UNIT 7 ETHYLENE LIBERATION AND ITS CONTROL

Structure

7.0 OBJECTIVES

After going through this unit, you should be able to:

- know the role of ethylene in fruit physiology;
- tell how ethylene is synthesized in fruit tissues;
- explain the mechanism of action of ethylene; and
- state the different methods to remove ethylene from storage rooms.

7.1 INTRODUCTION

Ethylene is the simplest chemical compound known to cause a significant physiological effect. It is also known as ripening hormone and is effective at very low concentration. Ripening is the result of complex changes; many of them probably occur independent of each other. Respiration and ethylene production are the two of the major processes occurring during ripening. Ethylene plays a role in post-harvest life of many horticultural crops, which is often deleterious, speeding senescence and reducing shelf life, but sometimes beneficial, improving the quality of the product by promoting faster, more uniform ripening.

Ethylene is a gaseous harmone with a characteristic suffocating sweetish odour. It is both an anaesthetic and asphyxiant. High vapour concentration can cause rapid loss of consciousness and perhaps death by asphyxiation. Removal to fresh air usually results in prompt recovery if the person is still breathing. When the gas is handled in liquefied form, skin and eye burns can result from contact with the liquid. Cases in which liquid ethylene contacts, a physician must see to the eye.

7.2 SOURCES OF ETHYLENE

Ethylene gas is relatively inexpensive industrial chemical, but it is often more convenient or safer to provide ethylene by means other than the gas. Regardless of the source of ethylene, the treatment conditions are important for the ripening process.

Explosion-proof Ethylene Mixtures

Using mixtures of ethylene with inert gases can eliminate the danger of explosions from oversupply of ethylene to a ripening room. The proportion of the inert gas should be such that at high concentrations of ethylene not enough oxygen remains in the ripening space to provide an explosive mixture. Ripegas, a commercial formulation of ethylene contains 6 percent C_2H_4 in CO_2 by weight.

Ethylene Generators

Ethylene generators, in which a liquid products ethylene when heated in the presence of a catalyst, are now widely used for supplying ethylene in ripening rooms. The liquid comprises of ethanol and agents that catalyze its dehydration.

Ethephon

Ethephon (2-chloroethyl phosphonic acid) is strongly acidic in water. In solutions above pH of about 5, the ethephon molecule spontaneously hydrolyses, liberating ethylene molecule. Ethephon is commercially available (Ethrel, Florel, Cepa) and is used for preharvest treatment on a variety of crops for controlling developmental processes, or inducing ripening. For enhancing postharvest ripening, it has the disadvantage as it has to be applied to the fruit in liquid form as a spray or as a dip. This extra step in handling may cause microbial infection. But compared to ethylene treatment, it has the advantage, as no special facilities are required to ripen fruit with ethephon, if the ambient temperatures are within the range required to ripen the commodity.

Calcium Carbide

Heating calcium oxide with charcoal, under reducing conditions, readily produces calcium carbide. When hydrolyzed, calcium carbide produces acetylene, containing trace amounts of ethylene that are sufficient to trigger fruit ripening. Simple generators can be used in partially vented spaces to ripen or degreen fruits. In some instances CaC_2 wrapped in newspaper can be used as the generator. Water vapour from the fruit releases sufficient ethylene from CaC_2 to cause ripening.

7.3 USES OF ETHYLENE

Post-harvest Uses

Post harvest uses of ethylene include its uses as ethephon, an ethylene-releasing chemical, as a growth regulator. Fruit ripening is by far the largest application of ethylene gas in post-harvest technology. Other uses of ethylene in crops include-

Flower and Sprout Induction

The stimulation of flowering of pineapples by ethylene treatment is important for pineapple industry. Japanese bulb growers discovered that iris bulbs from fields that had been burned at the end of the season to control leaf diseases flowered earlier and more prolifically than controls. It was found that smoke did the same for bulbs that had been harvested, and smoking of bulbs is still practiced in Japan. The active ingredient in the smoke is ethylene, and it has now been shown that ethylene treatment of the propagules of a number of flowering crops stimulates flowering. Narcissus bulbs normally do not flower without ethylene treatment, and treatment with ethylene for a few hours just after lifting induces almost 100 per cent flowering.

