACC that was taken up by 70% of the application was converted into a nonvolatile compound that they identified as MACC. Because this compound formed ACC upon acid hydrolysis, it was suggested that it was a conjugate of ACC (8). Apelbaum and Yang first reported that the ACC content decreased much faster than the amount of \( C_2H_4 \) formed, and they suggested that ACC was metabolized by some pathway other than \( C_2H_4 \) production (11). Hoffman et al. speculated that ACC also must be metabolized by some pathway other than \( C_2H_4 \), and independently, they identified MACC as the major nonvolatile metabolite of ACC in wheat leaves (53). The presence of this natural product suggested the possibility that \( C_2H_4 \) biosynthesis can be regulated through malonylation of ACC. Certainly, induction of ACC synthesis under water-stress conditions resulted in a marked increase in MACC level (55). Moreover, Amrhein et al. screened several tissues from various plants for their ability to metabolize administered ACC, and they showed that, except for ripe apples, all tissues were capable of producing MACC. Consequently, the enzyme responsible for MACC formation appears to be mostly constitutive, in a manner similar to that of the enzyme converting ACC to \( C_2H_4 \).
The following observations indicate that MACC is a biologically inactive end-product of ACC rather than a storage form of ACC. Germinating peanut seeds, which are rich in MACC, use methionine or ACC, but not MACC for \( \text{C}_2\text{H}_4 \) production (54), and MACC is a very poor ethylene-releasing agent and the conjugation of ACC to MACC is essentially irreversible. As a result, MACC is unlikely to serve as a precursor in \( \text{C}_2\text{H}_4 \) synthesis, and its formation may serve as a "safety valve" to dissipate excess ACC (9, 122, 124). It has been demonstrated that ACC can be translocated either by the xylem (24) or by the phloem (9); however, transport of MACC in plants has not been studied.

**Regulation of MACC formation**

Because ACC is the common precursor for both \( \text{C}_2\text{H}_4 \) and MACC, regulating the concentration of ACC also should regulate MACC formation.

**Stress-induced MACC formation** As soon as wheat leaves are stressed by wilting, there is a sharp rise, followed by a decline, of ACC and the production rate of \( \text{C}_2\text{H}_4 \). The content of MACC increases gradually until it reaches a plateau. The severity of water stress and the increase of ACC synthesis is correlated positively with MACC formation (55). The increase of MACC under stress also was investigated in chemically wounded mungbean hypocotyls and waterlogged tomato plants (9).
Hormones 1-Aminocyclopropane-1-carboxylic acid malonyltransferase, which converts ACC to MACC, is generally thought to be constitutive (9, 10, 53). The capability of converting ACC to MACC in preclimacteric tomato fruit was greatly promoted by C$_2$H$_4$ treatment. Ethylene treatment enhanced the development of ACC-malonyltransferase 6-fold (80). Indoleacetic acid (IAA) also was found to increase ACC-malonyltransferase activity (10).

**Interrelationship between ACC and MACC** Ethylene and MACC share ACC as a precursor (53). It is expected that ACC will accumulate when the conversion of ACC to C$_2$H$_4$ is inhibited, followed by a higher concentration of MACC. Several agents or environmental factors including cycloheximide, CoCl$_2$, n-propylgallate, DNP, anaerobiosis, high temperature, and cold-shock treatment inhibit the conversion of ACC to C$_2$H$_4$. Unfortunately, those agents and environmental factors also inhibited the conjugation of ACC to MACC in mungbean hypocotyls, although the extent of inhibition was not the same (124, 125). However, the enzymes that are responsible for C$_2$H$_4$ production and MACC formation are localized at different sites, and the synchronous inhibition of these two reactions by the same agents and factors suggest that the regulation of these two reactions may be interrelated. Currently, ACC-malonyltransferase, which catalyzes the following reaction, has been extracted and characterized in vitro (66, 80). The reaction is represented as follows: ACC + Malonyl-Co A $\rightarrow$ MACC +Co A. Both MACC and CoA are inhibitors of the enzyme. Co A is an uncompetitive inhibitor with respect to malonyl-CoA (66). A significant $K_i$ of 0.3 mM and 4.5 mM
for MACC caused a 50% inhibition of the reaction (66). However, this product inhibition needs further experimentation.

**Inhibition of D-amino acid on malonylation of ACC**

The physiological significance of N-malonylation is to inactivate foreign and potentially toxic substances. D-amino acids and herbicides might be harmful to plants (60, 68). Because ACC has no asymmetric carbon, it can be recognized as a D- as well as an L-amino acid. Therefore, an interrelationship between the malonylation of ACC and D-amino acids may exist. Certainly, various D-amino acids inhibited the formation of MACC from exogenously administered ACC in mungbean hypocotyl segments (78) and in cotyledonary segments of cocklebur seedling (99, 100). Consequently, this caused an increase in the free ACC content and $C_2H_4$ production rate (78). Reciprocally, ACC also inhibited the formation of N-malonyl-D-methionine from exogenously administered D-methionine (78).

The intimate relationship between the malonylation of ACC and D-amino acid can be demonstrated. Each of them may be catalyzed by the same enzyme or by separate enzymes that display cross-specificity for both D-amino acids and ACC (124). In addition, Satoh and Esashi have reported that D-amino acids stimulated $C_2H_4$ production and increased ACC content in cocklebur cotyledons (99, 100). The explanation for all of the above observations can be centralized on the point that D-amino acids inhibit malonylation of ACC, resulting in higher ACC levels and thereby higher $C_2H_4$ production rates (124).
SECTION I. INFLUENCE OF SILVER THIOSULFATE, (AMINOXY)ACETIC ACID, AND MALONATE PULSE TREATMENTS ON ETHYLENE PRODUCTION, RESPIRATION, VASE LIFE, AND SENECEENCE OF CARNATIONS
ABSTRACT

The postharvest life of commercially open cut carnations was studied in relation to the inhibition of the biosynthesis of ethylene ($C_2H_4$), 1-amino-cyclopropane-1-carboxylic acid (ACC), carbon dioxide ($CO_2$) production, and 1-(malonyl-amino)cyclopropane-1-carboxylic acid (MACC). Colorado-grown flowers were treated, after shipping to Iowa, with (aminoxy)acetic acid (AOA), silver thiosulfate (STS), or malonate (MA). (Aminoxy)acetic acid at 12 $\mu$mol/flower and STS at 1 $\mu$mol/flower extended flower longevity significantly, but MA at 1 $\mu$mol/flower reduced flower longevity in carnations harvested either in the bud stage or the mature stage.

