USE OF ETHEPHON IN ABSCISSION

by

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INTRODUCTION

Plant growth substances have an important role in plant growth and development. Though endogenous growth substances (naturally occurring) control plant growth and development, alteration in growth and development can be achieved by synthetic growth substances (69).

In apples, *Malus domestica*, Borkh, growth regulators such as gibberellins $A_4$ and $A_7$, cytokinin 6-benzyladenin (BA), succinic acid 2,2-dimethylhydrazide (daminozide), $\alpha$-naphthalene acetic acid (NAA), 2,4,5-trichlorophenoxycetic acid (2,4,5-T) have drawn the attention of many scientists in recent years.

Gibberellin induces growth, stimulates flowering in many species such as carrot, endive, cabbage, and turnip (69) increases fruit size (in grape and fig) (69) and increases the length-to-diameter ratio (L/D) in apples (65,67).

Cytokinin (BA) stimulates cell division, causes elongation of segments of etiolated stems, enlargement of leaves, delays senescence in plant tissues, increases fruit weight, L/D ratio (65,67,69,71).

Daminozide increases red color and flesh firmness, reduces fruit size, prevents fruit drop, decreases water core, induces flower bud formation, suppresses shoot growth, and delays ripening of fruit (23,30,31,41,42,45,66).

2,4,5-T and NAA cause ripening, prevent abscission, and improve fruit coloration. When applied with 2-chloroethylphosphonic acid (ethephon), NAA causes softening of fruit
after controlled atmosphere (CA) storage (14,29,32,34,35,43).

Ethylene has the capacity of promoting abscission. Over 100 years ago, German investigators correlated the phenomenon of abscission to the nearness of leaking illuminating gas pipes and Neliubov (54) revealed the nature of such gas as ethylene (3,10,11). Besides abscission inducing characteristics, short pre-treatment of ethylene induced sprouting in resting potato tubers and promoted seed germination in some plant species. Longer periods of treatments after germination or sprouting suppress the growth of leaves and shoots, prevents stem elongation, and causes stem swelling, abnormal curling of petiole and leaf blade and induces red color and ripening of fruit (34,35,36,55,58,67,69).

Since ethylene is a volatile gas and disperses rapidly, it is impossible to use under field conditions (69). Instead ethephon, as an ethylene producing material, has been used for its effect on fruit maturity, improvement of fruit color, abscission, etc.

The purpose of this study was to investigate the effect of ethephon on abscission, especially on apple fruit and leaf petioles, and to find an appropriate rate of ethephon application for leaf abscission on apple trees, 'Red Delicious'.
REVIEW OF LITERATURE

Plant Growth Regulators

"Plant growth regulators are defined as organic compounds which, in small amounts, promote, inhibit, or otherwise modify any plant physiological process. Plant hormones (synonym: Phytohormones) are regulators produced by plants which, in low concentration, regulate plant physiological process. Hormones usually move within the plant from a site of production to a site of action.

The term 'hormone' correctly used, is restricted to naturally occurring plant products. The term 'regulator,' however, is not necessarily restricted to synthetic compound but can also include hormones. The term 'regulator' can apply to any material that modifies a plant's physiological process (69).

Growth regulators, both natural and synthetic may be divided into five groups, based on differences in their structures and effects: (1) auxin, (2) gibberellins, (3) cytokinins, (4) ethylene and ethylene generators and (5) growth inhibitors." (70).

Use of Growth Regulators in Horticulture

Growth regulators are widely used in horticulture and, in fruit crops, summarized by Westwood (70).

Growth regulators such as daminozide and 2,3,5-triiodobenzoic acid (TIBA) are used for floral initiation.

DAMINOZIDE, TIBA, ethephon, 2-chloroethyltrimethyl/ammonium chloride (CCC) are used in order to increase flowering on young non-bearing and on heavy alternate-bearing trees. NAA and naphthaleneacetamide (NAAm) by thinning fruit early in the season, by removing much of the flowering inhibitors that are produced by young seeds, indirectly increase
flowering. 1,2-dihydro-3,6-pyridazinedion (MH), and 2,4,5-T also promoted flowering when used 10 or 50 ppm at weekly interval (69).

Although the mechanism of fruit set is not known, growth regulators, particularly auxin, gibberellins, abscisic acid, and ethylene, play a role in the phenomenon.

Growth regulators have been used as a thinning agent since 1930s. Being more selective in removing undesirable fruit than random hand thinning, growth regulators such as: 1-naphthyl N-methyl/carbamate (carbaryl), which is very potent if used 20-30 days after full bloom, 2,3-quin-oxalinedithiol cyclic carbonate (Morestan), and ethephon are used in chemical thinning of fruits. As a thinning agent growth regulators causes a better fruit quality, increase the size of fruit. Chemical thinning also causes an increase in cell number, at least in 'Golden Delicious'.

Use of NAA and 2-(2,4,5-trichlorophenoxy) propionic acid (2,4,5-TP) prevent pre-harvest drop and should be used 7 days before earliest estimated harvest.

Auxins, such as 2,4-dichlorophenoxyacetic acid (2,4-D); 2,4,5-TP, enhance maturity and ripening through ethylene production.

