THE PHYSIOLOGY OF ETHYLENE PRODUCTION BY CITRUS PEEL TISSUE

By

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To my husband, Carl, for his support and encouragement of my scientific endeavor.

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TABLE OF CONTESTS

Abstract of Dissertation Presented to the Graduate Council
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BY CITRUS PEEL TISSUE

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Citrus peel explants provided a convenient system for the study of the physiology of ethylene production. Untreated peel disks, 10 mm in diameter, produced wound ethylene at a maximum rate of 5-6 nl/hr/3 disks. Treatment with an abscission-accelerating chemical (Release: 5-chloro-3-methyl-4-nitro-1-H-pyrazole) greatly increased ethylene production by whole fruit and peel disks of both 'Hamlin' and 'Valencia' oranges (*Citrus sinesis* L. Osbeck). The response to Release was localized primarily in the flavedo part of the peel. Peel disks of mature 'Hamlin' were more sensitive to Release than disks from immature fruit; 'Valencia' disks of all stages of maturity tested responded similarly. Ethylene production by peel disks was temperature dependent; this dependence was especially marked in Release-treated disks.

Whole citrus fruit and citrus peel disks lacked an autocatalytic response to ethylene. The tissue failed to respond to propylene, an ethylene analog, indicating that ethylene is not autoinhibitory in this tissue.

Protein synthesis and RNA synthesis are required for high levels of ethylene production by orange peel tissue. Ethylene production at the lower rates found in untreated disks involves protein synthesis, but not RNA synthesis. Evidence is presented to indicate that ethylene is synthesized from methionine via a reaction involving a metal cofactor.

Disks from regreened fruit showed a reduced response to Release and Pik-Off (ethanedialdioxime), another abscission accelerating compound. Disks from non-regreening fruit responded to increasing concentrations of Release and Pik-Off (up to 1000 ppm) with increasing rates of ethylene production. Disks from regreening fruit responded to different concentrations of Release and Pik-Off with about the same low level of ethylene production, except at 1000 ppm Pik-Off, when the ethylene response increased to equal that of non-regreening disks.

Fruit damaged by citrus rust mite (*Phyllocoptruta oleivora* Ashmead) showed increased ethylene production and greater fruit loosening in response to Pik-Off when compared with undamaged fruit. Although uptake of ^{14}C-Pik-Off (^{14}C-ethanedialdioxime) was very rapid in both undamaged and mite-damaged fruit, the rate was slightly greater in mite-damaged fruit. The increased uptake of Pik-Off by mite-damaged fruit probably accounts in part for the observed changes in ethylene production and fruit loosening.

INTRODUCTION

The plant hormone ethylene is known to be involved in a number of developmental processes in plants, including fruit ripening and color development, abscission of leaves and fruit, and senescence (6). Despite extensive research on the physiology and biochemistry of ethylene production, it is still uncertain how plants regulate ethylene production and by what biosynthetic pathway ethylene is produced. There is considerable evidence showing that methionine is the precursor of ethylene (5, 162), but there is a possibility that alternate pathways coexist with or replace the methionine pathway in certain cases (17).

Citrus fruit produce small amounts of ethylene throughout development (132). Although fruit ripening or detachment from the tree do not result in increases in ethylene production, the fruit will produce considerable quantities of ethylene when stressed (57, 58, 67, 153). Compounds which cause chemical wounding are being used commercially to loosen oranges for mechanical harvesting (57, 65). There is, therefore, considerable interest in the physiology of ethylene production by citrus fruit, particularly in response to abscission chemicals.

In the present study, citrus peel explants were used to study some physiological and biochemical aspects of ethylene production, with special emphasis on the effects of abscission-accelerating chemicals.

LITERATURE REVIEW

Citrus fruit have for decades been considered the epitome of the non-climacteric fruit because the mature fruit do not produce a sharp increase in ethylene and respiration during ripening or following removal from the tree (14, 26, 67). Small amounts of ethylene are produced by citrus fruit throughout development (132). Detachment of small, immature orange or mandarin fruit from the tree results in a pseudoclimacteric rise in respiration and ethylene production, but this response declines with age until it is completely absent in fruit harvested in September or later (67, 89). Exogenous ethylene was shown by some researchers to accelerate respiration of citrus fruit at all stages of development tested (15, 152). However, McMurchie et al (115) found no increase in respiration or ethylene production in mature 'Valencia' or lemon fruit treated with the ethylene analog propylene. The discrepancy may be explained by the fact that the latter used lower, and much closer to physiological, concentrations.

Citrus fruit have a unique developmental pattern which is due at least in part to the low endogenous levels of ethylene production. Fruit may meet legal standards of maturity while retaining a green color, or may regreen after achieving good color (81). Ethylene is used commercially to degreen fruit.

Citrus fruit will produce large quantities of ethylene and show increased respiration rates when placed under various types of stress,

including chilling, dropping on hard surfaces, and physical or chemical wounding (57, 58, 152, 153). Since ethylene is the natural regulator of the abscission process, chemicals which cause wound ethylene production are being utilized to loosen fruit and facilitate mechanical harvesting (57). The physiology of ethylene production in citrus is therefore of practical, as well as scientific, significance.

The Physiology and Biochemistry of Ethylene Production

The physiology and biochemistry of ethylene production has been extensively researched. Reviews on the subject have been written by Abeles (5), Burg (31), Lieberman (105), Yang (162) and Yang and Baur (163).

The plant hormone ethylene is produced by a wide variety of plant tissues under numerous conditions. Small amounts of ethylene are regularly produced by healthy vegetative tissues (37, 38, 78), immature fruit (34, 111), and germinating seeds (30). Large quantities of ethylene are typically produced by stressed or wounded tissues (69, 91), climacteric fruits (32, 33), and senescing organs (84, 95, 149).

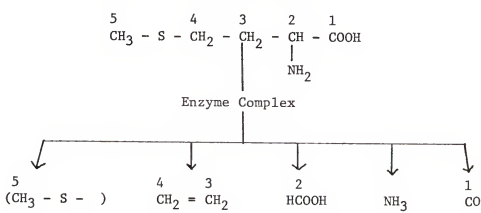
Biosynthesis

Experimental evidence favors the idea that methionine is the precursor of ethylene in higher plants (5, 162). Some tissues show increased ethylene production when methionine is added (22, 38, 108). The *in vivo* conversion of labeled methionine to labeled ethylene has been demonstrated for a variety of tissues, including citrus albedo tissue (1, 22, 38, 90, 108, 140).

The intermediates in the methionine-ethylene pathway are unknown. Mapson et al (116) obtained some evidence that the α-keto-γ-methyl thio-

butyrate (KMBA) was the intermediate in cauliflower florets, but other workers failed to confirm their results (20). The apparent discrepancy was resolved by Lieberman and Kunishi (106), who showed that the apparent production of ethylene from KMBA took place in an extracellular system produced by leaked peroxidase, KMBA, and cofactors.

There is a possibility that there are no methionine-ethylene intermediates in the strict sense. The activated enzyme complex could split methionine into its products in a single step:



A recycling system for the sulfur released during ethylene formation has been proposed by Baur and Yang (21).

The use of labeled substrate and rhizobitoxine, a substance which inhibits ethylene production from methionine (128), has allowed researchers to determine that methionine is the precursor for ethylene production due to stress (1), in auxin-treated vegetative tissues (140), in climacteric fruit (22), and in senescent flowers (84). The conversion of labeled methionine to ethylene in tissues producing very small amounts of ethylene is difficult to demonstrate, but relatively low production levels have been shown in citrus albedo tissue (90).

A cell-free ethylene producing system has yet to be isolated; the system is destroyed when tissues are homogenized (40, 106). Since the system is sensitive to disruption and osmotic shock, the site of ethylene production is believed to involve a membrane (40). Mattoo and Lieberman (118) have found that apple cells lose their ethylene synthesizing capability as the cell walls are digested, and regain it as the released protoplasts regenerate their cell walls. They hypothesized that the ethylene synthesizing system may be highly structured and located in a cell wall-cell membrane complex.

The endogenous control of ethylene production has yet to be elucidated. A proteinaceous inhibitor of ethylene evolution was isolated from a red alga (*Porphyra tenera*) that inhibited ethylene evolution and acrylate decarboxylase activity in the alga (154, 155). Acrylate could serve as an ethylene precursor in the alga.

Another proteinaceous inhibitor was obtained from mung bean seedlings (142). This inhibitor reversibly depressed ethylene production in mung bean hypocotyl segments, pea epicotyl segments, and less effectively, apple tissue slices (141).

The Effect of Hormones

Auxins. Auxin has been known as a stimulant of ethylene production since 1935, when Zimmerman and his colleagues compared the effects of ethylene with those of auxin on epinasty and adventitious root formation (63, 166). The plants treated with synthetic auxins behaved similarly to plants treated with ethylene, and in addition, showed increased production of epinasty-inducing gases.

Further information on the relationship between hormones came much later, after the development of gas chromatography. With the ability to measure extremely low concentrations of ethylene, scientists have confirmed that ethylene production may be regulated by auxin. Auxin responses mediated by ethylene include abscission (13), promotion of flowering in Bromeliads (35), inhibition of bud growth (37), plumular hook opening (94), and sex expression in cucurbits (146).

Other Hormones. Gibberellins may have no effect on ethylene production, as in pea seedlings and rape seed (73, 150), or, more often, may slightly increase ethylene production (13, 57, 97, 103). In citrus fruit, there is some evidence that gibberellins may be involved in the regreening phenomenon. Gibberellins are known to be present in citrus fruit throughout development (79) and were found to increase prior to regreening (130). Exogenous GA or potassium gibberellate increased regreening compared with untreated controls (47, 130).