(Aminoxy)acetic acid and STS inhibited $C_2H_4$, $CO_2$, and ACC production, but MA stimulated $C_2H_4$, $CO_2$, and ACC production. However, MA combined with AOA or STS overcame the stimulation of MA on $C_2H_4$, $CO_2$, and ACC production. Malonate could not conjugate ACC efficiently, although it produced higher malonyl-ACC than other treatments did in the petal tissues. The remainder of the ACC went toward $C_2H_4$ biosynthesis.

These results provide the possibility that these treatments could be developed for use in both the inhibition of $C_2H_4$ biosynthesis and the preservation of carbohydrates in preservative solutions.
INTRODUCTION AND REVIEW OF LITERATURE

Carnation flowers are susceptible to damage by \( C_2H_4 \), the major causal agent in the senescence of carnations (1, 26, 30, 31, 34).

During the senescence of carnation flowers, a climacteric rise in respiration and biosynthesis of \( C_2H_4 \) is associated with the rapid wilting of petals (15, 25). Ethylene biosynthesis is thought to proceed by conversion of L-methionine (MET) to S-adenosylmethionine (SAM), which is converted to ACC, the immediate precursor of \( C_2H_4 \) (2, 4, 5, 10, 21, 33). Ethylene biosynthesis can be regulated by many factors, including \( C_2H_4 \) itself (34).

Senescence in carnations can be delayed by the application of AOA or aminooethoxyvinylglycine (AVG) (6, 9, 13, 14, 24, 27, 28, 33). (Aminooxy)acetic acid inhibits many pyridoxal-phosphate-mediated reactions such as the conversion of SAM to ACC by inhibiting ACC synthase, a key enzyme in \( C_2H_4 \) biosynthesis (2, 19, 36). Pretreatment of carnations with STS prevents the climacteric rise in \( C_2H_4 \) production (3, 32) and delays senescence of the flowers (14, 28). Silver ion (\( Ag^+ \)) has been used commercially in cut carnations to extend their vase life (7, 32). Silver ion possibly could react with \( C_2H_4 \) to form a complex that removes \( C_2H_4 \) by a simple scavenging effect. However, this has been ruled out as a possible mechanism of its action, and the exact mechanism by which silver ions block or reduce \( C_2H_4 \) action is unknown (34, 35).

Malonate inhibits succinate dehydrogenase, an enzyme exclusively localized in mitochondria, and it has been shown that this inhibitor
partially blocks the tricarboxylic acid (TCA) cycle (about 50%) (11, 12).

The objectives of this research were to study the effects of STS, AOA, and MA on the reduction of ACC formation, the availability of ACC, the \( \text{C}_2\text{H}_4 \) production and respiratory rates, and then to investigate the possibility of mixing MA with either STS or AOA to extend the vase life and to improve the keeping quality of carnation flowers.
MATERIALS AND METHODS

Plant materials

Carnation (*Dianthus caryophyllus* L. cv. White Sim) were harvested around 3:00 p.m., shipped dry from Colorado by airplane and received at 11:00 p.m. that night. Stems were trimmed to 30 cm before pulse treatments in individual test tubes. Individually treated carnations absorbed 1 ml of STS, AOA, MA or combinations of these 3 reagents. Silver thiosulphate was applied at 1 μmol/flower, AOA at 12 μmol/flower, and MA at 1 μmol/flower. Flowers then were placed in a holding solution containing 1% dextrose, 60 mg/liter 8-hydroxyquinoline citrate, 4 mg/liter sodium hypochlorite (bleach), and 80 mg/liter citric acid in deionized water. The pH was adjusted to 3.5. Flowers were held at 20 to 22 °C under 11.3 Wm⁻² of cool white fluorescent light 24 hours per day until the time of sampling. Specific stages of senescence were determined by using patterns of respiration and \( C_2H_4 \) production and by visual parameters described by Mayak and Kofranek (23).

Carbon dioxide and \( C_2H_4 \) measurement

For experiments in which \( CO_2 \) and \( C_2H_4 \) production by the flowers were determined, flowers were held in 7.5 liter free space jars fitted with a 9.5 mm rubber septum. At the end of a 2 hr incubation period, a 2 ml sample was analyzed by using a Varian 3700 gas chromatograph equipped with a flame ionization detector for \( C_2H_4 \) and a thermal conductivity detector for \( CO_2 \).
ACC and MACC assay

Basal portions of petals (green portion) weighing 1 g were extracted twice with 5 ml of boiling 80% ethanol. The ethanol with the extracted residue was evaporated under vacuum at 55 °C. The residues were dissolved in 2 ml of H₂O. 1-Aminocyclopropane-1-carboxylic acid content in 0.30 ml aliquots of the aqueous solution was determined according to the method of Lizada and Yang (20). For quantification of MACC, the remaining extract was passed through an ion exchange resin column Dowex 50 (H⁺ form. Sigma Chemical Co., St. Louis, MO, USA) to remove free ACC. The effluent, containing MACC, was hydrolyzed with 2N HCl for 6 hrs, neutralized with NaOH, and the ACC liberated was assayed as described above. The ACC content in the hydrolysate was taken as a measure of MACC content (17).
RESULTS

Changing fresh weight patterns and vase life due to different pulse treatments

The fresh weight of all flowers, which were at the mature stage in all treatments, increased for a few days and then declined at a rate slightly smaller than the 1st day after pulse treatments (Fig. 1). Changing fresh weight patterns of cut carnation flowers play a major role in vase life (29). Water pulse treatments lose fresh weight rapidly 7 days after pulse treatment. The malonate pulse treatment loses fresh weight rapidly after 5 days, but AOA and STS kept the pattern smooth (Fig. 1). It is important to retain a constant fresh weight for vase life and salability. (Aminoxy)acetic acid at 12 μmol/flower and STS at 1 μmol/flower extended the vase life of the flower significantly at bud and mature stages, but MA at 1 μmol/flower reduced flower longevity at both stage. Malonate mixed with either STS or AOA extended the longevity of the carnations (Table 1).
Fig. 1. Changes in percentage of fresh weight of pulsed carnation flowers.
Table 1. Effect of pulse treatments with AOA, STS, and MA on vase life of carnation flowers. LSD=0.90