Growth regulators such as NAA and gibberellins (GA_{3}) have been found to be beneficial in reducing the physiological disorder, cracking in apple fruits. NAA (1 ppm) may reduce cracking as much as 60 percent if used 30 days before harvest (70).
Ethephon

Ethephon, is an ethylene-generating simple organic compound with the following structure:

\[
\begin{array}{c}
\text{O} \\
\text{ClCH}_2\text{CH}_2\text{P} - \text{OH} \\
\text{OH}
\end{array}
\]

Ethephon consists of about 46 percent 2-chloroethane phosphonic acid, 36 percent mono-2-chloroethylester of phosphonic acid, and 13 percent 2-chloroethane phosphonic anyhydride. The decomposition or breakdown of this composition results in production of phosphate, HCl and the generation of ethylene.

Ethephon is used instead of ethylene for ethylene treatment of field grown plants. Ethephon slowly releases ethylene into the plant tissues allowing plant regulator responses (25,47,69,70).

1. **Ethylene**

With the chemical formula, \( \text{CH}_2 = \text{CH}_2 \), ethylene, a volatile gas, is the simplest plant hormone in its chemical structure. It is produced by metabolic process in all plant tissues. The incomplete combustion of coal, petroleum, and other fossil fuels result in ethylene production (55,69).

2. **Ethylene synthesis**

Auxin is the most important ethylene producing substance. Other ethylene inducing materials include gibberellins, cytokinin, coumarine, defoliants, endothal, potassium iodide, abscisic acid, ascorbic acid, cobalt ion and cycloheximide (51).

Not under in vivo, but under in vitro conditions linolenic
acid, an 18-carbon unsaturated fatty acid, could have been converted to ethylene (55). Specific fatty acids possibly act as precursors of ethylene (55).

As already mentioned ethylene-stimulated materials, acetylene and propylene, are able to stimulate ethylene production (69). Application of any concentration (10, 50, 100, 500, 1000 ppm) on 'Red Delicious' apple fruits caused the induction of ethylene synthesis. The highest rate of produced ethylene occurred by application of the 50, 100, 500 ppm of propylene at 75 percent maturity (28). Low oxygen concentrations retarded the autocatalysis of ethylene production, induced by propylene. More than 6.5 percent oxygen was required in the atmosphere to induce the mechanism for autocatalysis of ethylene production when fruits at 83 percent of full maturity were treated with propylene 100 ppm. Thus, Sfakiotakis and Dilley (28) concluded that the induction of autocatalyses ethylene by propylene has some relation to the utilization of oxygen. Schneider (58) found that application of NAA as a spray increased ethylene evolution in leaves, fruits and pedicel of 'Red Rome', 'Golden Delicious' or 'Staymared' apples. The ethylene-producing ability of auxins also have been shown by Abeles and Rubinstein (5) using red kidney beans. Material treated with NAA, indoleacetic acid (IAA), indole propionic acid (IPA) and indole butyric acid (IBA) caused increased ethylene evolution. Weaker auxins, IPA and IBA, caused lesser ethylene production.

Cholodny and Went recited by Abeles and Rubinstein (5)
tried to show whether endogenous auxin affects the rate of ethylene evolution. From tropistic responses such as phototropism and geotropism, they found a higher rate of ethylene on the side which previously contained a higher amount of auxin. Reduction of endogenous auxin by decapitation or the inhibition of auxin transport by TIBA to bean plant or by using growth retardants such as 2,4-dichlorobenzyltributylphosphonium chloride (Phosphon), CCC and daminozide (which induce IAA oxidase and prooxidase) on cucumber seedlings, caused a reduction of endogenous auxin and, in turn, a reduction of ethylene. Gibberellic acid (GA) caused a high level of ethylene evaluation through destruction of IAA oxidase and by increasing endogenous auxin (5).

Hick and Brown (33) by treating apple leaves (through dipping) with 2,4,5-T and daminozide, found that 2,4,5-T stimulated ethylene production. The stimulation declined appreciably within 10 days after treatment and within 101 days after treatment, there was no indication of the ethylene production. Daminozide inhibited ethylene production mark-
ably within one day after treatment. This continued for 30 days. During the 30-day period, ethylene production increased and equaled the rate of the control. After the 30-day period, the rate of ethylene production continued to increase while the rate of the control declined. By 101 days, the rate of ethylene production in daminozide treated leaves was 4 times that of the control. The high initial production of ethylene by 2,4,5-T treated leaves may be attributed to the exis
of both endogenous and exogenous auxin which in turn may stimulate the production of ethylene. The low initial production of ethylene by daminozide treated-leaves may be related to a reduced supply of endogenous auxin (5). According to Chatterjee and Leopold (17) there is a decreased auxin sensitivity in aged leaves; thus, leaf age (by 101 days) is a possible reason for a shift from ethylene stimulation to inhibition by 2,4,5-T. The opposite occurred with daminozide an initial depression of ethylene production was followed by an increase (4 times that of control) 101 days after application.

A decrease in auxin sensitivity in mature apple fruits was reported by Looney (43). He applied 2,4,5-TP (20 ppm) on August 12, 25, and September 8 to the fruit. While the chemical induced endogenous ethylene production, the response decreased as fruit matured.

Harvest and isolation of fruit from the leaves causes a tremendous increase in ethylene level of fruit (27).