Ethylene is also used as a short treatment to enhance the sprouting of seed potatoes. Ethylene treatment breaks the dormancy of the buds, but prolonged treatment inhibits their extension growth.

Shuck Loosening and Fruit Release

Ethylene is also used to induce the abscission of leaves, flowers, and fruit from many plants. Pre-harvest application of ethylene to walnut and pecan trees induces shuck loosening and improves harvest efficiency. Similarly, these chemicals are used to loosen the abscission zone on the stalk of fruits that are mechanically harvested (e.g. sour cherries), for improving harvest yields.

Chlorophyll Destruction

In many plant tissues, ethylene treatment result in rapid loss of cholorphyll, the green colour in leaves and unripe fruit. This property is used for degreening of citrus, where the orange colour is revealed as the chlorophyll is destroyed during ethylene treatment. Ethylene also helps in bleaching of celery and accelerated curing of tobacco.

Fruit Ripening

The concentrations of ethylene (in the range of 0.1 to 1 ppm) are required for the ripening in most of climacteric fruits. The time of exposure to initiate ripening depends on type of fruit, but usually exposures of 12 hours or more are usually sufficient. Full ripening sometimes takes several days after the ethylene treatment. The effectiveness of ethylene in achieving faster and more uniform ripening depends on the type of fruit being treated, its maturity, the temperature and relative humidity of the ripening room, ethylene concentration and duration of exposure to ethylene.

Control of temperature is critical to good ripening with ethylene. Optimum ripening temperatures are 18° to 25°C. At lower temperatures ripening is slowed; at temperatures over 25°C bacterial growth and rotting may be accelerated, and above 30°C ripening may be inhibited. Fruit that have been cool-stored must be warmed to 20°C to ensure that ripening proceeds rapidly. As ripening starts, the burst of respiration can generates heat, therefore it is essential to ensure that this heat does not increase pulp temperatures to the point where ripening is inhibited

7.4 ETHYLENE AS RIPENING INDUCER

Ethylene is regarded as an agent that can induce ripening. It has been established that all the fruits produce minute quantity of ethylene during development, but climacteric fruits produce much larger amounts of ethylene as compared to non-climacteric fruits during ripening. Further, the internal ethylene concentration of climacteric fruits varies widely, but that of non-climacteric fruits changes little during development and ripening. A concentration of 0.1-1.0 μl/l ethylene for one day is sufficient to hasten ripening of climacteric fruits, but the magnitude of climacteric is relatively independent of the concentration of ethylene. In contrast, applied ethylene merely increases the respiration of non-climacteric fruits, and the magnitude of increase depends upon the concentration of ethylene. Further, the rise in respiration in response to ethylene may occur more than once in non-climacteric fruits in contrast to single respiration increase in climacteric fruits.

The significance of ethylene for fruit ripening was established during the early part of this century when heaters burning kerosene were used to degreen or colour yellow lemons. Ethylene was regarded as an external agent that could promote the ripening of fruit, and other plant tissues, produced extremely small quantities of ethylene.

7.5 BIOGENESIS OF ETHYLENE

It was shown that application of an amino acid methionine greatly stimulated ethylene production in apples, and this compound was then considered to be the starting point for ethylene biosynthesis. Then s-adenosyl-methionine (SAM) was identified as another important compound in pathway, which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is now regarded as the immediate precursor for ethylene and ACC synthase controls the rate at which pathway operates. ACC synthase is activated by an enzyme cofactor, known as pyridoxal phosphate. Inhibitors of enzyme that require pyridoxal phosphate, such as aminoethoxy vinyl Glycine (AVG) and amino-oxyacetic acid (AOA) can be used to inhibit ethylene production.