<table>
<thead>
<tr>
<th>Pulsing solution</th>
<th>Vase life (days)</th>
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<tbody>
<tr>
<td></td>
<td>Bud (1.25 cm)</td>
<td>Mature (2.50 cm)</td>
<td></td>
</tr>
<tr>
<td>H₂O</td>
<td>13.3</td>
<td>10.3</td>
<td></td>
</tr>
<tr>
<td>AOA (12 mol/flower)</td>
<td>21.2</td>
<td>15.1</td>
<td></td>
</tr>
<tr>
<td>STS (1.0 mol/flower)</td>
<td>22.8</td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td>MA (1.0 mol/flower)</td>
<td>11.6</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>MA+AOA</td>
<td>23.4</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>MA+STS</td>
<td>23.1</td>
<td>16.2</td>
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</table>

Ethylene production and ACC content of carnation flowers treated with different pulse treatments

The production of C₂H₄ fell as the petals of control flowers became severely wilted and dried. The ACC content of the flowers followed the same pattern (Fig. 2 and Fig. 3). Apparently, the ACC content of the petals remained high even when they were completely desiccated. Malonate did not inhibit the production of C₂H₄ and an increase in the ACC content, and the shapes of C₂H₄ production curves were similar to those of the controls except that the ACC content increased until the flowers died. Malonate stimulated C₂H₄ production up to its peak level (120 nl/flower) on the 6th day (Fig. 2), but when mixed with either STS
or AOA, it decreased the $C_2H_4$ and ACC production rates (Fig. 2 and Fig. 3). (Aminooxy)acetic acid and STS inhibited $C_2H_4$ evolution and ACC production (Fig. 2 and Fig. 3), but the AOA treatment had a second peak before the flowers died.

Effect of pulse treatment on MACC content

Malonate increased the rate of conversion of ACC to MACC when compared with the water control (Fig. 4). (Aminooxy)acetic acid and STS pulse treatments decreased the MACC content compared with the control. Because the yield of MACC is related to the production of ACC, the pattern of MACC changes over time are similar to the pattern for ACC (compare Fig. 3 and Fig. 4). Cut carnation flowers pulsed with AOA and STS contained low levels of MACC from the beginning until the end of the vase life period. On the 3rd day, both MA and the water control showed a first peak, and the MACC content then decreased rapidly. Subsequently, the water control kept increasing from the 5th day until 1 day before flowers died. The MA treatment kept increasing from the 6th day, and it remained elevated until the flowers died (Fig. 4).

Carbon dioxide production of carnations pulsed with AOA, STS, and MA

The production of $CO_2$ was elevated at the start of the experiment. The control and all of the MA treatments showed a climacteric peak on the 6th day. The rate of $CO_2$ production of those flowers treated with MA was significantly larger than that of the water control (Fig. 5). But mixing it with either STS or AOA prevented this elevated rate of $CO_2$. 
production from the 4th to the 7th day (Fig. 5). There was no climacteric peak for either the AOA or the STS pulse treatments. However, the rate of \text{CO}_2 production went down rapidly 6 days after pulsing with either AOA or STS pulse treatments (Fig. 5).
Fig. 2. Ethylene evolution of carnations pulsed with various combinations of AOA, STS, and MA
Fig. 3. Changes in the concentration of ACC in flowers pulsed with AOA, STS, MA or combinations thereof. Values are the means of 5 measurements each on 1 g of green parts of petals.
Fig. 4. Changes in the concentration of MACC over time after AOA, STS, and MA pulse treatments. Values are the means of 5 measurements each on 1 g of green parts of petals.
Fig. 5. Effect of AOA, STS, and MA pulse treatments on CO$_2$ production by carnation flowers.
DISCUSSION

The results of this research confirm that STS, AOA, STS+MA, and AOA+MA extend the longevity of flowers when compared with the water control (Table 1 and Fig. 1). An elevated level of turgidity is necessary for the complete development of flower buds to full-bloom maturity. It also is necessary for the continuance of normal metabolic activity in the cut flowers. Fresh weight or turgidity in plants and flowers is dependent upon a balance between the rate of water loss or utilization and water supply (22). Flowers can gain fresh weight only when the rate of water absorption is greater than the transpiration rate (29). Although the assumption that the effects of AOA and STS are solely related to their antiethylene properties probably is valid, it cannot be overlooked that STS and AOA may be effective in preventing senescence by some other mechanism.

The fresh weight of flowers treated with MA dropped rapidly the 6th day after pulse treatment, and the \( C_2H_4 \) and \( CO_2 \) production increased simultaneously (Fig. 2 and Fig. 5). However, AOA or STS combined with MA overcame this problem. Therefore, it seems that gains in flower fresh weight are manipulated by \( C_2H_4 \) and \( CO_2 \) production, which result in the senescence of the flowers.

In freshly harvested carnation flowers, the ACC content and \( C_2H_4 \) production rate were low (Fig. 2 and Fig. 3), and these results are similar to those of Bufler et al. (10), Hoffman and Yang (16), and Hsieh (18). During senescence and the accompanying autocatalytic rise in \( C_2H_4 \) production, the ACC content of the petal tissues increased rapidly. All
treatments produced increasing amounts of ACC during the first few days after harvest from the mother plant, and then the ACC content dropped rapidly. As senescence progressed, ACC gradually increased in the water and MA treatments. When the $\text{C}_2\text{H}_4$ production fell, the ACC content of the petal remained elevated. Silver thiosulfate and AOA inhibited ACC production during the holding period but AOA had a 2nd peak of ACC content before the flowers died.

Conjugation of ACC by using MA was hypothesized, but the results show that this does not occur. Malonate increased both the ACC and MACC when compared with the other treatments (Fig. 3 and Fig. 4). The increase in the MACC level is correlated positively with the increased synthesis of ACC. Yang and Hoffman has reported that the conjugation of ACC to MACC is essentially irreversible (34). Thus, the rapid decline in ACC, and consequently $\text{C}_2\text{H}_4$ production, in these carnation flowers can be attributed to the conjugation of ACC to MACC (4). Certainly, STS and AOA pulse treatments had lower MACC due to a reduction of ACC production (Fig. 4). Nevertheless, MA pulse treatments increased the MACC higher than the other treatments, but it also increased the level of ACC, which was not efficiently converted into MACC, and resulted in a higher $\text{C}_2\text{H}_4$ production rate. Consequently, the flowers were affected by an elevated rate of ACC production, which subsequently was converted to $\text{C}_2\text{H}_4$.