3. Inhibition of ethylene synthesis

Some methods limit ethylene synthesis in fruit. Growth retardants such as phosphon, chloromequate and daminozide have been used for this purpose. Daminozide most effectively hinders endogenous ethylene synthesis which in turn delays ripening of apple fruit. Phosphon and chloromequate induce IAA oxidase and prooxidase activity which causes a reduction of endogenous auxin and ethylene (60). Low levels of oxygen (5-7 percent) and high levels of carbon dioxide (10-20 percent) inhibit ethylene synthesis (5,28,51). Looney (43) using
daminozide (2500 ppm) on May 26, June 26, and July 28, and ethephon (500 ppm) on August 12, August 25, and September 8 to 'McIntosh' apple trees, found that daminozide inhibited the development of the respiratory climacteric and ethylene production.

To show the inhibitory property of AgNO₃ on ethylene synthesis, Beyer (10) used AgNO₃ (0, 15, 60, or 240 mg/l) as a foliar application on Alaska peas; at a rate of 0, 25, 50, 200 or 1000 mg/l on cotton (later treated with 12 µl/l ethylene); and at a rate of 750 mg/l on orchid (later treated with 0.2 µl/l ethylene). In peas, increasing the concentration of AgNO₃ caused the reduction in responses such as stem swelling growth retardation, and horizontal growth, which are known to be caused by ethylene. AgNO₃ at 240 mg/l completely inhibited the action of ethylene on the above patterns.

In cotton, all ethylene-treated leaves had abscised by seven days, while none of the controls had abscised by this time. When cotton was treated with AgNO₃ 200 mg/l just 9 percent of the leaves abscised. AgNO₃ protected the ethylene-stimulated senescence in orchids. AgNO₃ exerts its action through interfering with ethylene oxidation by making an ethylene-AgNO₃ complex.

AgNO₃ (250 ppm) applied to 'Golden Delicious' and 'Stay-mared' apple trees on 'M.7' previously treated with NAA (25 ppm) inhibited both ethylene synthesis and response of trees to ethylene. Though galactose increased ethylene evolution in mung bean tissues, when galactose (400 ppm) was used
on NAA (25 ppm) - sprayed apple trees, galactose did not affect fruit retention. Thus, either ethylene does not play a main role in initiating apple thinning by NAA or galactose does not promote ethylene synthesis by apple tissues (61).

Abscission

"The active separation of a leaf from a branch without injury to the branch is called leaf abscission. Plant parts other than leaf are shed by abscission likewise. Abscission is an adaptation that serves to remove senile leaves, ripe fruit, and flowers that did not set, and is a means of self pruning when excess of shoots are present.

Leaf abscission is commonly prepared near the base of the petiole by cytological and biochemical changes in cells along which the leaf eventually separates from the branch. The tissue region concerned is referred to as the abscission region or abscission zone. Two layers are discernible in the abscission zone: (1) an abscission or separation layer, through which the detachment occurs, and (2) a protective layer, which at leaf fall protects from desiccation and invasion by parasites the surface that becomes exposed.

In most leaves, flowers, fruits and some stems the preparation of the abscission zones occurs during ontogeny, but it may take place directly in response to conditions that provoke abscission" (26).

"Abscission process involves an orderly series of cellular changes including, usually, the differentiation of an abscission zone, cellular senescence in the tissue distal to the separation zone, and cell wall degradation. Cell wall degradation is the central and pivotal aspect of abscission process. The cell wall changes may be characterized as a hydrolysis of the cellulosic walls, a loss of cementing effectiveness of the middle lamella, and simple fracture of the vascular element" (58).
Abscission is defined also as a physiological phenomenon by which higher plants are able to throw off their organ by an active separation of cells. Generally there are 5 (morphological) stages in abscission:

a. Differentiation of an abscission zone (first stage).

b. Abscission zone in stage I - a static abscission zone during which there is no actual weakening of break-strength.

c. Abscission zone in stage II - failure of auxin to inhibit separation and ability of ethylene to promote separation are two changes which occur in this stage.

d. Separation - breaking of vascular cells, partial dissolution of the cellulosic wall and loosening of the cellulosic layer of middle lamella.

e. Healing - this process takes place after the separation on exposed surfaces as a protective layer (39).

1. Characteristic of abscission zone

"Abscission zone is characterized externally in most apple cultivars by a slight constriction where the apple pedicel joins the cluster base. Internally the zone shows a modified structure with specialized collenchyma beneath the constriction. This area is occupied by fibers and stone cells in adjacent parts of the pedicel. There is also a reduction in the number of fibers and vessels in the vascular cylinder as it extends through the zone as well as a reduction in the amount of sclerenchyma in the area where the normal cortical region joins the abscission zone.

A separation or fracture in the pith region of the zone usually marks the beginning of abscission. At the time of fruit drop the fracture extends from this layer in the pith through the vascular tissues and collenchyma" (39) (Figure 1).
Figure 1. Abscission zone in mature fruit at the time the initial separation in the pith is visible (from 39).
Pierik (56) working on in vitro cultivated apple flower pedicel without a primary abscission layer, found a secondary abscission layer, an abscission layer in an unusual place that normally does not occur, caused by application of auxin. To establish this abscission zone, the pedicel must be treated with an auxin-containing medium for at least 5 days. Unlike the fruit pedicel, no secondary abscission layer can be noticed in in vitro petiole of leaf by auxin. This can be an indication of petiole and pedicel abscission being, physiologically, two different patterns. Light, temperature, sugar and macroelements are also important in forming secondary abscission layers. Intensity of light is not important. The kind and concentration of sugar is of less importance than the existence of sugar which is essential in fulfillment of the secondary abscission layer. With application of high auxin concentration, which causes ethylene induction, and with application of ethephon, ethylene releasing material, there was no secondary abscission layer. Ethylene does not have a direct role in inducing of secondary abscission layers in apple pedicel.