It is generally agreed that the amino acid methionine is the precursor of ethylene in plants, with the conversion having an absolute requirement for molecular oxygen. Small amounts of ethylene probably can also be formed in plant tissues from the oxidation of lipids involving a free radical mechanism. As yet, the ethylene-producing system has not been isolated from fruit tissues for in vitro studies and the site or the organelle where ethylene is synthesized is still not very clear. The unravelling of the bio-chemical pathway of ethylene biosynthesis in plants has been one of the most interesting bio-chemical stories of recent years. Application of the amino acid, methionine greatly stimulated ethylene production in apples, and this compound was then considered to be the starting point for ethylene biosynthesis. Researchers at Davis, USA identified SAM (S-adenosyl-methionine) as another key compound in the pathway and then, almost simultaneously, Amrhein in West Germany and Adams and yang at Davis, USA discovered that SAM was converted to an unusual cyclic amino acid, ACC (1 aminocyclopropane-1-Carboxylic acid) which is now thought to be the immediate precursor for ethylene.

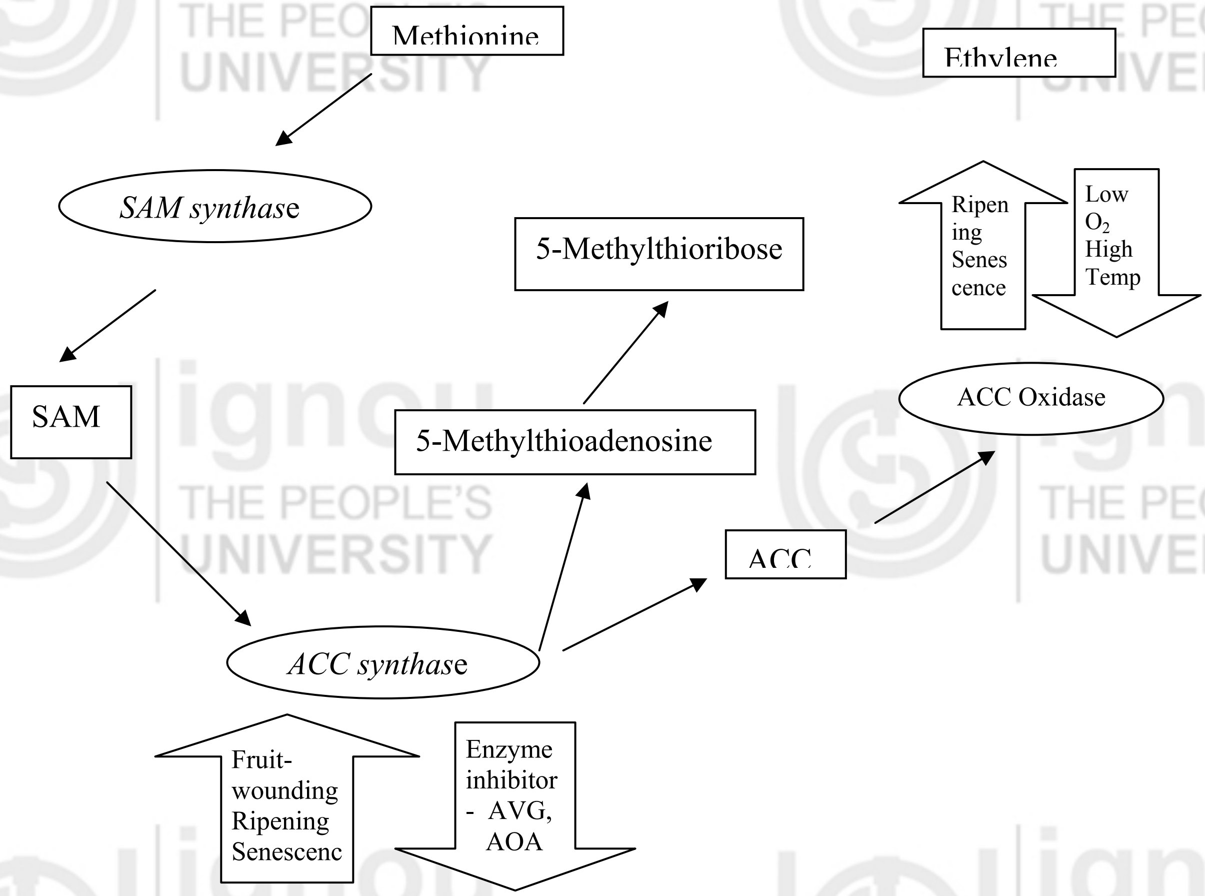

Figure 7.1: Biosynthetic pathway of ethylene

The enzyme which controls the rate at which the pathway operates, ACC synthase, is activated by a common enzyme co-factor, pyridoxoal phosphate such as AVG (amino-ethoxyvinyl gylcine) and AOA (aminooxyacetic acid) which inhibit ethylene production. Cobalt ion and low $O_{2,}$ which inhibit the final step in the pathway, the Acc oxidase can also reduce ethylene production.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.