Water (control) and MA pulse treatments had the same climacteric peak of $\text{C}_2\text{H}_4$ and $\text{CO}_2$ production (Fig. 2 and Fig. 5), but, once MA was combined with either STS or AOA pulse treatments, $\text{C}_2\text{H}_4$ and $\text{CO}_2$ production were inhibited (Fig. 2 and Fig. 5). These results confirm
that MA stimulated $C_2H_4$ production (Fig. 2) and also increased $CO_2$
production, which was unexpected (Fig. 5), because MA supposedly blocks
the TCA cycle and reduces $CO_2$ production in potato discs and pome fruits
(12). Unfortunately, carnations are sensitive to $C_2H_4$ and produce $C_2H_4$
autocatalytically (10). Malonate may first cause $C_2H_4$, and this may
result in an elevated rate of $CO_2$ production, as suggested by Beyer (8).
He reported that cut carnations incorporated $^{14}C_2H_4$ into an ethanol-
soluble material that metabolized and oxidized $^{14}C_2H_4$ to $^{14}CO_2$.
Consequently, MA pulse treatments combined with either STS or AOA may
first decrease $C_2H_4$, then $CO_2$ production declined further. Because of
the inhibition of $C_2H_4$ biosynthesis by STS or AOA, no extra $C_2H_4$ was
metabolized and oxidized into $CO_2$. We are led to believe that MA does
not block the TCA cycle in carnation flowers.
LITERATURE CITED


SECTION II. EFFECT OF SILVER THIOSULFATE, (AMINOXY)ACETIC ACID, AND MALONATE PULSE TREATMENTS ON 1-AMINOCYCLOPROpane-1-carboxylic ACID SYNTHASE ACTIVITY IN CARNATIONS
ABSTRACT

The 1-aminoacyclopropane-1-carboxylic acid (ACC) synthase activity in carnation flowers was determined in different portions of petals during the latter stages of development and senescence. Little ACC synthase activity existed in the upper portion of the petals. The basal portion of petals contained a much larger ACC synthase activity than did the upper portion of the petals. In the beginning of the holding period, ACC synthase activity was low in all flowers. This was followed by a large increase during the climacteric phase of the water control, and the activity then declined 2 days before the death of the flowers. (Aminoxy)acetic acid (AOA) and silver thiosulfate (STS) inhibited ACC synthase activity, and this resulted in a lower ACC content when compared with the water control. Malonate (MA), a competitive inhibitor of succinate dehydrogenase in the tricarboxylic acid (TCA) cycle, stimulated ACC synthase activity, and this increased the ACC content. Flowers pulsed with MA mixed with either AOA or STS showed low ACC synthase activity. The activity of ACC synthase, which was induced by MA, could be overcome by AOA or STS.
INTRODUCTION AND REVIEW OF LITERATURE

The association of ethylene ($\text{C}_2\text{H}_4$) with senescence of flowers is recognized widely, and carnation flowers (Dianthus caryophyllus L.) have been examined thoroughly in this regard (18). When certain cut flowers approach senescence, a dramatic rise in the rate of $\text{C}_2\text{H}_4$ production occurs, and this is followed soon by wilting of the petals (17, 18, 20). Once endogenous $\text{C}_2\text{H}_4$ production starts, $\text{C}_2\text{H}_4$ synthesis becomes autocatalytic (1). The pathway for $\text{C}_2\text{H}_4$ biosynthesis has been researched thoroughly and established (3). The formation of ACC from SAM is catalyzed by ACC synthase, and this enzyme is the rate-controlling enzyme in $\text{C}_2\text{H}_4$ biosynthesis (4, 21, 24). Recently, it was reported that ACC synthase and the content of ACC and conjugated ACC increased during the climacteric rise in $\text{C}_2\text{H}_4$ production associated with senescence in carnations (19).

Several factors influence ACC synthase activity (22). It has been suggested that ACC synthase is a pyridoxal-phosphate-mediated enzyme because it can be inhibited by aminoethoxyvinylglycine (AVG) and AOA (4, 24). Bohrer and Kende have reported that $\text{C}_2\text{H}_4$ production was inhibited 89% by application of 0.1 mM AVG during a 1-hr incubation period (5). Exogenous $\text{C}_2\text{H}_4$ can induce extractable ACC synthase activity and ripening in AVG-treated apples, and the removal of exogenous $\text{C}_2\text{H}_4$ caused a rapid decline in ACC synthase activity and $\text{CO}_2$ production (7). Calcium is responsible for membrane stabilization, and it has been shown that calcium increased the synthesis of ACC synthase in potato tissue (8, 9,
Antagonists of calmodulin, such as trifluoperazine (TFP), chloropromazine (CPZ), and phenothiazine can inhibit ACC synthase activity (14). Acaster and Kende showed that pericarp tissue of tomato, when cut and incubated in a solution containing $^{35}$S-methionine, incorporated radioactivity into protein. Following the addition of cycloheximide, the radioactivity incorporated into protein of pink fruit declined in parallel with the activity of ACC synthase. In green fruit, radioactivity incorporated into protein remained essentially at the same level for at least 5 hrs after administration of cycloheximide, and during this same time, the activity of ACC synthase declined (2). It was assumed that the ACC synthase activity turned over with the bulk protein in pink fruits but that it did not follow the same general pattern of protein turnover in green fruit. The activity of ACC synthase declined in both green and pink tomato tissue that was treated with cycloheximide, and the apparent half-life of ACC synthase in green fruit tissue was 30 to 40 min, while, in pink pericarp tissue, it was around 2 hrs (2).

The ACC synthase was reported to be unstable, and ACC synthase, which was induced by auxin in etiolated mungbean hypocotyls, was inactivated rapidly upon extraction (23, 24). Similar results were reported with tomato fruit (10). Acaster and Kende extracted ACC synthase from ripening tomato fruit by using Sephadex G-100 gel filtration, and they reported that the molecular weight of the native enzyme was 57 kilodaltons. Phenyl-Sepharose CL-4B hydrophobic chromatography improved purity of the enzyme 70-fold, but further
puriﬁcation was impeded by the lability of ACC synthase (2). Conversely, Nakajima and Imaseki found that ACC synthase from Cucurbita maxima Duch. mesocarp was relatively stable (16).