2. Factors affecting abscission

a. Environmental factors

(1) Respiration - according to Addicot (7) abscission will not occur unless oxygen is present. This is in accordance with the findings of other investigators (5, 28, 51) who found no ethylene synthesis, which is responsible for abscission process if there is a high level of CO₂ (10-20
percent) and low level of O₂ (6.5 percent or less). Carns recited by Addicot (7), found an increase in abscission by increasing the rate of oxygen. Abeles and Grahagam (1) reported that low oxygen rates, by inhibiting respiration, could hinder abscission. Furthermore abscission-inhibitory effects of a group of respiratory inhibitors on inhibition of a variety of respiratory enzymes, which in turn retarded or inhibited abscission, is another indicator of the role of respiration on abscission. CO₂ was able to block the abscission of 4 species explant (Cassia, Cotton, Coleus, Bean) but this abscission-inhibitory effect of CO₂ could be overcome by ethylene. It is noteworthy that high concentrations of ethylene are needed to overcome high CO₂ concentrations. Although CO₂ is usually known as an abscission inhibitor, in some cases it was able to stimulate abscission (7).

(2) Photoperiod - through direct effect of phytochrome on hormone levels, short photoperiods promote while long photoperiods inhibit leaf abscission. Under long photoperiods the rate of auxin and gibberellin production is higher than that of abscisic acid, a growth inhibitor, and this high level of auxin and gibberellin work against abscission. In short days the case reverses and high levels of abscisic acid cause abscission (7).

(3) Photosynthesis - by providing substrate for respiration and cell wall deposition, which results in a thicker cell wall, photosynthesis is able to prevent abscission (7).
(4) Ammonia and other atmospheric pollutants - high level of ammonia and other pollutants are toxic and cause abscission through leaf injury. On the other hand, probably by increasing the rate of nitrogenous substances in the plant, the lower rate of ammonia (non-toxic level) can hinder abscission (7).

b. Other factors

(1) Cell wall and respiratory enzymes - enzyme synthesis is necessary for abscission to occur. Both cell wall and respiratory enzymes appears to be of utmost necessity. Peroxidase, which increases ethylene synthesis from methionine and methionin analogues; and phosphates, which relate to aging also are associated with abscission phenomenon (58).

(2) Callose and lignin - though the effect of callose and lignin in the abscission phenomenon is not clear, accumulation of callose may inhibit the movement of intercellular materials including auxin, which is necessary in inhibition of abscission. An increase of these two substances in abscission zone of the bean leaf was reported by Poovaiah (58), but the author did not report an increase of these materials in the cherry fruit abscission zone.

(3) Xanthoxin (XA) and abscisic acid (ABA) have been shown as abscission-accelerating materials in senescent petioles of Coleus rehneltianus Berger, Phaseolus vulgaris L. 'Saxa', Acer pseudoplatanum, L. and Malus domestica Borkh. 'Golden Delicious' by Dörrflling et al (21). He found that these two abscission-accelerating substances caused abscission
directly, not through increased ethylene levels.

(4) Auxin - transport inhibitor - Morgan and Durham (50), working on cotton pointed out that morphactins, as a strong auxin-transport inhibitor, increased the rate of leaf abscission of vegetative cotton plants exposed to either ethylene or the ethylene releasing material, ethephon. Though morphactins increase the effect of ethylene or ethephon, when applied alone did not abscise the leaf.

Mode of Action of Ethylene

1. Stimulation of germination and sprouting

There are two possible methods through which ethylene enhances germination and sprouting (69). Ethylene stimulation of movement of hydrolytic enzymes in storage tissues (treatment of aparted barley aleurone layer with ethylene) induced the release of gibberellin-stimulated α-amylase from the cells into the endosperm, a storage tissue. Also, ethylene controls the movement of food reserves from adjacent tissues, synthesized during the growth of the buds (69).

2. Growth regulation

It is hypothesized that ethylene regulates growth by alteration of transport or metabolism of auxin. It may also aid in the secretion of specific enzymes important to growth from the cells by inducing important enzyme system correlated with the cell membranes (69).

3. Fruit ripening

Except by theory it is not known how ethylene stimulates
ripening. One theory is that ethylene stimulates ripening by altering the physical condition of cells or membranes, thus paving the way for previously-blocked reactions to take place. Possibly, ethylene causes ripening by inducing respiration and protein synthesis in immature fruits which in turn form a chain of biochemical events necessary for ripening (51).