Cytokinins usually cause a two- to fourfold increase in ethylene production (6, 73). The increase may be blocked with RNA and protein synthesis inhibitors. Cytokinins interact synergistically with auxins in enhancing ethylene production (37, 73, 98).

Abscisic acid increases ethylene production in leaves and fruit and inhibits it in cell suspension cultures and pea seedlings (2, 57, 61, 76, 77).

Ethylene. Ethylene is unusual in that it may trigger its own synthesis in certain tissues (82). Early workers established that ethylene can trigger the respiratory climacteric in a wide variety of fruits, and that ethylene is also a product of ripening (6, 26). It has since been

established that ethylene is required for fruit ripening (35). Fruits produce ethylene at a low level throughout their development; initiation of ripening may be brought on by an increase in ethylene production, an increase in sensitivity of the fruit to ethylene, or removal of the fruit from the parent plant (31, 32, 111, 145). In climacteric fruit, one response to the ethylene trigger is a huge increase in ethylene evolution.

Although the ethylene analog propylene is about 100 times less effective than ethylene itself (8, 36), it is useful in the study of autocatalytic ethylene production because its use permits the researcher to directly observe the quantity of ethylene produced. Propylene was shown to initiate ripening and ethylene synthesis in preclimacteric bananas (115), apples (145), and tomatoes (114). Propylene fails to induce ethylene synthesis in the non-climacteric citrus fruit (115).

There are a few instances where ethylene limits its own production. Pretreatment of preclimacteric bananas and chilled avocado fruit with ethylene supressed subsequent ethylene production (151, 164). Zeroni et al (165) found that ethylene acts as an autoinhibitor in figs in non-ripening, but not in ripening, stages.

The Effect of Metal Ions

Considerable attention has been given to the effects of metal ions on ethylene production, but it is difficult to separate specific ionic effects from secondary effects due to toxicity. Several model systems for ethylene production utilize Cu^{++} as a catalyst (108, 109). Ethylene production in hypocotyl segments, calamondin and orange fruit, orange branch segments, and bean and tobacco leaves was also stimulated by Cu^{++},

but these effects were attributed to stress (1, 23, 99, 135). Calcium ions, on the other hand, protect against copper-induced injury in bean hypocotyl segments, and promote ethylene production only in combination with copper or kinetin (99).

The monovalent silver ion Ag^+ has been reported to be a potent inhibitor of ethylene action in plants (24). This would indicate a role for silver in preventing autocatalytic ethylene production; this is apparently the case in postclimacteric apple and banana tissue (143). The silver ion has yet to be exploited as a tool for investigating autocatalytic ethylene production.

Metal ions may be directly involved in ethylene production from its substrate. The metal chelator EDTA has been shown to inhibit ethylene formation in apple slices (108, 147). In addition, two compounds which are specific copper chelators, cuprizone and sodium diethyl dithiocarbamate, were even more potent inhibitors of ethylene synthesis, suggesting that copper may be involved in the ethylene forming system (108).

Since the rules for structural activity of ethylene analogs resemble the rules for metal binding to olefins, it was proposed that ethylene must bind to a metallic receptor site (33). This hypothesis is supported by the fact that EDTA may inhibit ethylene action in some cases (5).

Other Factors

Gases. Oxygen appears to be required for ethylene production; its necessity has been confirmed in pears (83), broccoli (110), and avocado (22). One exception is a quasi-anaerobic ethylene producing system found in dormant cocklebur seeds (71).

High oxygen tensions can accelerate ethylene production (26). Ethylene synthesis and respiration require similar concentrations of oxygen, and are similarly affected by respiratory poisons, indicating that respiration may be required to produce energy or substrate for ethylene synthesis (39, 40). Murr and Yang (123) have obtained evidence that ATP is required for ethylene synthesis from methionine in apple tissue plugs.

Oxygen also appears to be involved in ethylene action as the activator of the ethylene receptor site (33).

CO_2 is a well-known ethylene antagonist (33), but its effects on ethylene production are limited to competition with ethylene in autocatalysis and depressing effects on respiration.

Light. Light is not an important regulator of ethylene synthesis in fruit (113, 137). In developing seedlings, however, light causes a decrease in ethylene production in the plumular hook; a single dose of red light will cause a transient decrease, and far red light will reverse the red effect (78, 94). It appears that ethylene intervenes in phytochrome control of plumular expansion.

Temperature. Fruits are unable to produce ethylene at temperatures above 35°C (39, 40, 83). The temperature effect is on ethylene synthesis directly, not via respiration, and is reversible (33). Prolonged high temperature treatments produced a more lasting depressive effect on ethylene synthesis in lettuce seeds (30).

Chilling temperatures enhance ethylene production during a subsequent warmer period in citrus fruit and in leaves of the deciduous peach

and trifoliate orange, but not in evergreen leaves of citrus, mango, avocado, or lychee (58, 59).

Pathogens. Stress ethylene is often produced in response to parasitic attack (159). Viral, fungal, and bacterial diseases may stimulate production of ethylene, which in turn stimulates development of disease symptoms such as yellowing, epinasty, and abscission (6, 16, 101). In addition to freeing the plant of diseased parts by abscission, ethylene may also play a role in stimulating synthesis of peroxidases and phenolic compounds, which may be involved in disease resistance. Ethylene sometimes assists in the progress of the pathogen through accelerated senescence or by increasing susceptibility of the tissue to pathogenic toxins (48, 64).

Insects may increase ethylene production by plant parts. Cotton weevil infestation results in boll abscission at a certain stage in the insect's life cycle (6). Red spider mites provoked increased ethylene production in infected rose leaves (159).

An important citrus pest is the citrus rust mite, *Phyllocoptruta oleivora* Ashmead. Heavy mite damage has been associated with reduced size of the damaged fruit, and reduced yields, vigor, and flowering during the following year (144). Very light feeding by mites on citrus fruit causes little damage, since the epidermal cells affected possess a self-healing mechanism (112). However, heavy feeding causes lignification and death of the affected cells. Appearance of visible injury coincided with a significant increase in ethylene production (112). Ethylene production often results in premature degreening of the damaged area of the fruit.

The Abscission Process

Morphology

The abscission zone is the region at the base of the abscising plant part through which separation occurs (3). The morphology of the abscission process is the same in leaves and fruit, the only difference being that fruit may abscise at any stage in development (72). In citrus, there are two abscission zones, one at the base of the fruit and another at the base of the pedicel (27). No visible histological differences between tissues of the abscission zone and adjoining tissue have been found in young citrus fruit or leaves (45, 85, 160). However, a darkening of the protoplasm in cells of the separation layer was noted as the tissues matured or when abscission was imminent. The abscission zone in citrus is not a structurally weak point prior to the onset of abscission.

Abscission of leaves and fruit is accomplished by dissolution of cell wall and/or middle lamellar material, a process which is distinguishable histologically (156, 160) and chemically (45, 121, 160). Actual separation occurs mechanically following enzymatic solubilization of cementing substances.

Biochemistry

The separation process involves any or all of the following events: 1) loss of cementing ability of the middle lamella between cells in the separation layer; 2) dissolution of the middle lamella and all or part of the cellulosic cell wall; 3) mechanical breakage of non-living elements (157). Loss of integrity of the middle lamella has been attributed

to activity of pectin methylesterase (126) and polygalacturonase (121) in *Phaseolus* leaf explants and in orange fruit (19, 80). However, other research has revealed no relationship between pectinase activity and abscission (4, 12, 129, 136).

The role of cellulase in abscission is well established. An increase in cellulase activity was correlated with a decrease in tensile strength in the abscission zones of bean leaves (4, 88), citrus leaves (136), citrus fruit (80, 129), and cotton and *Coleus* leaves (4). The promotion of abscission by ethylene is accompanied by an increase in cellulase activity; both effects are prevented by auxins (4, 92).

Hormonal Regulation of Abscission

It has long been known that leaves become increasingly susceptible to abscission with age (74). The time required for abscission of debladed petioles decreases with age in *Coleus*, cotton, bean, and lupine (43, 44, 100, 124). The decreased time for abscission has been linked with a decrease in diffusible auxin (124, 158). Abscission can be prevented if auxin is applied soon after excision of an abscission zone or debladed petiole, but a delay in the application of auxin allows development of sensitivity to ethylene and a loss of sensitivity to auxin (10, 44, 61, 138). Abscission is prevented in intact leaves if the petiole is ringed with an inhibitor of auxin transport (120). Conversion of auxin to indoleacetylaspartate and other conjugates has been shown by Craker et al (62) to be an important means of decreasing auxin levels in bean explants.

Fruit shows a similar prevention of abscission by various auxins; these are used commercially to prevent preharvest fruit drop (57).

Ethylene is perhaps the principal regulator of abscission in leaves and fruit. Ethylene production increases during maturation of fruit and leaves (25, 92, 103, 126). Internal levels of ethylene in fruit near the abscission zone are a reliable indicator of abscission in citrus (52, 57). Ethylene treatment produces an increase in RNA synthesis and protein synthesis in the abscission zones (9, 12, 86). One protein synthesized as a result of ethylene treatment is cellulase (4, 12, 88, 104, 136). It has been shown that ethylene regulates not only synthesis, but secretion of cellulases from their site of synthesis to their site of action (11, 12).

Light interacts with ethylene in the control of abscission in *Phaseolus* petiole explants. Explants from young, expanding leaves show a marked decrease in rapidity of abscission when incubated in the light compared with dark controls (28). Explants incubated in the dark behaved like light-incubated explants when sucrose was added, indicating that photosynthetates may be responsible for light inhibition of abscission.