The activity of ACC synthase often determines the developmental fate of plant tissues; therefore, there is of considerable interest in understanding how this enzyme is regulated. The objectives of this study were to: 1) determine the relationship between senescence and ACC synthase activity in carnation ﬂowers, and 2) determine the effects of STS, AOA, and MA on ACC synthase activity.
MATERIALS AND METHODS

Plant material

Carnation flowers (Dianthus caryophyllus L. cv. White Sim) were shipped dry from Denver, Colorado. Stems were trimmed to 30 cm before pulse treatments in individual tubes began. All carnation flowers absorbed 1 ml of H$_2$O (control), AOA, STS, or MA. The treatments were AOA at 12 µmol/flower, STS at 1 µmol/flower, and MA at 1 µmol/flower. Subsequently, flowers were placed in a holding solution (see SECTION I) in a room held at 20 to 22°C under 11.3 Wm$^{-2}$ of cool white fluorescent light 24 hours per day.

Extraction of ACC synthase

The extraction of ACC synthase was conducted according to Acaster et al. with slight modifications (2). Basal portions of petals from pretreated carnations were ground with a mortar and pestle in 1.5 M potassium phosphate buffer (pH 6 to 9 in the various experiments), containing 4 mM dithioerythritol (DTE) and 0.5 µM pyridoxal-5-phosphate, and then squeezed through one layer of nylon cloth. The homogenizing buffer solution was used at a ratio of 1 ml/g fresh weight of tissue. The homogenate was centrifuged at 20,000 g for 20 min. The clear supernatant was desalted by passing it through a Sephadex G-25 column that was preequilibrated with 20 mM potassium phosphate buffer containing 0.4 mM DTE and 0.5 µM pyridoxal phosphate. All of the above operations were performed at 0 to 4°C.
**Determination of ACC synthase activity**

The method for determining ACC synthase activity was that of Lizada and Yang, and it was used with slight modifications (13). A sample containing 100 μL of enzyme extraction was incubated with 400 μM SAM (hydrosulfate salt) in 100 mM phosphate buffer for 20 min in 13 x 100 mm test tubes. The enzyme reaction was terminated by addition of 500 μl ice-cold 20 mM HgCl₂. All tubes were sealed with a serum cap. Following this formation of ACC by this method, the ACC was converted to C₂H₄ by injecting 200 μL of an ice-cold mixture containing 5% NaOCl and 15 M NaOH (2:1 v/v). The sealed tubes were agitated for 30 sec, and a 2-ml sample from the headspace of each tube was drawn and injected into a gas chromatograph for the quantification of C₂H₄. The incubations were performed in a 30°C water-bath chamber. The specific activity of ACC synthase was expressed as nmole ACC per mg protein per hour, and the protein content of the enzyme extract solution was determined by the Bradford method (6).

**Effect of AOA, STS, and MA on ACC synthase activity**

Basal portions of petals were detached from flowers at the climacteric (determined by the pattern of CO₂ evolution). Flowers had been pretreated with AOA at 12 μmol/flower, STS at 1 μmol/flower, and MA at 1 μmol/flower upon arrival. Experimental materials were assayed for ACC synthase activity according to the above method.
RESULTS

Extraction of ACC synthase in carnation petals

pH influences ACC synthase activity (Fig. 1). The optimal pH was determined in 1.5 M phosphate buffer, and maximum activity occurred at a pH of 8.0.

Effect of incubation time on determination of ACC synthase activity

Enzyme activity in crude extracts was affected by the incubation period with 400 μM SAM, and a linear relationship between activity and incubation period did not exist when extract was incubated for more than 30 min (Fig. 2).

Effect of different portions of petals on determination of ACC synthase activity

Mor et al. showed that the ACC content varied in different portions of petals. The content of ACC in the basal portion of the petal is much higher than it is in the upper portion (15). To determine the extent of these variations in ACC synthase activity, various portions of the petals were tested daily during the vase life (Table 1).
Fig. 1. Influence of pH on ACC synthase activity
Fig. 2. Effect of incubation period on ACC synthase activity. Crude extract (0.1 ml) was incubated with 400 μM S-adenosyl-methionine in 100 mM potassium phosphate buffer, pH 8.
Table 1. Changes in ACC synthase activity of the upper and basal portions of carnation petals during the vase life period. Flowers were held in the holding solution, and they received no treatment. LSD (0.05)=0.54

<table>
<thead>
<tr>
<th>Time in holding solution (days)</th>
<th>ACC synthase activity (nmol ACC/mg protein/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petal portion</td>
<td>Collected</td>
</tr>
<tr>
<td>Upper</td>
<td>0.06 0.12 0.25 0.18 0.21 0.27 0.35 0.52 0.72 0.32</td>
</tr>
<tr>
<td>Basal</td>
<td>0.52 0.72 1.13 1.54 1.83 2.01 4.25 9.06 3.63 1.18</td>
</tr>
</tbody>
</table>

**Effect of STS, AOA, and MA on ACC synthase activity**

Application of STS, AOA, or MA in the pulsing solution affected the rate of \( \text{C}_2\text{H}_4 \) production (see SECTION I). The activity of ACC synthase was affected dramatically by STS, AOA, or MA (Fig. 3). The activity of ACC synthase was decreased dramatically by application of AOA or STS, while MA dramatically increased the activity of ACC synthase. These same chemicals affected \( \text{C}_2\text{H}_4 \) production in a comparable manner, and the effect of them on the ACC content showed a similar pattern, but it was less dramatic.
Suppression by AOA and STS of the ACC synthase activity that was induced by MA

(Aminoxy)acetic acid and STS inhibited ACC synthase activity, while MA increased it, especially during the climacteric (Fig. 4). The combination pulse treatment of AOA and MA decreased ACC synthase activity, and AOA overcame the stimulatory effect of MA (Fig. 4). Unfortunately, AOA could not suppress ACC synthase activity during the later portion of the vase life period, and it had a second peak of ACC synthase activity two days before the flowers senesced (Fig. 4). Silver thiosulfate inhibited the ACC synthase activity, and a combination of STS and MA also inhibited ACC synthase activity completely. Therefore, the increase in ACC synthase activity by MA could be suppressed completely with STS and partially with AOA when they were applied simultaneously.
Fig. 3. Effects of STS, AOA, and MA on ACC synthase activity, ACC formation, and C$_2$H$_4$ production on the 6th day. Activity of ACC synthase and ACC content was assayed in the basal portions of petals. Ethylene production was assayed by quantifying C$_2$H$_4$ production by pretreated flowers that were pulsed with AOA, STS, or MA.
Fig. 4. Suppression by AOA and STS of ACC synthase activity that was induced by MA. Arrows indicate the termination of the vase life period for the particular treatment.
DISCUSSION

The activity of ACC synthase is sensitive to small pH changes (11). In this study, the optimal pH was found to be 8, which is slightly different from the report (pH 8.5) by Yu et al. with tomato fruit (24).