4. Abscission

Because of the complicated nature of abscission, the exact role of ethylene in the process is not known (7,69). It is thought that ethylene causes abscission by controlling the release or secretion of cellulase to the cell wall, by speeding up the aging of plant tissue, and by turning on protein synthesis and the requisite RNA for the production of cell-wall-degrading cellulase (3,4,12). The stimulating ability of ethephon in abscission depends on the synthesis of RNA and protein. Ethephon, ethylene releasing chemicals, or ethylene itself affects abscission phenomenon on the stage II abscission zone by releasing structural destructive enzymes. However, the response of very young abscission zones to ethylene has been shown, thus ethephon's effects may not be confined to the stage II abscission zone (39). The fact that ethylene affects abscission on the stage II abscission zone has also been shown by ineffectiveness of ethylene in initiation of leaf abscission in beans unless at least some tissue senescence had occurred (3). But data given by Schneider (61) working on apples does not eliminate the initiative role of ethylene in abscission.
The abscission characteristic of ethephon was elucidated also by Edgerton and Blanpied (23) in their 3-year experiment on 'McIntosh' apple fruits. Preharvest foliar application of ethephon caused loosening in break-strength and an increase in harvest drop. The addition of an auxin counteracted this characteristic of ethephon. The preharvest application of ethephon on previously daminozide treated trees could offset the abscission delay effect of daminozide. Preharvest foliar application of ethephon caused an increase in respiration, which is in agreement with Addicott (7) and Weaver (69). When used in combination with ethephon, 2,4,5-TP caused a slight additional promotion in respiration. Lord et al (44) conducted a three year experiment to consider the effect of ethephon on fruit thinning of three apple cultivars. Ethephon (1000 ppm) alone or in combination with daminozide (1000 ppm) caused defoliation of the 'Cortland' trees, but ethephon (500 ppm) caused a 26 percent reduction in fruit set. With 'Mutsu' fruit, ethephon (500 ppm) used at 35 and 44 days after full bloom strongly reduced fruit set. Ethephon (250 ppm) could not cause thinning on this cultivar. On 'Early McIntosh' trees, ethephon (100 and 200 ppm) alone or in combination with daminozide (1000 ppm) applied 30 days after full bloom reduced fruit set.

Contrary to the findings by Edgerton and Blanpied (23) that daminozide suppressed the abscission-promoting effects of ethylene on mature fruits, Lord (44) found that daminozide increased the ability of ethephon to thin developing fruits.
Ethephon (200 ppm) benefited the size of fruit when used alone. As we know ethylene causes abscission through enzyme induction and secretion (3,4) and its ability to abscise leaves depends on the sensitivity of the plant tissues to ethylene. Also, ethylene through blocking auxin movement to abscission zones, increases sensitivity of tissues which are going to abscise.

Beyer (12) treated the third true cotton leaf petiole with 14 μl/l ethylene while keeping the leaf blade in the air. Since there was no inhibition in auxin synthesis in the blade, the auxin transport capacity blocked ethylene action preventing leaf abscission. No abscission occurred even after 7 days. But when the entire leaf was treated with ethylene, ethylene blocked the synthesis of auxin. The absence of auxin interaction with ethylene caused the leaf to abscise. As was proved by Beyer (12), it was the flow of auxin from leaf blade to abscission zone which prevented the total effect of ethylene in leaf abscission.

Optimal Concentration of Ethephon for Defoliation

In every part of the world there always existed a desire for nursery trees to be defoliated and be stored before the natural occurrence of abscission. Ethylene was found appropriate for this purpose (35). Ethylene was 60-100 times more effective than other ethylene-like materials (1). Since a volatile gas, application of ethylene is not possible, but an ethylene producing material is used for this purpose.
Different kinds of fruit trees show different responses to ethylene application as well as among different cultivars and species of the same kind of fruit. According to Larsen (35) a single application of ethephon (2000 ppm) was required for abscission of 'Spur Red Delicious.' Three applications of 2000 ppm was necessary for 'Golden Delicious' and 'French Crabapple' seedlings and only 80 percent defoliation was obtained three weeks after the last application.

In order to avoid bark or bud damage usually caused by ethephon (2000 ppm), Larsen (36) showed that if D-WK (Dupont-WK) (1-2%) was combined with ethephon and used at weekly intervals, 1 to 3 applications gave the same results in defoliation of nursery trees. Though D-WK by itself defoliates nursery trees, it seems that it helps the penetration of ethephon through cuticle layers.

Basak et al (8) using a mixture of ethephon (1000 mg/l), potassium iodide (KI) (0.15 percent), copper sulphate (CuSO₄·5H₂O) (0.75 - 1.0 percent) and magnesium chloride (Mg(ClO₃)₂·H₂O) (0.25 percent) found that the above mixture of inorganic defoliants, especially copper sulphate and magnesium chloride, and ethephon are the most promising defoliants for nursery apple trees. The mixture remarkably defoliated apple trees without injuring buds or bark and their influence on the reduction of winter hardiness of trees was not conspicuous.

Larsen (36) found bark or bud damage caused by ethephon (2000 ppm). Cummins and Fiorino (19), in order to get a proper concentration for defoliation of nursery trees, used ethephon
on October 21, 1968 at 2000 ppm, 3750 ppm and 5000 ppm on
2-year-old MM 106/seedlings. Ethephon (2000 ppm) met the three
required criteria for an optimum defoliant suggested by Larsen
(37): "(1) leaves should fall before or during the digging
process, (2) there should be no significant damage to bud
or bark and (3) growth after transplanting should be normal." 
Since all three concentrations enhanced root germ formation,
it was considered best to use ethephon at lower concentration
early in the fall (19). Although Basak (8) found a concen-
tration of 3000-4000 mg/l of ethephon as optimal for defoli-
ation of 'Yellow transparent,' 'McIntosh' and 'Jonathan,' if
nursery trees are to be sprayed after September 25.

Since different cultivars of fruit trees show varying
responses to ethephon (35), the concentration and timing for
ethephon for thinning apple fruits differs by the apple cul-
tivar. Application of ethephon (200 ppm), 28 and 42 days
after full bloom have been recommended on Jonathan by Veinbrants
and Hutchinson (68).