Other natural growth regulators have been shown to have effects on abscission in some plants under certain conditions. Kinetin was shown to accelerate abscission of young blue lupine leaflets, but to delay it in older leaflets (43). Osborne and Moss (127) found that cytokinins inhibit abscission only if applied directly to the abscission zone of bean explants. The effect of cytokinins is apparently due to deferral of senescence processes: decline in RNA, protein, and chlorophyll (10).

Gibberellins act to slightly accelerate abscission in cotton, bean, and citrus leaves, but not in citrus fruit (42, 44, 57, 102). This is

probably due to the fact that GA induces ethylene evolution from leaves, but not from fruit.

Abscisic acid has been shown to increase abscission of explants (2, 61) and leaves (57), an effect which was associated with increased ethylene production. ABA also accelerates abscission and ethylene production of orange fruit, and it has been demonstrated that ABA can accelerate abscission and increase cellulase activity even in the absence of ethylene (56, 131).

Artificial Regulation of Abscission

Since ethylene production by the fruit generally precipitates abscission, chemicals which induce ethylene production have been sought as potential abscission agents. Some compounds which cause ethylene production and abscission include ascorbic acid, citric acid, iodoacetic acid, maleic hydrazide, maleimides, 2-hydroxyethylhydrazine, ethephon, and cycloheximide (50). Ethephon and 2-hydroxyethylhydrazine decompose and release ethylene. A great deal of ethylene is released by 2-hydroxyethylhydrazine, but most of it escapes into the air (134). Ethephon enters the fruit and releases ethylene near the abscission zone, but has limited agricultural application due to variable effectiveness under different weather conditions and defoliation at effective concentrations (54, 68).

The other chemicals mentioned act by causing damage to the fruit, resulting in production of wound ethylene (1). Ascorbic and citric acids are safe, but are effective only at high concentrations, are expensive, and cause rind injury (51). Iodoacetic acid causes excessive

rind injury and defoliation. Cycloheximide is one of the most promising abscission agents. If applied directly to the separation zone, abscission is inhibited (129), but application at low concentrations to the fruit causes rind injury, ethylene production, increased cellulase activity, and abscission (51, 130). Cycloheximide has proven effective and economical in mechanical harvesting of early and mid-season oranges, but cannot be used on late season 'Valencia' oranges due to excessive young fruit drop (52, 55).

Another compound which has considerable promise as a commercial abscission agent is Release® (5-chloro-3-methyl-4-nitro-1-H-pyrazole). Release compared favorably with other abscission agents, including cycloheximide, in tests to determine effectiveness in removal of mature fruit while retaining immature fruit fruit and leaves (65). Like cycloheximide, it is more effective on early and mid-season oranges; like many abscission agents, its effectiveness and uniformity of action are affected by temperature, humidity, and rainfall (96).

^{14}C-Release is absorbed fairly well by the flavedo, and most of the radioactivity remains in that tissue, with small amounts being transported to the albedo and pulp (122). It has been suggested that the lysigenous glands act as accumulation sites for Release, where concentrated levels induce ethylene production by surrounding cells (29).

MATERIALS AND METHODS

Plant Materials

'Hamlin' and 'Valencia' oranges (*Citrus sinesis* (L.) Osbeck) were colected at commercial citrus groves in the Orlando area. The 'Hamlin' trees were on Carrizo citrange (*C. sinesis* X *Poncirus trifoliate*) rootstocks; the 'Valencia' trees were on rough lemon (*C. jambhiri* Luch) rootstocks.

Ethylene Determinations

Fruit were collected, brought into the laboratory, washed with a 5% Chlorox solution, and rinsed thoroughly.

When ethylene production from whole fruit was to be measured, the fruit were dipped in the test or control solutions and placed, three apiece, in airtight 3.8 liter containers with Swagelok fittings containing methylsilicone septa. Ethylene samples were collected at intervals, and the containers were flushed with air following each sampling to prevent depletion of oxygen and accumulation of carbon dioxide. Data thus acquired are termed external ethylene production.

Internal ethylene production by intact fruit was measured from beneath the point of stem attachment. Internal ethylene could be measured from fruit remaining on the tree or from detached fruit in the laboratory.

When ethylene production by peel disks was to be measured, explants 10 mm in diameter were cut from the peel of the washed fruit with a cork

borer. If appropriate, the flavedo and albedo were separated by cutting with a scalpel. The disks were treated with the appropriate solutions and placed three apiece in 10 ml vials. The vials were sealed with silicone septa and aluminum caps and covered with aluminum foil to maintain darkness. Gas samples were collected at designated intervals, and the vials were flushed with air after each sampling. Vials were incubated at room temperature unless otherwise specified.

The concentration of ethylene in the gas samples was measured with a Perkin-Elmer 990 Gas Chromotograph equipped with a glass column containing Poropak T. Results were recorded on a Hewlett-Packard Integrator, model 3380A, using an external standard. Calculations were made to report the results of ethylene production in units of µl/hr/sample.

Experiments with Mite Damaged Fruit

Field tests using Pik-Off

Selected fruit in a commercial grove were tagged and dipped in water or 150 ppm Pik-Off. Each fruit was given a numerical designation and rated visually for degree of mite damage, taking into account the density of damage and the proportion of the fruit surface affected. After 1, 2, 3, and 6 days, internal ethylene samples were taken; each fruit was sampled only once to avoid ethylene production stimulated by puncture. On the sixth day, the degree of loosening was determined for each fruit using an Amatek pull force meter. The experiment was performed twice during May, 1978.

Uptake of Pik-Off

Fruit to be used for Pik-Off uptake experiments were harvested, washed, and held overnight at room temperature. An area near the equatorial center of each fruit was chosen for uniform degree of mite damage. These selected areas were used to measure the degree of mite damage and for treatment with radiolabeled Pik-Off. The browning and loss of pigment in the mite damaged area of the fruit results in an alteration of the reflectance characteristics of the peel; these can be used to grade the peel on degree of mite damage (75). It was this characteristic that was used to establish the degree of mite damage on the preselected areas. The reflectance of 580 nm of the selected area of the fruit was measured with a Ratiospect (Agricultural Specialty Co.). The area was than marked with a 1 x 3 cm rectangular stamp. Typical Ratiospect values were 0.29 for undamaged fruit, 0.33 for light damage, 0.35 for medium damage, and 0.38 and up for heavy damage.

The fruit stems were recut and placed in water-filled Aquapiks® (Syndicate Sales, Inc.) to prevent excessive water loss during the six day incubation period. ^{14}C-Pik-Off was applied to the fruit within the stamped area at a rate of 25 µl of solution containing about 22,000 dpm. Non-radioactive Pik-Off was added to the solution to make the concentration 150 ppm. The fruit were incubated in growth chambers at 50% humidity, with a day temperature of 27°C and a night temperature of 16°C.

Fruit were removed at intervals and the treated area was washed first with a water-soaked cotton swab, then with a swab soaked in acetone. Using chloroform, a better solvent for citrus wax, for the second wash had no effect on the radioactivity removed by the swab. The treated area was cut from the peel and frozen.

The cotton swab and peel samples were oxidized in a Packard Sample Oxidizer, model 306, the $^{14}CO_2$ collected in Carbosorb ^{R} (Packard), and the radioactivity determined in a Packard Liquid Scintillation Counter, model 3320.

The uptake experiment was repeated *in toto* three times during May, 1978, with approximately 120 fruit used for each experiment.

Chemicals

Radioactive Pik-Off® (^{14}-ethanedialdioxime) was generously supplied by D. Ryskiewich of Ciba Giegy. The methoxy analog of rhizobitoxine (L-2-amino-4-methoxy-trans-3-butenoic acid) was a gift from A. Stempel of Hoffman LaRoche. Cordycepin (3'-deoxyadenosine) was obtained from Calbiochem. Commerical formulations were used of Release® (5-chloro-3-methyl-4-nitro-1-H-pyrazole) from Abbott Laboratories, Pik-Off® from Ciba Geigy, and Acti-Aid® (cycloheximide) from Upjohn Co.

Statistics

In the experiments measuring ethylene production, where significance is indicated in the text or figure legends, the data were tested for significant differences using the analysis of variance. In the Pik-Off uptake experiments, the data were analyzed by computer. The data curves shown represent linear or quadratic equations generated in the regression analysis.

RESULTS

Ethylene Production by Detached, Whole Fruit

Whole, detached 'Hamlin' fruit enclosed in 3.8 liter glass containers produced barely measurable quantities of ethylene (Figs. 1, 3). Treatment with Release resulted in evolution of considerable quantities of ethylene over a 3 to 4 day period, with increasing concentrations of Release resulting in increased rates of ethylene production (Figs. 1, 2, 3). In 'Hamlin' fruit, the degree of stimulation of ethylene production by 300 ppm Release was about the same in immature (harvested in August) and mature (harvested in November) fruit, but the response of immature fruit to 1000 ppm Release was about twice that of mature fruit.

Acti-Aid, applied at a rate of 25 ppm, was also effective in stimulating considerable production of ethylene by 'Valencia' fruit (Fig. 3). Acti-Aid concentrations used approximated those applied in the field on 'Valencia' trees for reduction in bonding force of the fruit (65). The Release concentrations used are higher than necessary to produce fruit loosening in the field. Effective concentrations of Release for field applications range from 150-250 ppm for 'Hamlin' fruit and 250-500 ppm for 'Valencia' fruit (65).