The content of ACC synthase, in terms of protein, is low, but it increased during ripening of tomatoes (4). Apparently, low ACC synthase exists in the upper portion of carnation petals (Table 1). A much higher ACC synthase activity existed in the basal portions of the petals. It is evident that ACC synthase activity increased during the climacteric (Table 1), and its activity paralleled the \( \text{C}_2\text{H}_4 \) production rate (see SECTION I).

Yu et al. showed that AOA inhibited ACC synthase activity (24). In this study, it has been shown that AOA and STS inhibited ACC synthase activity, while MA increased ACC synthase activity (Fig. 3). In addition, the ACC content and \( \text{C}_2\text{H}_4 \) production rate followed a pattern similar to the one for ACC synthase activity (Fig. 3). The greater the ACC synthase activity, the more ethylene production there is. Consequently, the mode of action of ACC synthase is clear, and it is the key enzyme for \( \text{C}_2\text{H}_4 \) biosynthesis.

(Aminoxy)acetic acid is a potent inhibitor of ACC synthase. These results show that AOA stimulated ACC synthase activity only slightly in the beginning of the vase life period, but it inhibited ACC synthase activity at the climacteric (Fig. 4). A possible cause for this slight increase after pulsing may be osmotic or chemical damage as a result of the pulse treatment.
Silver thiosulfate also inhibited ACC synthase activity, and its effects are similar to those of AOA (Fig. 4). The mechanism by which STS inhibited C$_2$H$_4$ biosynthesis still is unknown. However, in this study, STS inhibited ACC synthase activity and resulted in the inhibition of C$_2$H$_4$ biosynthesis (22). Malonate increased ACC synthase activity, but when combined with either AOA or STS, it showed low ACC synthase activity. The increase in ACC synthase activity by MA could be suppressed with AOA or STS due to the characteristic of inhibition of C$_2$H$_4$ biosynthesis by AOA or STS. Therefore, C$_2$H$_4$ production induced by MA could be inhibited (see SECTION I). Consequently, flowers pulsed by MA mixed with either AOA or STS avoided the side effect of MA on C$_2$H$_4$ production.
LITERATURE CITED


Physiol. 69:1150-1155.


SECTION III. IDENTIFICATION AND CONTROL OF ETHYLENE BIOSYNTHESIS IN THE ETHYLENE-FORMING-ENZYME SYSTEM OF CARNATIONS
ABSTRACT

The ethylene-forming-enzyme (EFE) in carnation flowers was assayed by determining the maximum rate of ethylene ($C_2H_4$) production. Various sets of conditions were studied during the development of this procedure. The maximum rate of $C_2H_4$ production was found to occur during a 2-hr submergence following a 4-hr aerobic condition with the administration of 1 mM 1-aminocyclopropane-1-carboxylic acid (ACC). The following inorganic ions are not cofactors for EFE, and they showed an inhibitory effect on EFE. The inhibitory function sequence, from most powerful to least powerful, is as follows: $Co^{2+}$, $Fe^{2+}$, $Mn^{2+}$, $Ca^{2+}$, $Mg^{2+}$, and $K^+$. 

Flowers pulsed with (aminooxy)acetic acid (AOA) and silver thiosulfate (STS) showed a low EFE activity as measured by $C_2H_4$ production. A malonate (MA) pulse treatment increased the EFE activity after the 4th day of the holding period. Even after the flowers died, the EFE activity remained elevated.
INTRODUCTION AND REVIEW OF LITERATURE

Senescence of carnation flowers (Dianthus caryophyllus L. cv. White Sim) can be modified by various treatments (6, 7, 12, 17). The senescence of carnation flowers usually is accompanied by an increase in the rate of C\textsubscript{2}H\textsubscript{4} biosynthesis and a concomitant climacteric rise in respiration (14, 19). The pathway of C\textsubscript{2}H\textsubscript{4} synthesis in several higher plants has been established as L-methionine (L-MET) $\rightarrow$ S-adenosylmethionine (SAM) $\rightarrow$ 1-aminocyclopropane-1-carboxylic acid (ACC) $\rightarrow$ C\textsubscript{2}H\textsubscript{4} (1). In young petals of carnation flowers, C\textsubscript{2}H\textsubscript{4} production is low because the petals have a limited ability not only to convert SAM to ACC but also to convert ACC to C\textsubscript{2}H\textsubscript{4} (18). It has been shown that ACC synthase, which converts SAM to ACC, is the main site of control of C\textsubscript{2}H\textsubscript{4} biosynthesis. Ethylene-forming-enzyme, which converts ACC to C\textsubscript{2}H\textsubscript{4}, also is an important control site of C\textsubscript{2}H\textsubscript{4} production (20).

Because the presence of EFE has not been demonstrated in a cell-free system, its activity can be measured only in situ (20). The EFE in flower tissues was assayed by determining the maximum rate of C\textsubscript{2}H\textsubscript{4} production in the presence of saturating levels of ACC at 25 °C. Preliminary experiments showed that the maximum EFE activity was obtained when the plant tissues were incubated in 1 mM ACC. Ethylene-forming-enzyme is an oxygen-dependent enzyme. Therefore, the possibility of inducing C\textsubscript{2}H\textsubscript{4} production when assaying EFE could be avoided by restricting incubation times to less than 6 hrs (10). However, attention needs to be paid to the consideration of the slow
diffusion of ACC into the tissue. Moreover, the roles that inorganic ions play also need to be considered.