Pollard (57) in his study shows that preharvest applica-
tion of ethephon in a low concentration (50 ppm) combined
with daminozide improved desirable color in 'McIntosh' with
only 4 percent abscission of the fruit, while ethephon (250
to 500 ppm) resulted in a heavy abscission of the fruit.

Fruit drop is correlated to ethephon concentration.
Ethephon caused more fruit drop of 'McIntosh' apple fruits
with higher (500 ppm) than lower (250 ppm) concentration.
When ethephon (250 ppm) was combined with daminozide, the
later reduced the effect of ethephon significantly. Ethephon (125 ppm) did not affect fruit drop (30).

Uniform coverage of all parts of the tree and temperature after application also play, if not an important, a role in response to ethephon (25).

With 300 to 350 gallons per acre and a sufficient coverage, 'Cortland,' 'R.I. Greening,' 'Golden Delicious,' and 'Twenty Ounce' apples have shown satisfactory response to ethephon (250 ppm). In the case of cool day and night temperatures, 500 ppm was suggested. For effective loosening, 'Rome Beauty,' 'Idared' and 'Ben Davis' required higher ethephon concentration (25).

Green et al (31) found an erratic response to ethephon on 'Delicious' apple fruits. While ethephon (1000 ppm) caused abscission when applied at 2 stages (between July 15 and August 21 and early September through September 23) in 1974, the same concentration eliminated all the fruit soon after application in 1975.

Edgerton and Greenhalgh (24) reported that ethephon (200 ppm) during bloom or prebloom on 'McIntosh' and 'R.I. Greening' caused a reduction in fruit set, ethephon (2000 ppm) eliminated fruit completely. When applied 10 days after full bloom, ethephon (200 ppm) reduced fruit set. Concentration up to 250 ppm did not reduce fruit set on 'McIntosh' when applied 28 and 44 days after full bloom. Depending upon the concentration of ethylene in the air and exposure periods to
ethylene, developing 'Golden Delicious' fruit abscised a few days after fertilization (24).

Factors Affecting Ethylene Action

The mode of action of ethylene in loosening break-strength depends upon the level of juvenility factors (auxin, cytokinin) and other materials. An increase in ethylene levels in the cell promotes abscission by reducing the rate of juvenility factors. As long as the level of juvenility factors are enough to undermine the action of ethylene, no abscission occurs (3,11). Other metal ions such as Co, Cd, Cu, Pd, Hg, Rh, Zn, Ni, have the ability to offset ethylene action. None of these except Ag(NO₃)₂ show the expected effectiveness when they were used to intact plant foliarly. AgNO₃ was found to have the ability to reduce, at least slightly, the growth inhibitory characteristic of ethylene (10).

Materials or circumstances which hinder ethylene synthesis also are effective in blocking ethylene action. Therefore, high CO₂ concentration and low oxygen concentration and low temperature are also counted as factors inhibiting ethylene action (51). CO₂ probably acts by slowing down or preventing the initiation of the stage of abscission which is ethylene sensitive. Actinomycin D and Cycloheximide presumably by blocking RNA and protein synthesis necessary for the formation of destructive enzymes, inhibited abscission (19).
Acceleration of abscission by exposing plants to ethylene may be caused by inhibiting auxin synthesis, by altering auxin levels in the abscission zone, by promoting auxin destruction and by reducing auxin transport capacity. Three auxin-transport inhibitors—TIBA, 3,3α-dihydro-2-(p-methoxyphenyl)-8H-pyrazole-(5,1-alpha)isoindol-8-one(DPX-1840) and N-1-naphthylphthalamate (ALANAP) — caused a noticeable increase in abscission with comparison to the control (receiving ethylene but not auxin-transport inhibitors) (11,49,60). The time interval between applications of ethrel and auxin-transport inhibitors did not have a major role in abscission. There was a decrease in enhancement of abscission by DPX-1840 when ethylene levels increased. These materials act in enhancement of abscission by limiting auxin supply at the abscission zone.

Neither abscission nor remarkable inhibition of auxin took place when cotton plants were exposed to 14 μl/l of ethylene for 24 hours at 21.5°C or below. There was approximately 10 percent abscission at 27°C, and 70 percent of abscission occurred at 35°C (11).

Whereas increased temperatures affected ethylene in its auxin-transport inhibitory effect, pre-treatment auxin inhibited ethylene induced abscission. When 3-week old cotton was treated with NAA (250 mg/l) only a low percentage of abscission occurred, the corresponding third true leaf of control had a 100 percent abscission (11).

Most abscission-accelerators cause abscission by affecting ethylene activity through ethylene production. Schneider
(62) used 14-year old 'Golden Delicious,' 'Staymared' or 'Red Rome' trees on M.7 stock. Carbaryl and NAA were applied to foliage, fruit and pedicel. Carbaryl did not affect the evolution of ethylene in fruit and foliage. It increased ethylene evolution in pedicel and in fruit of 'Red Rome' and 'Golden Delicious' attached to the pedicel. The author expressed doubt that carbaryl acts through ethylene synthesis.

NAA, applied as a spray, caused an increase in ethylene levels in fruit, leaves and pedicel in 3 of the cultivars and the greatest increase was observed by samples collected 48 hours after application.