b) Compare your answers with those given at the end of the unit.

1. Name the precursor of ethylene biosynthesis.

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2. How ethylene effect differs in climacteric and non-climacteric fruits?

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3. What is the role of ethylene in post-harvest life of horticultural produce?

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4. Name the factors regulating action of ACC synthase enzyme?

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7.6 MECHANISM OF ETHYLENE ACTION

Although applied ethylene will initiate the ripening of climacteric fruits and will cause some ripening like changes in non-climacteric fruits similar to those in senescing tissue, it has not been possible to prove that ethylene acts in vivo as a true plant hormone, that is, acts in fruit tissues at trace levels, in promoting and controlling fruit ripening. A considerable amount of circumstantial evidence is available which suggests that ethylene, probably in concert with the other plant hormones (auxins, gibberellins, kinins and abscisic acid) exercises hormonal type control over the fruit ripening process. The relationship of the other plant hormones to ripening is as yet not clearly defined.

In some fruits, such as banana, avocado and melons, there is a small rise in endogenous (internally produced) ethylene concentration preceding the commencement of the respiratory climacteric. For example, the internal ethylene concentration of Honey Dew melon rises from the pre-climacteric level of 0.04 microlitre/litre to 3.0 microlitres/litre at which concentration the fruit commences to ripen. Other fruits, such as mango and apple, do not show this rise in internal ethylene concentration before ripening. Once ripening has commenced, the large amount of ethylene synthesized by climacteric fruits is thought necessary to promote all of the aspects of ripening.

It is well known that many fruits as they develop and mature, become more sensitive to ethylene. For sometime after anthesis (flowering) young fruit can have high rates of ethylene production. Early in the life of fruit can have high rates of ethylene production. Early in the life of fruit the concentration of applied ethylene required to initiate ripening is high and the length of time to ripen is prolonged, but decreases as the fruit matures. The tomato is an extreme case of tolerance to ethylene. Banana and melons, in contrast, can be readily ripened with ethylene even when immature. Nothing is known about the factors that control the sensitivity of the tissue to ethylene.

There is also no clear evidence to suggest the mechanism by which ethylene initiates and controls fruit ripening and little is known about the site of action of ethylene and the mechanism by which ethylene either promotes ripening or increases respiration in non-climacteric fruits or in other tissues such as the potato tuber. Ripening has long been considered to be a process of senescence and to be due to a breaking down of the cellular integrity of the tissue; some ultrastructural and bio-chemical evidence supports this view. There is now considerable evidence for ripening being a phase in the differentiation of plant tissue, with altered nucleic acid and protein synthesis occurring at the commencement of the respiratory climacteric. Both views fit in with the known degradative and synthetic capacities of fruit during ripening, but this type of study is unlikely to determine how ethylene initiates ripening.

Although applied ethylene initiates ripening in fruits, it is not proved how ethylene acts in vivo as a true plant hormone. Some evidence suggests that probably in combination with other hormones it exercises hormonal type control over the fruit ripening process. Some fruits show a rise in internal ethylene concentration before ripening, whereas others do not. The factors that control the sensitivity of the tissues to ethylene are yet to be known. But once ripening has commenced the large amount of ethylene produced by climacteric fruits is thought to be necessary to promote all aspects of ripening. It has been proposed that two systems exist for the regulation of ethylene biosynthesis. System I is initiated or perhaps controlled by an unknown factor, probably involved in the regulation of senescence. System I then triggers system II, which is responsible for production of large amounts of ethylene in climacteric fruits. Non-climacteric fruits lack an active system II.