Figure 4 compares ethylene production by Release-treated 'Valencia' fruit measured as evolution from enclosed fruit and as internal ethylene. The pattern of ethylene production is similar, but when ethylene is measured externally there is a delay before peak ethylene production is

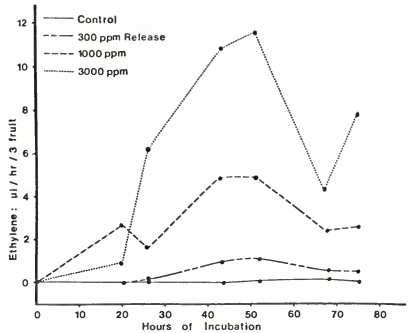


Figure 1. Ethylene production by enclosed, immature 'Hamlin' oranges dipped in water or one of three concentrations of Release.

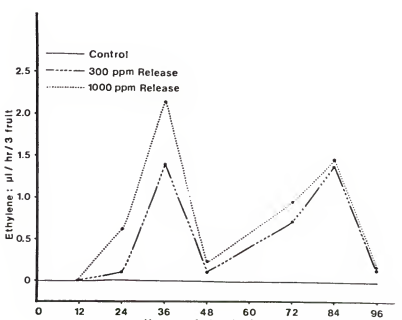


Figure 2. Ethylene production by enclosed, mature 'Hamlin' oranges dipped in water or Release.

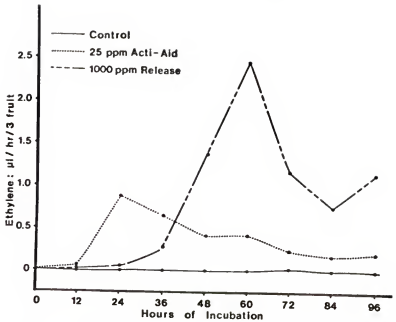


Figure 3. The effects of 25 ppm Acti-Aid and 1000 ppm Release on ethylene production by enclosed 'Valencia' fruit which have achieved an orange color but are internally immature.

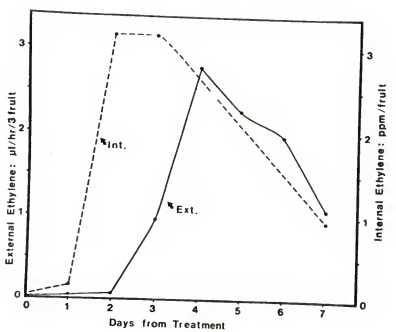


Figure 4. Internal and external ethylene production by 'Valencia' oranges treated with 1000 ppm Release.

detected. This is consistent with data showing that the flavedo does present a barrier to diffusion of ethylene (18). The delay in this particular experiment is longer than in some others (see Figs. 1, 2, 3).

Ethylene Production by Peel Disks

Explants of orange peel provided a versatile system for the study of ethylene production by citrus tissue. Untreated peel tissue from 'Hamlin' or 'Valenica' fruit produced considerable quantities of ethylene, much of it probably due to damage received when the tissue was cut (Figs. 5,6). The disks produced the same amount of ethylene whether or not they were moistened with a few drops of water, so it is unlikely that extracellular ethylene production was occurring.

Peel disks were cut between the pigmented outer layer (flavedo) and the white, spongy inner layer (albedo) to determine the rates of ethylene production of these tissues separately. In mature 'Hamlin' peel, the flavedo, albedo, and whole tissue showed similar rates of ethylene evolution for the first 48 hrs (Fig. 5). The whole disks of mature 'Valencia' peel produced the greatest quantity of ethylene, followed closely by the albedo (Fig. 6). The flavedo produced considerably less ethylene than either the albedo or the whole disks. In neither the 'Hamlin' nor the 'Valencia' peel does the combined ethylene production of the flavedo and the albedo equal that of the whole disks; additional wounding of tissue when the flavedo and albedo are cut apart complicates ethylene production rates.

Effect of Release

Release was applied to the peel disks as a 15 µl drop of the appropriate concentration on the flavedo side. Release applied to the albedo

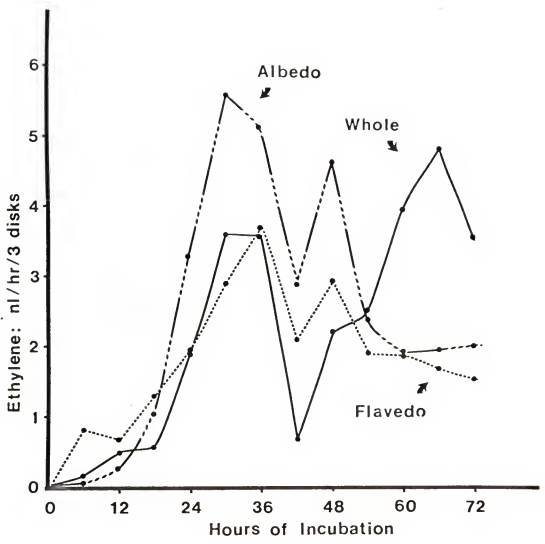


Figure 5. Ethylene production by untreated, whole peel disks, flavedo, and albedo tissue from 'Hamlin' oranges.

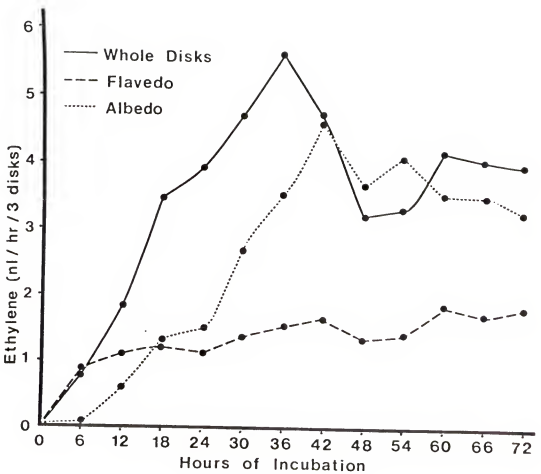


Figure 6. Ethylene production by untreated whole peel disks, flavedo, and albedo tissue from 'Valencia' oranges.

side gives significantly higher ethylene production, probably due to greater and more rapid tissue penetration. Release at 300 ppm (4.5 µg/disk) increased ethylene production in 'Hamlin' peel explants three- to fourfold (Fig. 7). The highest rate of ethylene production occurred on the second day after treatment. Release-treated peel disks from fruit which were still green, but beginning to turn yellow, produced significantly less ethylene than disks from mature, orange colored fruit between 24 and 42 hrs of incubation (Fig. 7). Disks from untreated mature fruit also produced slightly more ethylene than those from immature fruit. Since the fruit labeled "green" were harvested in early November, and "orange" fruit were harvested in late November, there is probably no radical difference between the two in internal fruit maturity. The observed differences are mainly due to the physiological state of the peel, possibly to the hormone balance associated with the presence of photosynthetic units.

'Valencia' peel disks harvested in October, when the fruit is completely green, and March, when the fruit is orange, responded to Release with a three- to fourfold increase in ethylene production (Fig. 8). The differences between similarly treated disks are not significant. Why 'Valencias' do not become increasingly sensitive to Release during maturation is unclear.

The response to Release in citrus peel is localized in the flavedo. Isolated flavedo disks respond to Release with a 5-fold increase in ethylene production, while albedo disks show no significant increase in ethylene production in response to Release (Fig. 9).

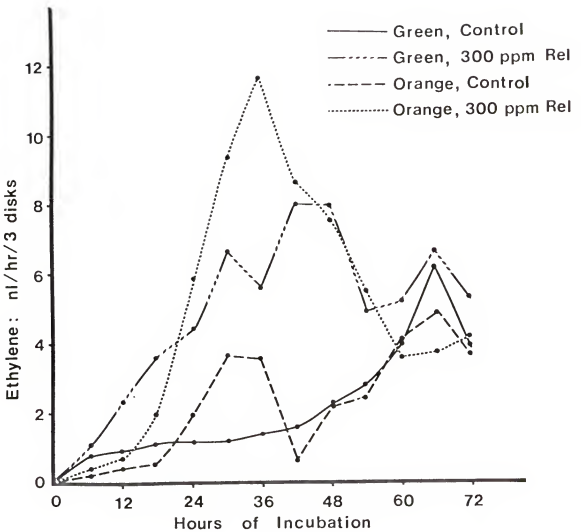


Figure 7. The effect of 300 ppm Release on ethylene production by peel disks from green and orange colored 'Hamlin' fruit.

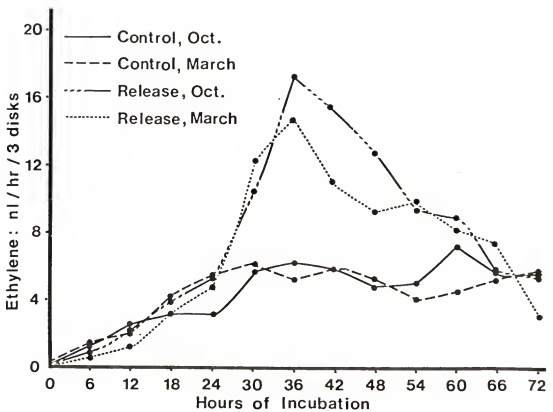


Figure 8. The effect of 1000 ppm Release on ethylene production by peel disks from green colored (harvested in October) and orange colored (harvested in March) 'Valencia' fruit.

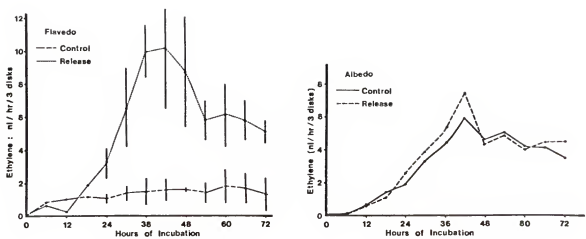


Figure 9. The effect of 1000 ppm Release on ethylene production by flavedo and albedo tissue from 'Valencia' oranges. The differences in ethylene production in control and Release-treated albedo disks are not significant.

