

Ethylene Effects

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Introduction: Ethylene (C₂H₄) is a simple naturally occurring organic molecule that is a colorless gas at biological temperatures.

Biological Attributes of Ethylene:

- Colorless gas at biological temperatures.
- Naturally occurring organic compound.
- Readily diffuses from tissue.
- Produced from methionine via ACC by a highly regulated metabolic pathway.
- Key enzymes are ACC synthase and ACC oxidase.
- C₂H₄ synthesis is inhibited by C₂H₄ in vegetative and immature reproductive tissue.
- C₂H₄ synthesis is promoted (autocatalytic) by C₂H₄ in mature reproductive climacteric tissue.
- Effective at ppm and ppb concentrations (1 ppm = 6.5 x 10⁻⁹ M at 25 °C).
- Requires O₂ to be synthesized, and O₂ and low levels of CO₂ to be active.

Many biotic and abiotic sources contribute to the presence of C₂H₄ in the postharvest environment. Ripening and diseased plant tissues are a significant source of C₂H₄, as are industrial sources, the most prominent ones being internal combustion engines and fires.

Ethylene is biologically active at very low concentration measured in the ppm and ppb range. Most plants synthesize small amounts of C₂H₄ that appear to coordinate growth and development. Because it is a gas, C₂H₄ readily diffuses from sites of production, and continuous synthesis is needed to maintain biologically active levels in the tissues. Barriers to diffusive loss not only include the commodity's epidermis, but also postharvest coatings and packaging. Under biotic or abiotic stress, or during climacteric ripening, C₂H₄ production can increase dramatically, and emanations from stimulated tissue can accumulate in packages or storerooms and produce unwanted effects in adjacent tissue. Other molecules with specific configurations can mimic C₂H₄, but are less effective. For example, C₂H₄ analogs propylene (C₃H₆) and acetylene (C₂H₂) require 100- and 2,700-fold, respectively, the concentration of C₂H₄ to elicit the same effect.

Plants produce C₂H₄ through an actively regulated biosynthetic pathway in which the amino acid methionine is converted to ACC (1-aminocyclopropane-1-carboxylic acid) and then to C₂H₄ through a series of biochemical reactions. O₂ is required for the synthesis of C₂H₄ and both O₂ and CO₂ are required for its biological activity. Each reaction in the synthesis and action of C₂H₄ involves a biological catalyst, i.e., an enzyme, that focuses the reaction into producing the next specific chemical for that pathway. Enzyme activity is regulated either through its synthesis and/or destruction, or by interactions with substrates and products. These interactions can create a positive or a negative feedback of C₂H₄ on its synthesis (Fig. 1).

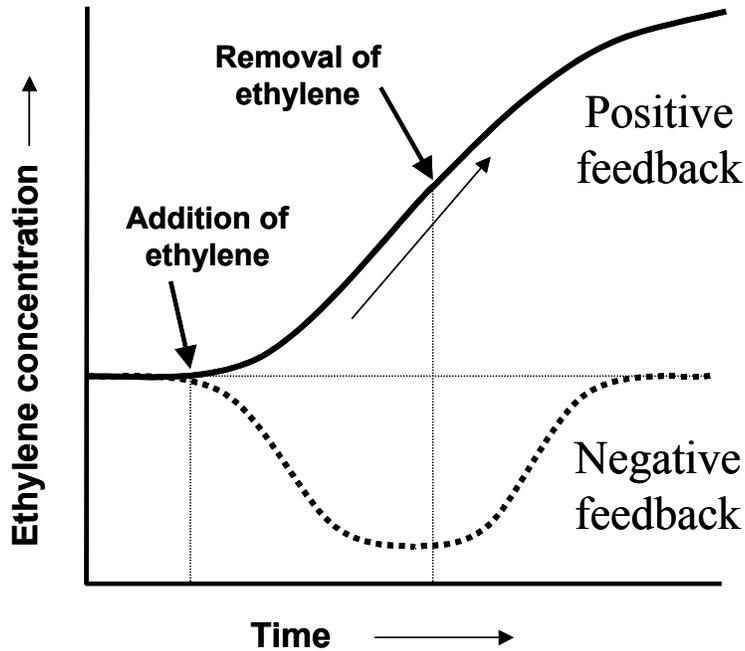


Figure 1. Effect of adding and removing ethylene from the atmosphere surrounding tissues that respond with a positive (ethylene promotes its own synthesis) or negative (ethylene inhibits its own synthesis) feedback (modified from Saltveit, 1999).