Physiologically proper temperature and oxygen are required for promotion of ethylene action. Ethylene causes other ethylene-induced events by reducing auxin supply to abscission zone tissues. Therefore, ethylene action probably is promoted by circumstances which induce senescence or limit auxin supply. Applied auxins such as IBA and IPA caused plant sensitivity to ethylene, but not as much as IAA or NAA. Therefore they caused the promotion of ethylene action in plants. Although auxin itself makes tissues such as immature tomato and immature apple fruit sensitive to ethylene, ripening fruit showed the reverse or opposite effect of auxin either in mature tomato fruit or mature apple fruit (5,25,51).
MATERIALS AND METHODS

Initial research (3 August to 22 August 1978) was conducted on 18 year old 'Red Delicious' apple trees and was to determine optimum concentration of Ethrel (ethephon, Amchem Products, Inc., Ambler, Pennsylvania 19002) to cause leaf abscission.

The second phase (26 August to 16 September 1978) was conducted on 8 year old 'Red Delicious' apples (Wellspur Strain) to determine factors affecting ethephon activity on leaf abscission.

Ethephon was applied with and without Cittowet (BASF) Wyandotte Corp., Parsippany, New Jersey 07054) surfactant using a hand applicator to the drip point on selected shoots of experimental trees. Leaves were counted prior to application and again at selected intervals until effect on abscission was achieved. Climatic conditions (temperature, relative humidity, sun condition, and wind speed) and soil moisture condition were recorded at application time during the second phase or main experiment. Temperature ranged between 24.4°C and 32.2°C, relative humidity (RH) from 43 to 63 percent, wind speed from 0 to 5 km/hr and sun condition from hazy to clear sky. Soil moisture ranged between an estimated fair to poor.
RESULTS AND DISCUSSION

The percentage of abscission was dependent on the used ethephon concentration (Table 1). Ethephon (12800 ppm) caused all leaves to abscise. The percentage of leaves treated with ethephon (6400 ppm) abscised ranged from 41.8% to 100%; at 3200 ppm, from 21.9% to 75.5%; at 1600 ppm, from 11.4% to 75.5%. There was only one treatment with 800 ppm and 8.2% abscission was recorded.

The concentration of ethephon selected for the main experiment was 4800 ppm. At this concentration, significant leaf abscission would occur yet abscission would be less than complete. This was considered desirable for testing factors of climate and soil moisture on abscission.

The addition of an adjuvant (Cittowet) did not affect abscission (Table 2). It did not appear necessary to use an adjuvant in the main field of experiment.

Response to ethephon generally differs by different cultivars of apples, so different concentrations have been recommended by investigators (19,23,35,36,44). From my experiment, 4800 ppm was a convenient concentration for studying leaf abscission on 'Red Delicious' apple (Table 1).

Taking environmental factors into consideration, the highest (87.2%) abscission occurred at the lowest temperature (24.4°C) and highest humidity (63%), the poor soil moisture and 0 km/hr wind, which is in accordance with findings of Edgerton (25) who worked with ethephon on cherries and apples.
and found 23.9°C the most suitable temperature for loosening cherries and calm weather which in turn reduces evaporation. But it is not in agreement with the findings of Basak et al (8) from the point of view of weather. He found sunny days suitable for ethephon application while in my experiment the most abscission occurred when the weather was hazy. Or, it is possible that high humidity (63%) and poor soil condition were an alternative for sunny days. Since stems and root under moisture stress produce abscisic acid which in turn causes abscission (7) it is possible the poor soil moisture by promoting the roots in producing ABA causes abscission of the leaf. Lower temperature, high humidity, and calm weather are factors related to slow evaporation rate. Under slow evaporation, more chemical penetrates into the leaf which results in more abscission.

Furthermore, this experiment supports the findings of other authors (19,48,49,51) working bean or apples, that aged leaves abscised first and younger leaves later. As indicated in Table 3 the percentage of abscission tended to be higher when treatments were applied towards the approach of the natural occurrence of abscission.
SUMMARY

Growth regulators as modifiers for plant growth and development are being used widely.

Ethephon (ethylene releasing material) as a growth regulator has been used for defoliating nursery trees, improving fruit color, loosening the break strength of fruit for mechanical harvesting, etc. on apple trees as well as other kinds of fruit. The response to ethephon varies with cultivar, concentration of ethephon, the time of application, and environmental conditions. Other growth regulators and organic or inorganic compounds, if used with ethephon, are other factors affecting ethephon activity.

In my experiment 4800 ppm ethephon was found to be the appropriate concentration for abscission of leaf of 'Red Delicious' apple trees. The effectiveness of ethephon was higher when used at lower temperature (24.4°C) and higher humidity (63%).
Table 1. Effect of ethephon concentration on 'Red Delicious' apple leaf abscission.