Regulation of Ethylene Production in Peel Disks

Temperature has a considerable effect on ethylene production by untreated and Release-treated 'Valencia' peel disks (Fig. 10). Maximum ethylene production was achieved at 25°C in both cases. Very low amounts of ethylene were evolved at 10°C and 15°C (Table 1). The response to Release was strongly affected by temperature; above and below the maximum temperature of 25°C, ethylene production by Release-treated disks approximated control rates. This may be due in part to reduced uptake of Release at lower temperatures (122).

It has been established that citrus fruit do not possess the capacity for autocatalytic ethylene production (14, 67), but no evidence has been presented to show whether ethylene has an inhibitory effect on its own synthesis in citrus. Since the monovalent silver ion, in the form of $AgNO_3$, has been reported to inhibit ethylene action in a variety of plant tissues (24), silver nitrate was applied to peel disks to ascertain the effects on ethylene production. As shown in Figure 11, $AgNO_3$ alone or in combination with Release increases the rate of ethylene production; the combination apparently induces a synergistic response. Although mature fruit are more sensitive to Release, they are slightly less sensitive to silver than the immature fruit.

Increasing the concentration of silver nitrate results in a large increase in ethylene production (Fig. 12). Increasing the concentration of Release to 1000 ppm produced little change in the rate of ethylene production, but the increased rate combined with 50 ppm $AgNO_3$ caused release of very large quantities of ethylene. The combination of 1000 ppm Release and 100 ppm $AgNO_3$ gave lower rates of ethylene production

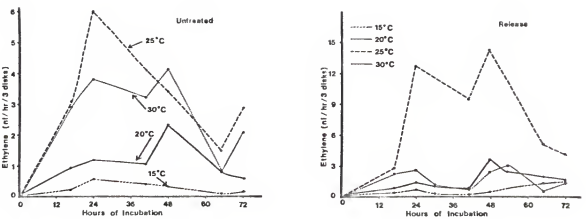


Figure 10. The effect of temperature on ethylene production by untreated and Release (1000 ppm) treated 'Valencia' peel disks.

Table 1. Ethylene production (nl/hr/3 disks) by untreated and Release-treated peel disks incubated at different temperatures.

Temperature	Treatment	Hours of Incubation					
		17	24	41	48	65	72
10°C	Control	0.06	0.14	0.20	0.39	0.15	0.03
	Release	0.12	0.08	0.32	0.51	0.09	0.23
15°C	Control	0.21	0.56	0.40	0.29	0.09	0.14
	Release	0.41	0.76	0.28	0.52	1.3	1.5*
20°C	Control	0.94	1.2	1.05	2.3	0.79	0.60
	Release	0.86	1.4	0.83	3.6	2.0*	1.7
25°C	Control	3.0	6.0	4.1	3.4	1.5	2.8
	Release	2.3	13.4*	9.8	14.5*	4.6	4.0
30°C	Control	2.9	3.8	3.2	4.1	0.81	1.7
	Release	2.3	2.6	0.84	2.5	0.62	1.3

* Indicates that the value is significantly different at the 5% level from the corresponding control value.

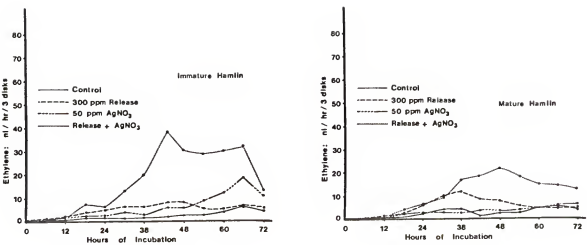


Figure 11. The effects of Release and $AgNO_3$ on ethylene production by disks from immature and mature 'Hamlin' oranges.

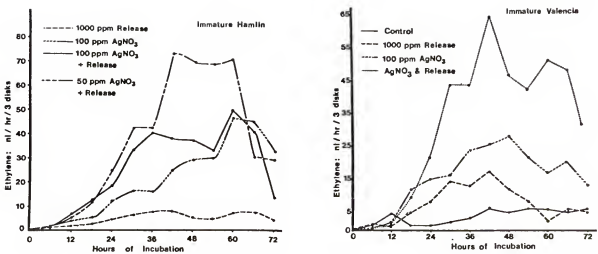


Figure 12. The effects of 1000 ppm Release combined with two concentrations of $AgNO_3$ on disks of immature 'Hamlin' fruit, and combined with one concentration of $AgNO_3$ on disks of immature 'Valencia' fruit.

than the 1000 ppm Release + 50 ppm $AgNO_3$ combination, perhaps due to excessive tissue damage.

'Valencia' peel disks respond similarly to silver and Release (Fig. 12).

Propylene is an ethylene analog which is about 100 times less effective than ethylene (36). Propylene was added to jars containing whole fruit and to vials containing peel disks, and the effects on ethylene production were observed. Propylene had no significant effect on ethylene production by whole fruit or disks (Figs. 13, 14). This was true regardless of the maturity of the fruit. The data on propylene effects indicate that ethylene has neither an autocatalytic nor an autoinhibitory effect on its own synthesis in citrus. The silver effect is probably a toxicity effect; heavy metals are known to cause stress which results in ethylene production (1, 59).

The methoxy analog of rhizobitoxine was added to peel disks to determine whether methionine is the precursor of ethylene in citrus peel. Rhizobitoxine at 10 µg/disk nearly blocked ethylene production altogether (Fig. 15) in both untreated and Release-treated peel disks. Rhizobitoxine also blocked ethylene production in flavedo and albedo tissue (data not shown). This indicates that methionine is probably the precursor of ethylene in these tissues.

In some cases, it has been shown that addition of methionine to an ethylene-production system will accelerate ethylene production (108). Efforts to stimulate ethylene production in citrus tissue with methionine were inconclusive. Methionine at 10^{-7} M had no effect on ethylene production by whole disks, flavedo, or albedo tissues. When 10^{-5} M methionine was added to the peel disks, ethylene production was increased

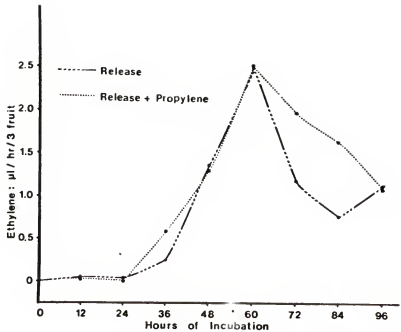


Figure 13. Ethylene production by whole enclosed 'Valencia' fruit treated with Release and incubated with or without 250 ppm propylene. The fruit were harvested in late January.

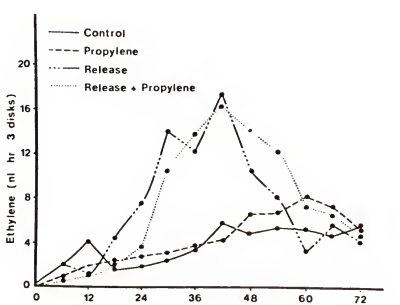


Figure 14. The effects of 150 ppm propylene on ethylene production by untreated and Release (1000 ppm) treated 'Valencia' peel disks. The differences between air and propylene treatments were not significant.

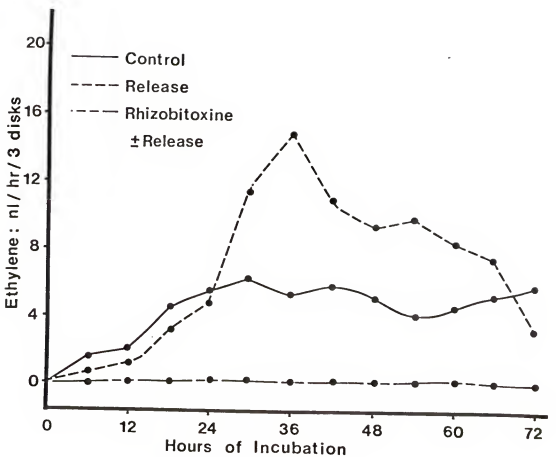


Figure 15. The effect of rhizobitoxine (10 µg/disk) on ethylene production by untreated and Release (1000 ppm) treated 'Valencia' peel disks.

significantly between 24 and 36 hrs of incubation (Fig. 16). However, ethylene production from flavedo and albedo tissues was actually reduced.

Hyodo (90) was able to demonstrate conversion of labeled methionine to labeled ethylene is aged, but not fresh, albedo tissue. The conversion was demonstrated during the period when the tissue produced the maximum quantity of ethylene. Since maximum ethylene production occurred between 24 and 48 hrs in our system, peel disks were aged 18 hrs, then treated with water or 10^{-5} M methionine. The addition of methionine increased ethylene production significantly during the first four hours after treatment, but produced erratic results thereafter (Fig. 17). It is possible that the methionine pool in the tissue is small and rapidly turned over; in this case, additional methionine would have only a limited effect.

One of the lines of evidence supporting the hypothesis that a metal cofactor is required for ethylene synthesis from methionine is the inhibiting effect of metal chelators such as EDTA (108, 147). Disodium EDTA applied to the albedo side of the citrus peel disks strongly inhibited ethylene production (Fig. 18). Inhibition increased as the concentration of EDTA was increased.