In vegetative tissue and in non-climacteric and immature climacteric fruit tissue C_2H_4 suppresses its own synthesis and in ripening climacteric fruit C_2H_4 enhances its own synthesis. This positive feedback of C_2H_4 on C_2H_4 synthesis is called autocatalytic C_2H_4 production.

Plants respond to C_2H_4 in a number of ways:

Ethylene stimulates:

- Synthesis of C_2H_4 in ripening climacteric fruit
- Ripening of climacteric fruit and some non-climacteric fruit.
- Anthocyanin synthesis in ripening fruit.
- Chlorophyll destruction and yellowing (eg., degreening of citrus).
- Seed germination.
- Adventitious root formation.
- Respiration & phenylpropanoid metabolism.
- Flower initiation in bromeliads, eg., pineapple.
- Abscission and senescence.

Ethylene inhibits:

- Ethylene synthesis in vegetative tissue and non-climacteric fruit.
- Flowering and flower development in most plants.
- Auxin transport.
- Shoot and root elongation, ie., growth.

Depending on a number of variables, C_2H_4 has both beneficial and deleterious effects on harvested fruits,

vegetables, and ornamentals:

Beneficial effects:

Promotes color development in fruit.
Stimulates ripening of climacteric fruit.
Promotes de-greening of citrus.
Stimulates dehiscence in nuts.
Alters sex expression (cucurbitaceae).
Promotes flowering in (eg., pineapple).
Reduces lodging of cereals.

Detrimental effects:

Accelerates senescence.
Enhances excessive softening of fruits.
Stimulates chlorophyll loss (eg., yellowing).
Stimulates sprouting of potato.
Promotes discoloration (eg., browning).
Promotes abscission of leaves and flowers.
Stimulates phenylpropanoid metabolism.

Often an C_2H_4 -induced change in one commodity is viewed as beneficial, while the same change in another commodity is viewed as detrimental. For example, C_2H_4 is used to promote: ripening of bananas, melons, and tomatoes; degreening of oranges; and synthesis of pigments in apples. Yet the same changes are unwanted when C_2H_4 promotes: over-ripening of fruit; yellowing of broccoli; development of brown russet spot lesions in lettuce; and senescence of flowers. Because of these diverse and often opposite effects of C_2H_4 , controlling its action in plants is of great economic importance to producers, wholesalers, retailers and consumers of fresh fruits, vegetables, and ornamentals.

In most vegetative tissues, C_2H_4 is only produced in biologically active amounts during early stages of development, or in response to a biotic or abiotic stress. Mutant plants that do not respond to C_2H_4 often grow normally, with only a few insignificant alterations in development. Most of the effects of C_2H_4 on vegetative tissue are therefore the result of the tissue's response to a stress or to the intentional or unintentional exposure of tissue to active levels of C_2H_4 .

In contrast to vegetative tissue, biologically produced C_2H_4 plays a crucial role in the development of reproductive tissues and in the ripening of certain climacteric fruit. The rates of C_2H_4 production and its internal concentration often vary by orders of magnitude during early stages of development and during the initiation and development of reproductive structures. Increased rates of C_2H_4 production are especially pronounced during the ripening of climacteric fruit such as apples, avocados, bananas, melons, pears, and tomatoes. In these fruit, the autocatalytic production of C_2H_4 heralds the onset of ripening and is required for many of the reactions associated with ripening to continue. The ethylene production rates of many fruits and vegetables is summarized in the "General Introduction" section of this handbook.

Once internal C_2H_4 exceeds a level characteristic for the species, tissue and developmental stage, the further production of C_2H_4 is stimulated by presence of previously produced C_2H_4 . In this way, autocatalytic positive feedback can increase rates of C_2H_4 production and internal concentration of C_2H_4 by a 1000-fold during ripening. External application of C_2H_4 can promote the ripening of climacteric fruit, eg., avocado, banana, honeydew, and tomato, and beneficial quality changes in non-climacteric fruit, eg., degreening of lemon and orange. Once autocatalytic C_2H_4 production has started in climacteric fruit, lowering its external concentration has an insignificant effect on its internal levels, rates of production, or action.