Recently a model for the way in which ethylene induces a host of effects was introduced. According to this ethylene binds to a protein, called a binding site, and stimulates the release of a so called second message instructing the DNA to form mRNA molecule specific for the effects of ethylene. These molecules are translated into protein by polyribosomes, and the proteins so formed are the enzymes that cause the actual ethylene response.

7.7 ETHYLENE TREATMENT SYSTEMS

For ethylene treatment ripening rooms or specially built chambers with automatic control of temperature, humidity, and ventilation are used. It is not essential that the rooms be hermetically sealed, but they should be as tight as practicable to prevent leakage. Because of the rapid increase in respiratory heat production following ethylene treatment, ripening rooms should be equipped with refrigeration systems adequate to hold the temperature.

Several methods are used to provide the proper ethylene concentration in the ripening room.

The "shot" System

In the shot system, measured quantities of ethylene are introduced into the room at regular intervals. The shots may be applied by weight, or by flow. The required ethylene application is made by adjusting the regulator to give an appropriate flow rate and, the time of delivery of the gas. Any piping leading into the ripening room should be grounded to prevent possible electrostatic ignition of the explosive concentrations of ethylene that are always present near the orifice when ethylene is being introduced. Sometimes the ethylene is administered by weight also.

Because the room containing the product being ripened is sealed in the shot system, CO_2 accumulates in the room and may inhibit the ripening process. Therefore the room should be well ventilated before each application, particularly if it is well sealed, by opening the doors for about half an hour. In large ripening rooms, ventilating fans should be provided. Where the ripening rooms are near rooms used for storage or handling of ethylene-sensitive commodities, the room should be ventilated to the exterior to prevent contamination.

The Trickle or Flow-through System

The ethylene is introduced into room continuously, rather than intermittently. As the flow of ethylene is very small, it has to be regulated carefully. This is usually done by reducing the pressure using a two-stage regulator and passing the gas into the room through a metering valve and flowmeter.

To prevent a build up in either CO_2 or C_2H_4 fresh air is drawn into the ripening room at a sufficient rate to ensure a change of air every 6 hours. The air is vented through a small exhaust port to the rear of the room.

A convenient way of monitoring gas being supplied in a trickle system is a simple "sight glass" in which, the ethylene bubbles through a water trap on its way to the ripening room. As in the shot system, correct temperature maintenance and adequate air circulation are essential for good ripening.

7.8 CONTROL

Ethylene is produced whenever organic materials are stressed, oxidized or combusted. There are many sources of ethylene pollution during post-harvest handling of perishables, but the most important are internal combustion engines, ripening rooms and ripening fruits. Other sources are aircraft exhaust, decomposing produce and sometimes fungi growing on it, cigarette smoke, rubber materials exposed to UV light and virus infected plants. Sometimes ethylene contamination of flowers takes place from propane-powered floor polishers. The undesirable effects of ethylene that must be controlled are as follows:

Accelerated Senescence

In green tissues, ethylene commonly stimulates senescence, as indicated by loss of chlorophyll, loss of protein, and susceptibility to desiccation and decay. Ethylene pollution can result in yellowing of leafy vegetables (spinach, fresh herbs, parsley, broccoli and other green vegetables). The senescence of some flowers is also stimulated by ethylene at very low concentrations. These effects occur in flowers where increased ethylene production is part of natural senescence (sweet peas) and in others where it is not a part of natural senescence (roses).

Accelerated Ripening

Although acceleration of ripening is a beneficial use of ethylene, it can also be undesirable, as the presence of ethylene in the cucumber causes premature yellowing. Most climacteric fruits senescence faster if ethylene is present in the atmosphere, therefore ethylene in the storeroom may reduce storage life. The firmness of kiwi fruit in storage was dramatically reduced at 20 ppb ethylene concentration in the cool store.

Induction of Leaf Disorders

In many plants, exposure to ethylene results in darkening or death of portions of their leaves. This response is commonly seen in foliage plants and is of major economic consequence in lettuce, where ethylene causes the disorder known as russet spotting. In lettuce, the browning results from collapse and death of areas of cells following increased synthesis of phenolic compounds in response to ethylene.