<table>
<thead>
<tr>
<th>Date Applied</th>
<th>Date Rated</th>
<th>Concentration ppm</th>
<th>Leaves treated No.</th>
<th>Leaves abscised %</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 3</td>
<td>August 20</td>
<td>6400</td>
<td>67</td>
<td>41.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3200</td>
<td>64</td>
<td>21.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1600</td>
<td>88</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>800</td>
<td>73</td>
<td>8.2</td>
</tr>
<tr>
<td>August 10</td>
<td>August 26</td>
<td>12800</td>
<td>61</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6400</td>
<td>73</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3200</td>
<td>55</td>
<td>41.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1600</td>
<td>45</td>
<td>26.6</td>
</tr>
<tr>
<td>August 14</td>
<td>August 26</td>
<td>12800</td>
<td>11</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6400</td>
<td>48</td>
<td>45.8</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>46</td>
<td>39.1</td>
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<td></td>
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<td>1600</td>
<td>76</td>
<td>15.9</td>
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<tr>
<td>August 18</td>
<td>August 26</td>
<td>12800</td>
<td>56</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6400</td>
<td>52</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>62</td>
<td>75.5</td>
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<td></td>
<td></td>
<td>1600</td>
<td>53</td>
<td>19.0</td>
</tr>
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</table>
Table 2. The ineffectiveness of surfactant (Cittowet) with ethephon to promote apple leaf abscission.

<table>
<thead>
<tr>
<th>Concentration (ppm)</th>
<th>Ethephon only</th>
<th></th>
<th>Ethephon and Cittowet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf No</td>
<td>Abscised %</td>
<td>Leaf No</td>
<td>Abscised %</td>
</tr>
<tr>
<td>12800</td>
<td>53.3</td>
<td>100</td>
<td>12800</td>
<td>55.7</td>
</tr>
<tr>
<td>6400</td>
<td>50.8</td>
<td>75.7</td>
<td>6400</td>
<td>62.6</td>
</tr>
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<td>3200</td>
<td>56.8</td>
<td>45.0</td>
<td>3200</td>
<td>54.3</td>
</tr>
<tr>
<td>1600</td>
<td>57.5</td>
<td>18.3</td>
<td>1600</td>
<td>49.5</td>
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</tbody>
</table>
Table 3. Effect of ethephon (4800 ppm) on 'Red Delicious' apple leaf abscission as related to the date of application and climatic and soil moisture conditions.

<table>
<thead>
<tr>
<th>Date of Application 1978</th>
<th>Conditions at application time</th>
<th>Leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature °C</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>26 August</td>
<td>33.9</td>
<td>46%</td>
</tr>
<tr>
<td>2 September</td>
<td>35.6</td>
<td>45%</td>
</tr>
<tr>
<td>9 September am</td>
<td>24.4</td>
<td>63%</td>
</tr>
<tr>
<td>9 September pm</td>
<td>33.3</td>
<td>43%</td>
</tr>
<tr>
<td>16 September am</td>
<td>32.2</td>
<td>50%</td>
</tr>
<tr>
<td>16 September pm</td>
<td>36.7</td>
<td>43%</td>
</tr>
</tbody>
</table>
LITERATURE CITED


USE OF ETHephON IN ABSCISSION

by

S. WAKEEL MAYHENMAHR

B.S., Kabul University, 1972

AN ABSTRACT OF A MASTER'S REPORT

submitted in partial fulfillment of the

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Department of Horticulture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1980
This paper relates to the effect of 2-chloroethylphosphonic acid (Ethephon), an ethylene-generating growth regulator, on abscission and finding an appropriate concentration to be used for this purpose.

Ethylene, the simplest plant hormone \((\text{CH}_2 = \text{CH}_2)\) is produced in all plant tissues, causes germination and sprouting, regulates growth, causes fruit ripening, and is an abscission promoting material. Being a volatile gas, usage of ethylene is not practicable under field conditions. Instead ethephon is used to fulfill the aim. Ethephon slowly releases ethylene into the plant tissues allowing plant regulator responses.

Other materials such as auxin, gibberellins, cytokinin, coumarine, endothal, potassium iodide, and abscisic acid also have ethylene producing ability. Growth retardants such as phosphon, chloromequate and daminozide inhibited ethylene synthesis.

Ethylene causes abscission through enzyme induction and secretion. Its ability to abscond leaves depends on the sensitivity of the plant tissue to ethylene. Also, ethylene through blocking auxin movement to abscission zone, increases sensitivity of tissues which are going to abscond. Ethephon (2000 ppm) met the three criteria for leaf abscission of nursery stock: (1) leaves should fall before or during the digging process, (2) there should be no significant damage to bud or bark and (3) growth after transplanting should be normal, necessary for an optimum defoliation. Concentrations
of 3000 to 4000 mg/l of ethephon have been found as optimal where nursery trees are sprayed after September 25.

Application of ethephon (200 ppm) 28 and 42 days after full bloom have been recommended on Jonathan for thinning purposes. Concentrations up to 250 ppm is appropriate for 'McIntosh' apple trees if applied 28 and 44 days after full bloom.

The mode of action of ethylene in loosening break-strength depends upon the level of juvenility factors (auxin, cytokinin) and other materials such as Co, Cd, Cu, Pd, Hg, Rh, Zn, Ni. As long as the level of juvenility factors are enough to undermine the action of ethylene, no abscission occurs.

In my experiment the percentage of leaf abscission also was dependant on the used and ethephon concentration. Ethephon (12,800 ppm) caused all leaves to abscise. The percentage of leaves treated with ethephon (6400 ppm) abscised ranged from 41.8% to 100%; at 3200 ppm, from 21.9% to 75.5%; at 1600 ppm, from 11.4% to 75%. The addition of an adjuvant (Cittowet) did not affect abscission.

In this study ethephon (4800 ppm) was a convenient concentration. Taking environmental factors into consideration, the highest (87.2%) abscission occurred at the lowest temperature (24.4°C), highest humidity (63%) poor soil moisture, and 0 km/hr wind.