Dinitrophenol (DNP), an uncoupler of oxidative phosphorylation, at concentrations which reduced respiration and ethylene production in apple tissue, also reduced ethylene production in citrus peel disks (Fig. 19). The differences among the concentrations used were not significant, with the exception of the sharp rise at 72 hrs for 10^{-4} M

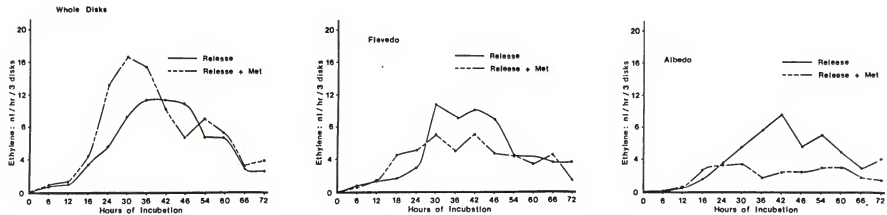


Figure 16. The effects of 10^{-5} M methionine on ethylene production by Release (1000 ppm) treated whole disks, flavedo, and albedo from 'Valencia' oranges.

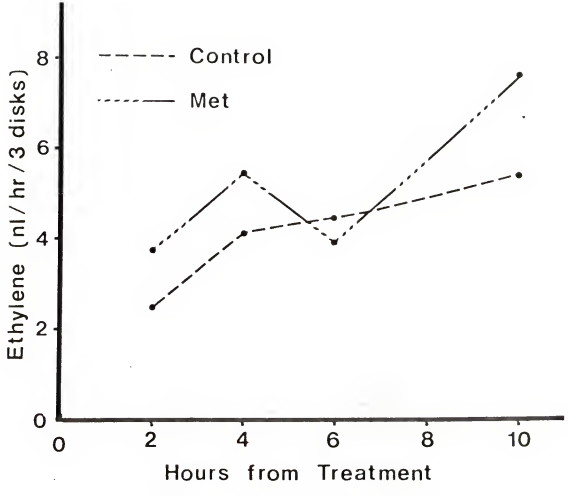


Figure 17. The effect of 10^{-5} M methionine on ethylene production from 'Valencia' peel disks which were aged for 18 hrs before treatment.

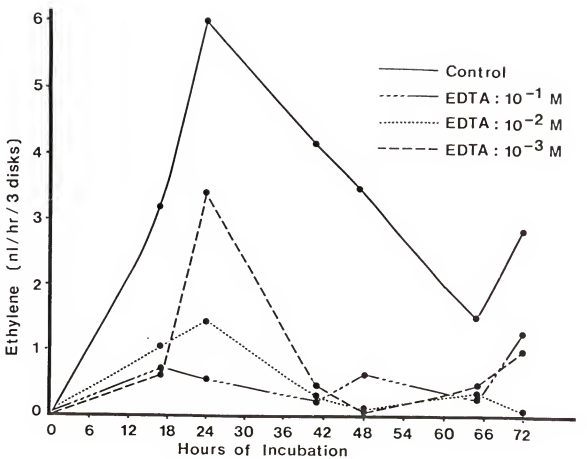


Figure 18. The effects of three concentrations of EDTA on ethylene production by 'Valencia' disks.

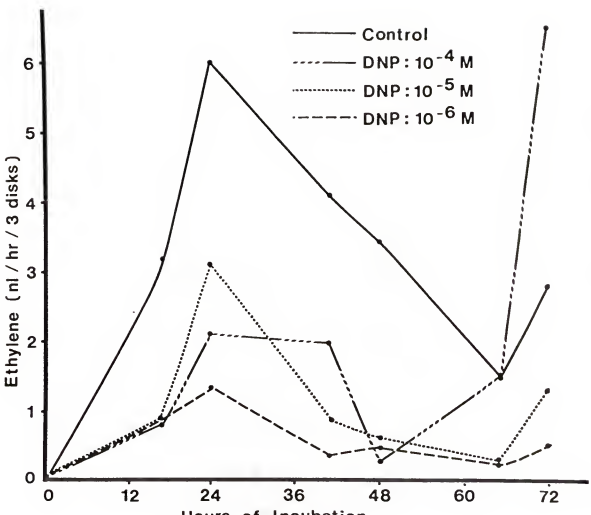


Figure 19. The effects of three concentrations of dinitrophenol (DNP) on ethylene production by 'Valencia' peel disks.

DNP. This rise could signal a recovery from the DNP effects with an "overshoot" in ethylene production like that observed when tissues are removed from anaerobic to aerobic atmospheres (39).

It is known that protein synthesis is required for ethylene production (1, 107). Cordycepin, an inhibitor of post-transcriptional RNA processing, had an inhibitory effect on ethylene production by Release-treated peel disks, but had no significant effect on ethylene production by untreated tissue (Fig. 20). It seems that the higher rates of ethylene production elicited by Release require synthesis and processing of RNAs which are not required for the lower rate of ethylene synthesis. The protein synthesis inhibitor cycloheximide, in the form of 25 ppm Acti-Aid, blocked ethylene synthesis almost completely in untreated and Release-treated citrus peel disks (data not shown). An ethylene producing system in apple reacted similarly to cycloheximide (107).

Effects of Regreening

'Valencia' fruit which have achieved a good orange color during the early spring may undergo regreening during May or June. The extent and time of regreening varies from grove to grove and from season to season. Treatments with abscission accelerating chemicals on regreened fruit generally fail to produce adequate fruit loosening (46, 53, 87). Holm and Wilson (87) found that fruit were relatively unresponsive to abscission compounds early in the regreening period, but that responsiveness increased later, even though the fruit continued to regreen.

The effects on ethylene production of various concentrations of Release and Pik-Off are shown in Figure 21. Fruit at a commercial grove

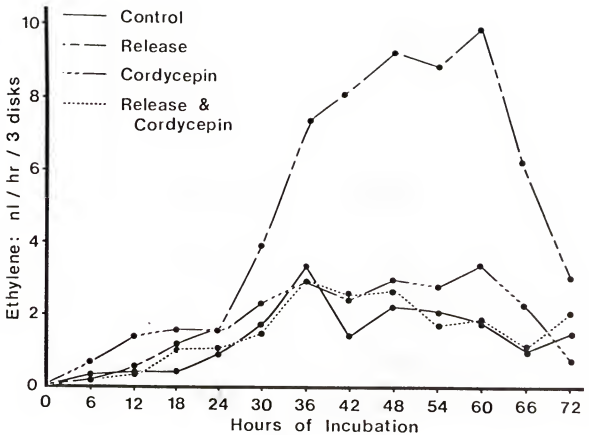


Figure 20. The effects of 10 µg/disk cordycepin on ethylene production by control and Release (1000 ppm) treated 'Valencia' disks.

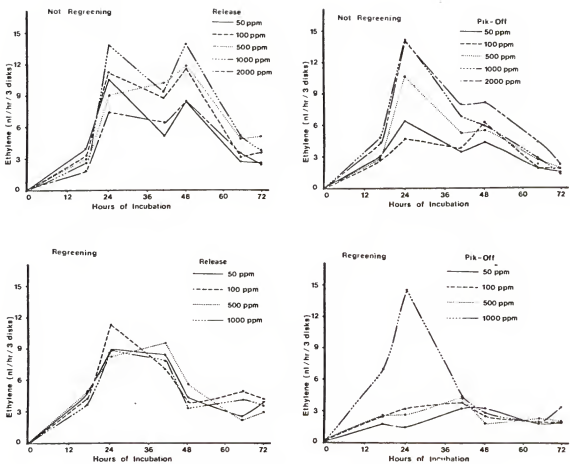


Figure 21. The effects of several concentrations of Release and Pik-Off on ethylene production by peel disks from regreening and non-regreening 'Valencia' fruit.

were observed to be regreening in early June; disks from these fruit and from non-regreening fruit from another location collected on the same dates were used in the experiment. Disks from fruit which were not regreening showed increasing ethylene response to increasing concentrations of Release and Pik-Off. The highest concentration (2000 ppm) of Release and Pik-Off reduced ethylene production compared with 1000 ppm; this may be due to excessive tissue damage and cell death at the highest concentration. Regreening peel disks showed about the same response to all concentrations of Release used; this response was generally lower than that in non-regreening fruit (Fig. 22). The reduced response to Pik-Off in regreening fruit was even more dramatic, especially at the lower concentrations (Fig. 21, 23). The fact that regreened fruit treated with abscission compounds produce less ethylene than non-regreened fruit may be partially responsible for the reduced loosening. However, those hormonal changes associated with the onset of regreening may also alter the sensitivity of the abscission zone to ethylene.

The Effects of Mite Damage on Ethylene Production and Uptake of Pik-Off by 'Valencia' Fruit

Heavy feeding on citrus fruit by rust mites is associated with morphological changes in the peel, including lignification and death of the most heavily affected areas (112). The presence of damaged tissue could affect the uptake and effectiveness of abscission chemicals applied to the peel surface. The effect of mite damage on ethylene production by peel disks is shown in Figure 24. These data were rather

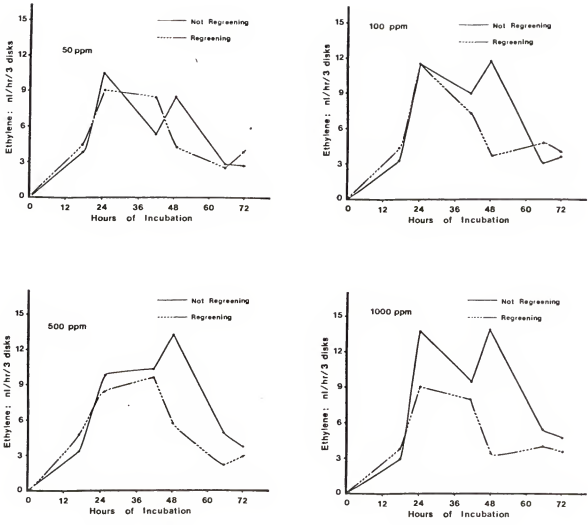


Figure 22. Differences in ethylene production by peel disks from regreening and non-regreening 'Valencia' fruit treated with several concentrations of Release.