Sprouting

The ethylene-stimulated sprouting is useful in propagules, but undesirable in commodities intended for consumption. Sprouting of potatoes increases water loss, leading to early shriveling and makes them unmarketable.

Abscission of Leaves, Flowers and Fruits

Ethylene induced abscission is most often a problem in ornamental plants, where low concentrations can cause complete loss of flowers or leaves. The Christmas cactus is sold when the first flowers are open, but exposure to ethylene may cause all the flowers fall in the bottom of the box during transportation.

Toughening of Asparagus

Ethylene stimulates the lignification of xylem and fiber elements in the growing asparagus spear, leading to undesirable touchiness and reducing the portion of the spear that is edible.

Induction of Physiological Disorders

Ethylene sometimes induces or hastens the appearance of physiological disorders of stored commodities. Rapid ripening of apples with low calcium contents induces high levels of the bitter pit storage disorder. Similarly, high ethylene levels in the storage chamber reduce the effectiveness of controlled atmospheres in maintaining quality of apples. While useful in inducing flowering in bulbs and other propagules, ethylene damages these propagules after the flowers have started to develop.

A number of techniques have been developed to protect sensitive commodities from the effects of ethylene. Selection of the appropriate method obviously depends on the commodity and the handling techniques used in its marketing.

Removing ethylene from the atmosphere around the commodity is the preferable method of preventing deterioration of ethylene sensitive products. Most of the times, removing the sources of ethylene can do it. Combustion gases exhaust should be avoided from handling and storage rooms. A good sanitation by removing overripe and rotting produce will help in reducing ethylene levels. Ventilation of storage rooms can also help in reducing ethylene concentration.

Eliminating Sources of Ethylene

High levels of ethylene in storage and handling areas can be avoided by removing sources of ethylene. In particular, commodities sensitive to ethylene, should be handled using electric forklifts. Internal combustion engine vehicles should be isolated from handling and storage areas and engines should never be left idling in an enclosed space during loading and unloading operations. Where these techniques are not feasible, it is possible to fit combustion engine exhausts with catalytic converters, which will reduce C_2H_4, emissions by 90 percent. Rigorous attention to sanitation will remove overripe and rotting produce which can be a source of ethylene.

Ventilation

Where the air outside storage and handling areas is not polluted, simple ventilation of these areas can reduce ethylene concentrations. An exchange rate of one air change per hour can readily be provided by installing an intake fan and a passive exhaust.

Chemical Removal

Ethylene can be removed by a number of chemical processes; the most important are described below:

- Using potassium permanganate
- Using ultraviolet lamp (by ozone)
- Activated or brominated charcoal
- Catalytic oxidizers

Potassium permanganate: Commercial materials, such as Purafil, utilize the ability of potassium permanganate ($KMnO_4$) to oxidize ethylene to CO_2 and

H_2O. The requirements for such materials are a high surface area coated with the permanganate and ready permeability to gases. Many porous materials have been used to manufacture permanganate absorbers, including vermiculite, pumice and brick. The type of material may depend on the purpose for which the absorber is required. For removing ethylene from room air, the absorber should be spread out in shallow trays, or air should be drawn through the absorber system.

Ultraviolet lamps: Commercial equipment for ultraviolet lamps draws the air from the storage room through the lamps. Ultraviolet lamps produce ozone, which is an active ethylene-removing agent. As the ozone produced by the lamps is very toxic to fresh produce, it must be removed from the produce.

Activated or brominated charcoal: Charcoal air purifiers, especially if brominated, can absorb ethylene from air. These systems are largely confined to use in the laboratory, as potassium permanganate absorbers are cheaper and more widely available.

Catalytic oxidizers: If ethylene and oxygen are combined at high temperature in the presence of a catalyst (e.g. platinized asbestos) the ethylene will be oxidized. Ethylene scrubbers overcome the difficulty of heating the incoming air by using the bed of ceramic as a heat sink and reversible flow of gas through the bed. These scrubbers are very efficient in reducing the ethylene concentration in the air to 1/100th of original concentration.