Ethylene is an important plant growth regulator that has pronounced effects on many aspects of plant growth and development. Regulating its effectiveness is commercially important for many crops. Controlling its effectiveness can mean either increasing its beneficial effects or decreasing its detrimental effects. There are a number of ways to accomplish either objective.

Reducing Effectiveness of Ethylene:

Use ethylene tolerant cultivars.
Keep atmosphere free of C_2H_4 .
Maintain at coldest possible temperature.
Store under CA or MA, or in MAP.
Minimize time between exposure and use.

Increasing Effectiveness of Ethylene:

Use C_2H_4 sensitive cultivars.
Keep an active level of C_2H_4 in the air.
Maintain at optimum temperature.
Store under adequate levels of O_2 and CO_2 .
Allow sufficient time for plant response.

Ethylene Interactions in Plants: There are some significant interactions between the plant and its environment that are important in understanding how to control biological activity of C_2H_4 in plants (Fig. 2).

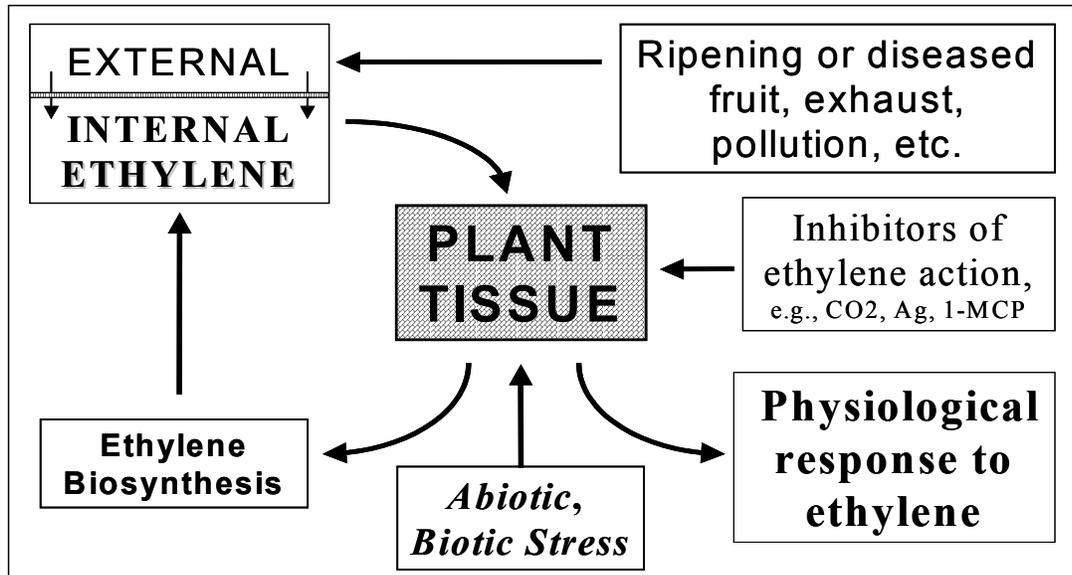


Fig. 2. Interactions among the plant and ethylene in its environment (Saltveit, 1999).

Ethylene in the atmosphere can have a direct effect on plant tissue by raising the internal concentration to an active level. Sources of atmospheric C_2H_4 include exhaust from trucks and forklifts, pollution from industrial activity and the burning of fuels, and biosynthesis by diseased plants or ripening fruit. In some cases C_2H_4 - whether applied as a gas or as an C_2H_4 releasing compound such as ethephon - is intentionally added to the plant's environment to stimulate desirable changes. The changes can include promotion of flowering in pineapple, ripening of avocado, banana, melon, and tomato fruit, degreening of citrus, altering sex expression in cucurbits, defoliation, promotion of latex secretion, and many others.

The activity of C_2H_4 inside plants is not only regulated by the absolute level of C_2H_4 , but also by the responsiveness of tissues and the presence of CO_2 , the natural antagonist of C_2H_4 action. The response of plants to C_2H_4 , therefore, depends on a number of factors, only one of which is the rate of C_2H_4 production by the plant. Tissue sensitivity is dependent on species, cultivar, cultural practices, and stage of development.

Prior and current stresses have a significant effect on modulating the effect of C_2H_4 . For example, wounding stimulates both C_2H_4 production and a host of plant defense responses such as increased phenylpropanoid metabolism. Some of these responses involve C_2H_4 , and others do not. Increased phenolic metabolism greatly increases the susceptibility of some crops like lettuce to develop browning, eg., russet spotting, when exposed to C_2H_4 and/or mechanical injury.