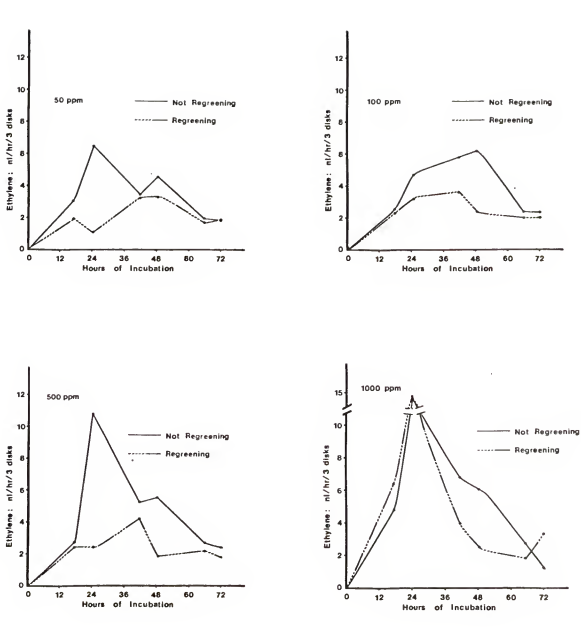


Figure 23. Ethylene production by peel disks from regreening and non-regreening 'Valencia' fruit treated with several concentrations of Pik-Off.

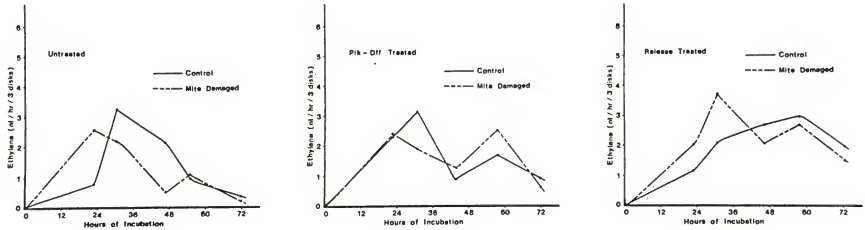


Figure 24. The effect of mite damage on ethylene production by untreated, Release (125 ppm) treated, and Pik-Off (150 ppm) treated 'Valencia' peel disks.

variable and consequently there was no significant difference in ethylene production due to mite damage.

The response of whole fruit to Pik-Off is affected by mite damage. When internal ethylene was measured from Pik-Off treated fruit in a commercial grove, fruit with medium and heavy mite damage showed more than double the rate of ethylene production on the first day after treatment (Fig. 25). The degree of fruit loosening, measured as the force required to detach the fruit from the stem, was also determined for the mite damaged fruit. Mite damage did not affect the bonding force of untreated fruit, but the attachment force in fruit treated with 150 ppm Pik-Off was considerably less in mite damaged fruit (Table 2).

Fruit were brought into the laboratory to determine whether mite damage had any effect on uptake of Pik-Off. When ^{14}C-Pik-Off was applied to the peel, much of the radioactivity was taken into the peel tissue during the first day after application (Fig. 26). The degree of mite damage, as measured by the Ratiospect reading, did have a significant effect on the proportion of ^{14}C-labeled material isolated from the tissue. The differences were very small on days 2, 3, and 6; only on the first day was the effect large enough to be important (Fig. 27). The data indicate that mite damaged fruit take up Pik-Off more quickly than undamaged fruit.

The total amount of radioactivity recovered, i.e. tissue cpm + surface washes cpm, was greater in mite damaged fruit than in undamaged fruit (Fig. 28). This is probably because the loss in total counts as the incubation period progressed was largely due to the Pik-Off rubbing off the surface onto other fruit or the holding trays.

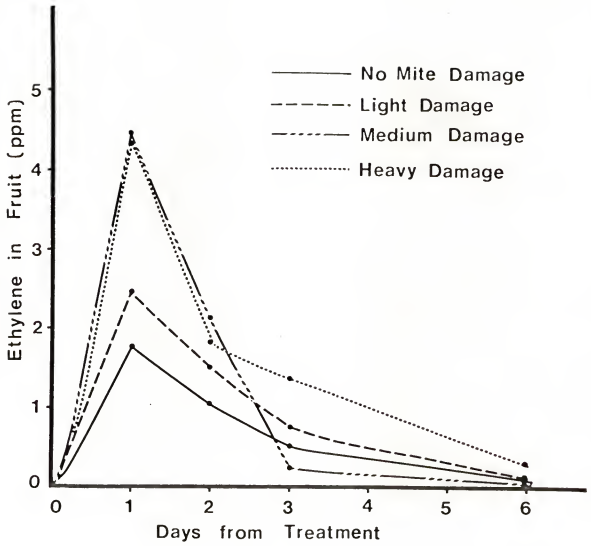


Figure 25. The effect of various degrees of mite damage on internal ethylene in Pik-Off (150 ppm) treated 'Valencia' oranges in the field.

Table 2. Pull force (in kg) of untreated and Pik-Off treated 'Valencia' oranges exhibiting various degrees of mite damage.

Mite Damage	Treatment	
	Untreated	150 ppm Pik-Off
None	2.7 a*	2.1 a
Light	3.4 a	1.4 ab
Medium	3.5 a	0.7 b
Heavy	3.1 a	1.3 b

*Numbers within columns followed by the same letter are not significantly different at the 5% level according to Duncan's New Multiple Range Test.

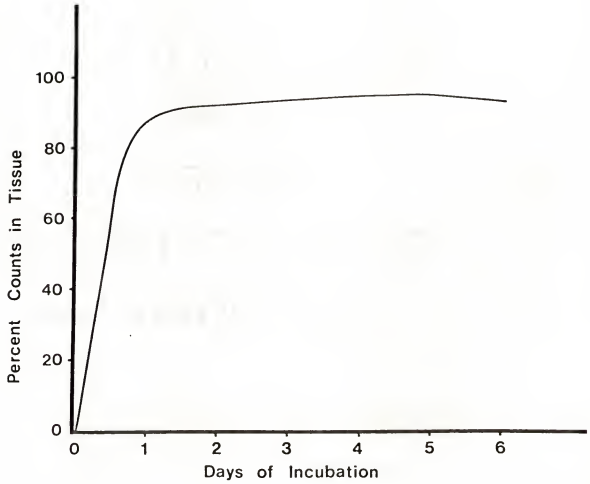


Figure 26. Uptake of ^{14}C-Pik-Off by 'Valencia' peel tissue over a six day period. The percent counts in the tissue was calculated by dividing the cpm found in the washed tissue by the total counts recovered (tissue cpm + two washes with cotton swabs).

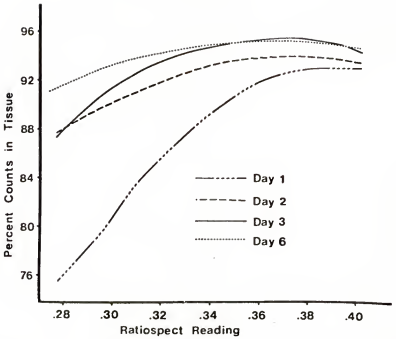


Figure 27. Dependence of ^{14}C-Pik-Off uptake on degree of mite damage during a six day incubation period. Higher Ratiospect Readings indicate a greater degree of mite damage.

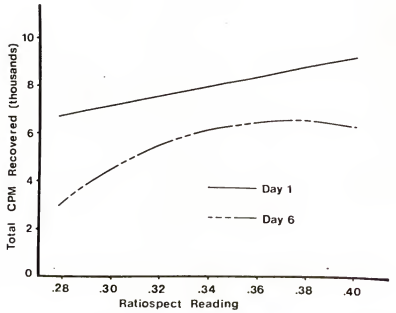


Figure 28. The effect of mite damage, measured by Ratiospect readings, on total radioactivity recovered in the first and sixth day after treatment of 'Valencia' fruit with ^{14}C-Pik-Off. Total radioactivity recovered (cpm) is the sum of the cpm found in washed peel tissue and cpm found on the cotton swabs used to wash the tissue.

DISCUSSION

The physiology of ethylene production by citrus peel disks in many respects resembles that of other systems which have been investigated. Cut peel tissue produced easily measurable quantities of ethylene, most of which was probably due to wounding. Intact, undamaged oranges produced very little ethylene. Hyodo (89, 90) obtained a similar response from whole Satsuma mandarin (*Citrus unshiu* Marc,) fruit and from excised flavedo and albedo tissues.

Treatment of whole fruit and peel disks with Release caused evolution of greater quantities of ethylene. The response to Release was localized primarily in the flavedo tissue. 'Hamlin' peel disks were slightly more sensitive to Release after achieving an orange color, while 'Valencia' disks were not.

Protein synthesis was required for ethylene production by citrus peel; this was also true of etiolated pea seedlings (73), albedo tissue from Satsuma mandarin (90), apple tissue slices (107) and mung bean hypocotyl segments (139). The role of RNA in ethylene synthesis is less clear. Lieberman and Kunishi (107) found that ethylene production by apple slices and pea subhook segments was inhibited by actinomycin D, which inhibits DNA-directed RNA synthesis, α-amanitin, which inhibits nuclear RNA polymerase II, and cordycepin, which inhibits post-transcriptional processing of RNA. Our data demonstrated that cordycepin had an inhibitory effect on ethylene production by Release-treated, but

not untreated 'Valencia' peel disks. Hyodo (90) found that actinomycin D had no effect on ethylene production in aged albedo disks of Satsuma mandarin. Hyodo measured the rate of ethylene production due only to wounding. The pea and apple tissues Lieberman used were producing high quantities of ethylene, the pea tissue because it was auxin-treated, the apple tissue because it was post-climacteric. In the present study, cordycepin did not inhibit ethylene production stimulated by cutting the tissue, but did inhibit ethylene production stimulated by Release. RNA synthesis is probably required for the higher levels of ethylene synthesis induced by treatment with Release. The tissue probably contains a stored system which is capable of directing synthesis of the lesser quantities of ethylene produced as a result of wounding.