Bacterial systems: Approximately 30,000 metric tons of ethylene is liberated into the atmosphere each day from internal combustion engines, but the concentration of ethylene in air remains very low. The bacteria that use ethylene as a bio-chemical substrate are able to remove ethylene from the atmosphere.

Hypobaric Storage

Hypobaric storage helps in reducing the levels of ethylene as the relative concentration of all the gases in storage room goes down. Many of the benefits of hypobaric storage are due to reduction in the partial pressure of oxygen, which accompanies reduction in the atmospheric pressure.

Inhibition of Effects of Ethylene

Sometimes it is not possible to ensure low concentration of ethylene. In such cases attempts should be made to inhibit the effect of ethylene. Different techniques used are:

- Controlled atmosphere storage
- Use of antiethylene compounds (NBD, STS)
- Inhibition of ethylene biosynthesis

Controlled atmospheres: Low concentrations of O_2 and high concentrations of CO_2 in the storage atmosphere reduce the rates of respiration, ethylene production and other metabolic processes. CO_2-enriched atmospheres also may inhibit the action of ethylene on tissues sensitive to it. Accumulation of CO_2 produced by the fruits may help in preventing the action of ethylene.

Use of anti-ethylene compounds: Compounds that inhibit the action of ethylene include silver ion and 2, 5 norborneadiene (NBD). Complex between silver and thiosulfate (STS) is being used for ornamental commodities. It has a very low stability constant and therefore it moves readily from the vase solution to the head of cut flowers. Flowers pulsed with this material last two to three times as long as control flowers. Potted flowering plants do not lose their flowers during transportation if they are first sprayed with STS.

Inhibition of Ethylene Biosynthesis

Ethylene may reduce quality even when it is not present as a pollutant if the tissue itself produces ethylene. Inhibitors of ethylene biosynthesis, such as AVG and AOA have been used in laboratory experiments to extend flower vase life and fruit storage life but not on commercial scale.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Write the mechanism of ethylene action?

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2. How hypobaric storage helps in controlling ethylene?

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3. Name the chemical processes that can remove ethylene from storage environment.

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4. How one can minimize the deleterious effects of ethylene?

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7.9 LET US SUM UP

Ethylene, a ripening hormone plays an important role in post-harvest life of many commodities. Sometimes its effect are beneficial where it is used for uniform ripening or degreening, but mostly it is considered deleterious as it hastens senescence and reduces post-harvest life. Simple practices of good sanitation and ventilation of storage room can help in reducing ethylene concentration. Use of chemicals or advanced storage techniques can also reduce the deleterious effects of ethylene.

7.10 KEY WORDS

Physiology : Study of the functions and vital processes of living organisms or their parts.

Ethylene : A colourless flammable gas which stimulates ripening.

Ripening : The advance stage in the development at which fruit and vegetable are suitable for consumption/ utilization.

Climacteric : Fruits/vegetables showing a sudden upsurge in respiration couples with ethylene evolution.

Non-climacteric : Fruits/vegetables who do not show a sudden upsurge in respiration couples with ethylene evolution.

Biosynthesis : The formation of chemical compounds by the enzyme action of living tissues.

Hormone : A substance which is synthesized at one tissue and has specific effect on another tissue.

Ventilation : To circulate fresh air or drive out foul air.

Hypobaric : Less than normal air pressure.

Degreening : Removal of chlorophyII pigment form the tissue.

7.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answers should include following points:

   - Amino acid
   - Methionine

2. Your answers should include following points:

   - Ethylene concentration
   - Increase in respiration

3. Your answers should include following points:

   - Senescence
   - Decolourization

4. Your answers should include following points:

   - Wounding
   - Ripening
   - Enzyme inhibitors

Check Your Progress Exercise 2

1. Your answers should include following points:

   - Binding site
   - Polyribosomes

2. Your answers should include following points:

   - Relative concentration
   - Oxygen level

3. Your answers should include following points:

   - Potassium permanganate
   - UV rays
   - Charcoal

4. Your answers should include following points:

   - Controlled atmosphere
   - Antiethylene compounds

7.12 SOME USEFUL BOOKS

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