The effect of tissue susceptibility is most clearly seen in fruit tissue. Immature climacteric fruit respond to C_2H_4 with increased respiration and reduced C_2H_4 production. Once the tissue has reached a certain stage of maturity, however, C_2H_4 not only promotes increased respiration, but also increased C_2H_4 synthesis.

Controlling the effectiveness of C_2H_4 does not always involve a reduction in its activity. There are many beneficial effects of C_2H_4 that can be enhanced (see above). The techniques used to increase the effectiveness of C_2H_4 are almost the mirror image of techniques used to reduce its effectiveness.

Ethylene action can be enhanced by using cultivars that are sensitive and respond uniformly to C_2H_4

rather than cultivars that are C₂H₄ insensitive. An effective concentration of C₂H₄ should be maintained around the tissue for a sufficient time to elicit the full response. However, since the response to C₂H₄ is log-linear (a log increase in C₂H₄ concentration results in a linear increase in the response), there is an extremely large range over which the concentrations are effective. The application of C₂H₄ must be at the proper stage of development and at the proper temperature for the desired effects to be induced. Ethephon, and similar C₂H₄ releasing chemicals, permit the commercial application of C₂H₄ in the field. After harvest, C₂H₄ gas, either from compressed gas cylinders or catalytically generated from alcohol can be used in enclosed storage rooms.

Controlling Ethylene Action: There are roughly three ways to control the action of C₂H₄ in plants. The first is to prevent the plant from being exposed to biologically active levels of C₂H₄. The second is to prevent the plant tissue from perceiving the C₂H₄ that is in its surrounding atmosphere or that is being produced by the tissue. The third is to prevent the plant from responding to the perceived C₂H₄ by controlling exposure to C₂H₄. It is important to:

- Keep the air around the commodity C₂H₄ free.
- Use fresh, C₂H₄-free air from outside.
- Scrub C₂H₄ from the storage atmosphere.
- Use sachets of C₂H₄ absorbers inside packages to reduce levels.
- Segregate C₂H₄ producing commodities from sensitive ones.
- Keep exposure to a minimum (duration, level).
- Inhibit C₂H₄ synthesis (AVG, ACC synthase; Low O₂, ACC oxidase)

This is not much of a problem in the field, since the levels of C₂H₄ found even in polluted air rarely reach biologically active levels. However, in greenhouses, cold-storage-rooms, and transportation vehicles C₂H₄ can frequently accumulate to biologically active levels. Ethylene found in these enclosed spaces comes from varied sources, but the two most prominent are from diseased, stressed or ripening plant tissue, and from the incomplete combustion of organic fuels.

With proper ventilation of enclosed spaces, and persistent attention to the condition of adjacent plants and the operation of heaters and gas-powered fork-lifts, C₂H₄ can be kept below biologically active levels. Sometimes, the C₂H₄ that we are concerned with comes from the plant itself. Application of inhibitors of C₂H₄ biosynthesis, eg., AVG and AOA, to the tissue before or after harvest can significantly reduce this source of C₂H₄ exposure. For example, tissue can be prevented from making either stress or autocatalytic C₂H₄ by blocking the biosynthetic pathway for C₂H₄ synthesis. If exposure cannot be prevented, or has already occurred, then the duration of exposure and the level of C₂H₄ in the atmosphere should both be kept as low as possible.

Prevent Perception of Ethylene: If significant amounts of C₂H₄ are in the immediate environment, certain methods can be used to block the perception of C₂H₄ by the plant:

- Store at coldest possible temperature.
- Use inhibitor of C₂H₄ perception.
 - CO₂
 - Silver (eg., silver thiosulfate)
 - 1-Methyl cyclopropene (1-MCP)
- Use C₂H₄-insensitive cultivars.
- Interrupt the C₂H₄-induced signal.

Since perception is a metabolic process, holding the tissue at the lowest possible temperature will effectively reduce perception. Specific chemical inhibitors can also be used that directly interfere with the

perception event.

A gaseous inhibitor like CO₂ or 1-MCP (1-methylcyclopropene) can be introduced into the atmosphere. The tissue can be dipped or fed a nonvolatile inhibitor like silver; but this treatment is limited to non-food crops. Ethylene resistant cultivars can be selected or the tissue can be genetically engineered to lack the necessary biochemical receptors for ethylene or the signal pathway necessary to transduce the signal into a physiological event.