Anaerobic or low-oxygen conditions interfere with the conversion of ACC to \( \text{C}_2\text{H}_4 \) (1). Inhibitors such as 2, 4-dinitrophenol (DNP) and cobaltic ion \((\text{Co}^{2+})\) also interfere (25). Cobaltic ion retards senescence of detached leaves (4, 8, 23), but other inorganic ions have not been studied adequately as to their roles as inhibitors or cofactors of EFE. (Aminoxy)acetic acid and STS delayed carnation senescence by inhibiting \( \text{C}_2\text{H}_4 \) production (6, 7, 15, 16), and changes in the ACC content of naturally senescing and STS-pretreated carnations have been studied (3). The mechanism by which silver inhibits \( \text{C}_2\text{H}_4 \) biosynthesis still is unknown (22). The effect of STS pulse treatments on EFE activity has not been studied in carnation petals. (Aminoxy)acetic acid acts as an inhibitor of ACC synthase (24), and the effect of AOA pulse treatments on EFE activity also have not been studied. Malonate, an inhibitor of succinate dehydrogenase in the tricarboxylic acid (TCA) cycle (5), stimulates \( \text{C}_2\text{H}_4 \) production (see Section I), but its effect on EFE activity also is not known.

The purpose of this research was to identify the immersion period needed and the roles of several inorganic ions on EFE activity. Ultimately, the effect of STS, AOA, and MA on EFE activity will emerge, and the mechanism or mode of action of AOA, STS, and MA on the vase life of carnations can be understood.
MATERIALS AND METHODS

Plant material

Carnation flowers (Dianthus caryophyllus L. cv. White Sim) were shipped dry from Denver, Colorado. Stems were trimmed to 30 cm before pulse treatments in individual tubes. Individually treated carnations absorbed 1 ml of STS, AOA, and MA solution. Silver thiosulfate was applied at 1 μmol/flower, AOA at 12 μmol/flower, MA at 1 μmol/flower. Following the pulse treatment, flowers were held at 20 to 22°C under 11.3 μmol/m² of cool white fluorescent light 24 hours per day until the time the flowers reached the climacteric (see SECTION I).

Test solutions

Basal and upper portions of flower petals were dissected and weighed individually 2 g for each test. Petal tissue was placed in 15 x 125 mm tubes with a septum cap. To this was added 3 ml of 1 mM ACC and 2 ml of the test solutions of the various concentrations of several salts such as KCl, MgCl₂·6H₂O, MnCl₂·4H₂O, CaCl₂·2H₂O, FeCl₂·6H₂O, and CoCl₂ at 1, 2, or 3% (w/v) and H₂O as the control.

EFE assay method

Effect of incubation period and tissue position on EFE activity

The petal tissues were immersed for 2, 4, or 6 hrs in a solution of 1 mM ACC and the tubes were sealed. The incubation solution was removed by syringe according to particular timetable set for each experiment. A 2-
ml sample was analyzed by using a Varian 3700 gas chromatograph equipped with a flame ionization detector for C$_2$H$_4$ analysis.

**Effect of several inorganic ions on EFE activity related to ethylene production**  
Petal tissue was incubated in a test solution of KCl, MgCl$_2$·6H$_2$O, MnCl$_2$·4H$_2$O, CaCl$_2$·2H$_2$O, FeCl$_2$·6H$_2$O, or CoCl$_2$ at 1, 2, or 3% (w/v). All treatments were soaked for 2 hrs and then removed the incubation solution from the tubes. The petal tissues were agitated with a high-frequency shaker until the petal tissues were separated, then waited for 4 hrs without any immersion to allow the activity of EFE to produce C$_2$H$_4$ in the aerobic condition. A 2 ml sample was assayed for C$_2$H$_4$ by gas chromatography. The EFE assay was performed at 22°C.

**Effect of STS, AOA, and malonate pulse treatments on EFE activity**  
Plant materials that were pulse-treated were analyzed daily until the flowers died. Petals weighing 2 g were immersed for 2 hrs in a solution containing 3 ml of 1 mM ACC and 2 ml distilled water. Subsequently, the incubation solution was removed, the petal tissues were separated by a high-frequency shaker, and after 4 hrs in aerobic conditions the tissue was assayed for EFE activity by the production of C$_2$H$_4$. A 2-ml gas sample was assayed for C$_2$H$_4$ by a gas chromatography and the activity of EFE was determined by C$_2$H$_4$ production.
RESULTS

Effect of incubation period and position within petal on EFE determination

The EFE in the flower should be assayed by the most abundant part of flowers and efficient methods. Results have shown that the optimum incubation period for EFE activity can be determined by the maximum rate of \( C_2H_4 \) production (Table 1). The maximum rate of \( C_2H_4 \) production is obtained when a 2-hr immersion is followed by 4 hrs of aerobic conditions. Treatments immersed longer than 2 hrs did not show a greater \( C_2H_4 \) production rate for EFE. The basal portion of the petals produced many-fold more \( C_2H_4 \) than did the upper portion.

Table 1. Effect of incubation period on ethylene production by different petal parts of carnations. LSD (0.05)=3.15

<table>
<thead>
<tr>
<th>Portion of petal</th>
<th>Incubation period (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Basal portion</td>
<td>46.03</td>
</tr>
<tr>
<td>Upper portion</td>
<td>5.37</td>
</tr>
</tbody>
</table>
Effect of inorganic ions on EFE activity

All inorganic ions tested (Ca$^{2+}$, Co$^{2+}$, Fe$^{2+}$, K$^+$, Mg$^{2+}$, and Mn$^{2+}$) inhibited EFE activity as measured by $C_2H_4$ production (Table 2). The sequence of the inhibitive function is as follows: Co$^{2+}$ > Fe$^{2+}$ > Mn$^{2+}$ = Ca$^{2+}$ > Mg$^{2+}$ = K$^+$. There is no significant difference in the effect of the concentration as it changed from 1 to 2 to 3% (w/v) for Co$^{2+}$, Fe$^{2+}$, Mg$^{2+}$, and Mn$^{2+}$, but there is a concentration effect for Ca$^{2+}$ and K$^+$ (Table 2).