Even after the molecular perception event has occurred, blocking the transduced signal will effectively prevent perception. However, effective methods to do this will require a far greater understanding of the signal pathway than is currently available.

Prevent Response by the Plant: The third way to control C₂H₄ is to prevent the plant from responding to the perceived C₂H₄. This can be done by interfering with the metabolic machinery that is induced by exposure to C₂H₄:

Store at coldest possible temperature.

Store under CA or MA, or in MAP.

Inhibit or reduce specific enzyme activities using chemical inhibitors (eg., AIP) or genetic engineering (eg., antisense or other gene knockout techniques).

Divert protein synthesis, eg., heat-shock.

Minimize time before use, eg., consumption.

Since all the effects of C₂H₄ on plants that we are interested in involve metabolic changes, reducing the rate of metabolism by lowering the temperature, withholding a vital reactant (eg., O₂), or by inhibiting a specific enzyme (eg., with a chemical or through genetic engineering) will prevent the response to C₂H₄.

For example, ripening promoted by C₂H₄ often entails tissue softening that significantly reduces shelf-life.

Using antisense technology to reduce the activity of enzymes involved in tissue softening has produced fruit that remain firmer longer. Ethylene also promotes phenylpropanoid metabolism in many tissues that use stress produced C₂H₄ as a signal to induce defense mechanisms. Interfering with synthesis or activity of phenylalanine-ammonia lyase (PAL; first enzyme in phenolic metabolism) with chemical inhibitors or heat treatment eliminates tissue response to C₂H₄, preventing development of postharvest disorders.

Application of Ethylene: The quality of some fruit is increased when they are harvested at a mature stage that can withstand the rigors and duration of transport and then treated with C₂H₄ to promote ripening before sale. These fruit include avocados, bananas, honeydew melons, lemons, oranges and tomatoes. An effective atmosphere of 100 to 150 μL L⁻¹ C₂H₄ in air can be produced by a number of methods. The 'shot' method introduces a relative large amount of gaseous C₂H₄ into a ripening room by metering C₂H₄ from compressed gas cylinders. Ethylene and air mixtures between 3.1% and 32% are explosive. While these concentrations are more than 200-fold higher than recommended, they have been reached when metering equipment has malfunctioned. Use of compressed gas containing around 3.1% C₂H₄ in N₂, ie., 'banana gas,' eliminates this problem. Catalytic converters are instruments that use a heated metal catalyst to convert alcohol into C₂H₄. They deliver a continuous flow of low C₂H₄ into the storage room. Ethylene can also be applied in aqueous form from decomposition of compounds such as Ethrel. While stable at acidic pH, Ethrel quickly breaks down to C₂H₄ as temperature and pH increase. Field application is approved for many food crops, but postharvest application is not approved.

Treatment with C₂H₄ stimulates many metabolic pathways, including respiration. Oxygen use is increased, as is the production of CO₂ and heat. Rooms designed to hold produce being exposed to C₂H₄ must be designed with extra air moving capacity to insure that an optimal ripening environment is maintained around the crop. Exposure to C₂H₄ must be uniform through out the room and within packages.

Heat of respiration and excessive CO₂ must be removed to maintain the proper environment. Loss of water by the crop will be increased by the rise in respiratory heat production. Maintaining a high RH can lessen water loss, but too much water vapor can decrease the strength of cardboard boxes and promote pathogen

growth. Judicious maintenance of proper ripening environments will insure production of high quality fruit. Care must be exercised in venting and opening ripening rooms to prevent release of sufficient amounts of C₂H₄ to adversely affect other commodities stored in the same warehouse.

Conclusion: Ethylene can be both beneficial and detrimental to the storage of horticultural crops. Practical uses for C₂H₄ and treatments to minimize its adverse effects have slowly accumulated over almost a century of study. The three general methods used to modulate C₂H₄ activity involve controlling exposure, altering perception, and varying the response of the tissue. An understanding of ethylene's synthetic pathway and mode of action has greatly expanded the ability of Postharvest physiologists to devise treatments and storage conditions to control C₂H₄ during the commercial storage and handling of horticultural crops. Simple methods like ventilation and temperature management can be combined with more sophisticated treatments like MAP and inhibitors of specific induced enzymes to provide conditions that optimize both storage-life and product quality.

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