Table 2. Effect of several inorganic ions on EFE activity as measured by $C_2H_4$ production in the basal portion of carnation petals. The water control treatment was 46.03 nL/g/hr. LSD (0.05)=2.95

<table>
<thead>
<tr>
<th>Concentration (% w/v)</th>
<th>Ca$^{2+}$</th>
<th>Co$^{2+}$</th>
<th>Fe$^{2+}$</th>
<th>K$^+$</th>
<th>Mg$^{2+}$</th>
<th>Mn$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.57</td>
<td>0.85</td>
<td>15.54</td>
<td>29.00</td>
<td>27.03</td>
<td>21.49</td>
</tr>
<tr>
<td>2</td>
<td>30.32</td>
<td>0.42</td>
<td>13.27</td>
<td>24.92</td>
<td>27.51</td>
<td>19.04</td>
</tr>
<tr>
<td>3</td>
<td>25.24</td>
<td>0.49</td>
<td>12.33</td>
<td>22.36</td>
<td>25.64</td>
<td>19.59</td>
</tr>
</tbody>
</table>
Effect of STS, AOA, and MA pulse treatments on EFE activity

(Aminoxy)acetic acid and STS inhibited EFE activity significantly (Fig. 1). Malonate decreased EFE activity as compared with the control (H₂O) before the climacteric, but later, on the 7th day, the EFE activity of the MA-treated flowers increased greatly (Fig. 1). Consequently, the flowers died on the 9th day (see SECTION I). When the flowers died, the EFE activity remained high, as did the ACC content.
Fig. 1. Effect of STS, AOA, and MA pulse treatments on EFE activity as measured by $C_2H_4$ production.
DISCUSSION

Ethylene-forming enzyme, which converts ACC to \( C_2H_4 \), is largely constitutive (21) and an oxygen-dependent enzyme (1). The period of incubation in elevated concentrations of ACC (1 mM) is an important aspect of the determination of the maximum rate of \( C_2H_4 \) production. These results show that petals immersed for periods longer than 2 hrs lack oxygen, and therefore, there is an interference with EFE activity by exposure to anaerobic conditions (Table 1). The EFE activity in the upper portion of the petal is lower than the activity in the basal portion of the petal (Table 1). It also has been reported that the basal portion of the petal had a much higher ACC content than the upper portion (13). Therefore, our finding that EFE activity in the basal portion of petals is higher than the level of activity in the upper portion of petal is a probable conclusion.

The determination of the role of inorganic ions in the activity of EFE also was studied. These results showed that \( Co^{2+} \) inhibited EFE activity (Table 2), and this is similar to the results obtained for mungbean hypocotyls (23) and rice leaves (8). It has been reported that \( Ca^{2+} \) retards senescence and senescence-dependent \( C_2H_4 \) production (9). It was suggested that the effect of \( Ca^{2+} \) on the inhibition of \( C_2H_4 \) biosynthesis was "indirect" (21), and that \( Ca^{2+} \) preserved membrane structure from deterioration, and that the onset of \( C_2H_4 \) production was delayed. In this research, evidence was provided that \( Ca^{2+} \) inhibited EFE activity, which related to the conversion of ACC to ethylene (Table
These results show that Ca$^{2+}$ "directly" inhibited C$_2$H$_4$ production in carnation petals during the climacteric, and they are similar to the results for K$^+$ (11). Other inorganic ions tested in this research, such as Fe$^{2+}$, Mg$^{2+}$, and Mn$^{2+}$, also play an inhibitive role in EFE activity in carnation petals during the climacteric, but their effects are smaller.

(Amiooxy)acetic acid, STS, and MA showed different effects on EFE activity (Fig. 1). (Aminoxy)acetic acid inhibited EFE activity, and its response was similar to the response reported previously (13). (Aminoxy)acetic acid decreased ACC production (see SECTION I). Consequently, EFE activity was low due to the constitutive character of EFE. Silver thiosulfate showed the same pattern as AOA. Previously, it has been shown that silver ion did not affect the conversion of ACC to C$_2$H$_4$ (2), and it was suggested that ripening, climacteric tissue had a positive feedback system. Additional research is needed to distinguish between the possible modes of action of silver. In this research, STS caused a low EFE activity (Fig. 1), and previous experiments showed that STS inhibited ACC production (see SECTION I). Therefore, this research has solved partly the unknown, complicated, mechanisms by which silver affects C$_2$H$_4$ biosynthesis. Also, it was shown that MA increased EFE activity during the climacteric (Fig. 1), because, when flowers that were treated with MA died, the EFE activity remained elevated. This suggested that MA increased the concentration of ACC, which then enhanced the EFE activity, which then produce an elevated concentration of C$_2$H$_4$. 
LITERATURE CITED


GENERAL SUMMARY AND CONCLUSIONS

Senescence in carnation flowers is typified by an increase in the rates of production of \( C_2H_4 \) and \( CO_2 \), and the influence of AOA, STS, and MA on \( C_2H_4 \) biosynthesis and \( CO_2 \) production was studied. (Aminooxy)acetic acid and STS inhibited both \( C_2H_4 \) biosynthesis and \( CO_2 \) production, whereas MA caused an opposite response. In addition, AOA and STS suppressed the increased production of \( C_2H_4 \) that was induced by MA.

The basal portion of carnation petals at the climacteric stage were incubated in 400 \( \mu \)M SAM-potassium phosphate buffer to determine the degree of inhibition of ACC synthase activity by AOA or STS. Both inhibitors decreased the activity of ACC synthase, and they also overcame the increase in ACC synthase activity that was induced by MA. In addition, carnations pulsed with AOA or STS showed decreased amounts of EFE activity when compared with the control, but MA increased the EFE activity.

These results suggest that \( C_2H_4 \) biosynthesis can be manipulated through the inhibitory effect of AOA or STS on both ACC synthase activity and EFE activity. The preservation of carbohydrates by pulse treatments with MA to block the TCA cycle causes side effects that increase \( C_2H_4 \) biosynthesis. Malonate combined with the \( C_2H_4 \) biosynthesis inhibitors AOA or STS allowed a decreased rate of activity of ACC synthase and EFE, along with a decreased rate of utilization of carbohydrates.
LITERATURE CITED FOR THE GENERAL INTRODUCTION 
AND THE GENERAL LITERATURE REVIEW


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stimulation of ethylene release from leaves of *Gomphrena globosa* L. Plant Physiol. 71:588-593.


Physiol. 16:777-787.


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