Our results with rhizobitoxine, and Hyodo's (90) results demonstrating conversion of labeled methionine to ethylene in Satsuma mandarin albedo tissue, indicate that methionine is the precursor of ethylene in citrus tissue. The fact that exogenous methionine caused very little increase in ethylene production in our system is not surprising. Baur et al (22) found that the size of the methionine pool was not a controlling factor in ethylene production by avocado tissue. The small pool of methionine had to be actively synthesized and utilized during periods of rapid ethylene synthesis. Sakai and Imaseki (140) were able to demonstrate efficient conversion of ^{14}C-methionine to ^{14}C-ethylene in auxin-treated mung bean hypocotyl segments, even though the addition of cold methionine did not enhance ethylene production.

The inhibition of ethylene production in citrus peel by DNP, an uncoupler of oxidative phosphorylation, indicates an ATP requirement

for ethylene production. DNP also inhibited ethylene production in apple fruit tissue and mung bean hypocotyl sections (40, 123, 147). Burg and Thimann (40), but not Murr and Yang (123), were able to reverse the DNP inhibition by adding ATP to the tissue. These results have been questioned on the charge that DNP contains an impurity which may interfere with ethylene synthesis in an unknown manner.

Ethylene synthesis from methionine is thought to involve a metal cofactor. Most model systems for ethylene production utilize a heavy metal in the reaction mixture (20, 109, 148, 161). EDTA, a strong metal chelator, inhibited ethylene synthesis in the citrus peel system and in apple tissue slices (108, 147). Since compounds which specifically chelate copper also inhibited ethylene production in apple tissue, it was suggested that the metal involved was copper (108). Additional work is needed to establish the involvement of a metal cofactor, since EDTA would be expected to inhibit all metal-requiring enzymes, including many not directly involved with ethylene production.

The effects of temperature on ethylene production by peel disks were consistent with other systems studied. The temperature optimum for ethylene production in the citrus peel explants was between 20°C and 30°C; the optimum was 30°C for apples (39), and 20°C for pears (83) and tomatoes (117). Ethylene evolution by Release-treated disks was more strongly dependent on temperature than untreated disks. Release-treated disks did not produce greater quantities of ethylene than controls except at 25°C, when ethylene production by Release-treated disks was considerably higher than at the other temperatures. The

strong temperature dependence may be due to changes in uptake of Release at higher temperatures (122). Other metabolic events involved in Release-induced ethylene production may be especially temperature dependent, such as Release breakdown in the tissue, or metabolism of RNAs for the increased ethylene synthesis.

Citrus fruit differ from many other fleshy fruits in that they do not exhibit a respiratory climacteric. The fruit ripen over a period of several months; internal events associated with ripening are unaccompanied by increased respiration or ethylene production. Ethylene produces neither an autocatalytic nor an autoinhibitory response in citrus. Ethylene has no known effect on internal quality of citrus during ripening (6, 125), but it is involved in peel color development and abscission. Ethylene production enhanced by chilling (58) may be responsible for degreening, which occurs during the winter in mature early varieties, such as 'Hamlin', and in immature late varieties, such as 'Valencia' (70). Early varieties may also abscise during the winter, but immature 'Valenica' fruit normally do not; these fruit may remain on the tree until the following winter. Immature 'Hamlin' fruit from a late bloom will also remain on the tree, indicating that fruit maturity, rather than varietal differences, is the controlling factor in natural abscission. Fruit of any age can be induced to abscise by chemical treatment or stress.

There are other fruit in which ethylene regulates abscission but not internal ripening. Grapes are non-climacteric fruit in which the onset of a series of ripening changes termed "veraison" is controlled by growth regulators. Ethylene concentration does not increase during

veraison, and although all exogenous hormone treatments resulted in increased ethylene production, some hastened and some delayed veraison (49). Ethylene had no effect on ripening in olives, but it did increase the rate of cholorophyll destruction (119). Color development in cranberries was controlled by ethylene and light, but ethylene had no effect on respiration or sugar and acid content (60,66).

Regreened fruit had reduced sensitivity to abscission chemicals, both in the field (87) and as treated peel disks. Factors involved in onset of regreening include temperature (41, 70), nitrogen nutrition (93), and an increase in levels of gibberellic acid (130). The increase in GA, combined with events of the regreening process, could alter the balance of other hormones as well, resulting in decreased efficiency of the ethylene-producing system and decreased sensitivity of the abscission zone to ethylene.

Mite damaged fruit produced more ethylene and had reduced bonding force in response to Pik-Off when compared with undamaged fruit. The difference in ethylene production was greatest on the first day after treatment; this was also the time when differences in uptake of Pik-Off were greatest between mite-damaged and undamaged fruit. It is possible that, in addition to allowing greater uptake of the abscission chemical, the damaged area of the peel also responds more readily to Pik-Off by producing larger quantities of ethylene.

Response of fruit in the field to Pik-Off has been variable; often Pik-Off alone will not produce adequate fruit loosening (65, 133). In this experiment, adequate fruit loosening was obtained with both mite damaged and undamaged fruit. Efficient mechanical harvesting requires that fruit removal force be 5 lbs (2.2 kg) or less (133). Since this

is not always the case, the disproportionate loosening of the mite damaged fruit may be a factor which has to be considered when using abscission chemicals in a damaged grove. The altered morphology and physiology of mite damaged fruit may also cause hypersensitive responses to other chemical treatments, for example, fungicides and insecticides.

SUMMARY

Peel disks from citrus fruit produced easily measurable quantities of ethylene, most of it probably due to wounding of the tissue when the disks were cut. The rate of ethylene production could be increased by treatment with abscission accelerating chemicals such as Release or Pik-Off, or by treatment with toxic compounds such as silver nitrate.

Ethylene had neither an autocatalytic nor an autoinhibitory response in citrus tissue. Ethylene evolution by citrus tissue was temperature dependent; the temperature optimum for ethylene production by both untreated and Release-treated disks was about 25°C. The ethylene producing system in Release-treated disks was more sensitive to temperature than that of untreated disks.

Ethylene evolution was almost completely blocked in the peel disks by cycloheximide, indicating that protein synthesis is required for ethylene synthesis. Our results with cordycepin indicate that RNA synthesis is required for the higher rates of ethylene synthesis resulting from Release treatment, but not for ethylene production due only to cutting the tissue.

Ethylene production by untreated and Release-treated citrus peel disks was blocked by rhizobitoxine, indicating that methionine is the probable precursor to ethylene in this tissue. Addition of exogenous methionine to the tissue produced only a small increase in ethylene

production. Methionine is probably not the limiting factor in ethylene production.

An ATP requirement for ethylene synthesis is shown by the depressing effect of dinitrophenol on ethylene production. Ethylene evolution was also reduced by EDTA, supporting the hypothesis that a metal cofactor is required for ethylene synthesis.

Citrus peel disks, like whole fruit, show a reduced response to abscission chemicals during the regreening period. Several concentrations of Release and Pik-Off were applied to peel disks from regreening and non-regreening fruit; ethylene production by disks of regreening fruit was consistently lower. It is suggested that the hormonal balance is responsible for the reduced sensitivity to abscission chemicals.

Whole citrus fruit which show visible citrus rust mite damage responded to Pik-Off with a greater ethylene production and fruit loosening than undamaged fruit. Uptake of ^{14}C-Pik-Off was more rapid in mite damaged fruit than in undamaged fruit. Uptake of Pik-Off in both undamaged and mite damaged fruit was very rapid furing the first 24 hrs after treatment, with very little uptake occurring after that time.

LITERATURE CITED

1. Abeles, A. L. and F. B. Abeles. 1972. Biochemical pathway of stress-induced ethylene. *Plant Physiology* 50: 496-498.

2. Abeles, F. B. 1967. Mechanism of action of abscission accelerators. *Physiologia Plantarum* 20: 442-454.

3. Abeles, F. B. 1968. Role of RNA and protein synthesis in abscission. *Plant Physiology* 43: 1577-1586.

4. Abeles, F. B. 1969. Abscission: role of cellulase. *Plant Physiology* 44: 447-452.

5. Abeles, F. B. 1972. Biosynthesis and mechanism of action of ethylene. *Annual Review of Plant Physiology* 23: 259-292.

6. Abeles, F. B. 1973. Ethylene in Plant Biology. Academic Press.

7. Abeles, F. B., L. E. Craker, and G. R. Leather. 1971. Abscission: the phytogerontological effects of ethylene. *Plant Physiology* 47: 7-9.

8. Abeles, F. B. and H. E. Gahagan. 1968. Abscission: the role of ethylene, ethylene analogues, carbon dioxide, and oxygen. *Plant Physiology* 43: 1255-1258.

9. Abeles, F. B. and R. E. Holm. 1966. Enhancement of RNA synthesis, protein synthesis, and abscission by ethylene. *Plant Physiology* 41: 1337-1342.

10. Abeles, F. B., R. E. Holm, and H. E. Gahagan. 1967. Abscission: the role of aging. *Plant Physiology* 42: 1351-1356.

11. Abeles, F. B. and G. R. Leather. 1971. Abscission: control of cellulase secretion by ethylene. *Planta* 97: 87-91.

12. Abeles, F. B., G. R. Leather, L. E. Forrence, and L. E. Craker. 1971. Abscission: regulation of senescence, protein synthesis, and enzyme secretion by ethylene. *HortScience* 6: 371-376.

13. Abeles, F. B. and B. Rubenstein. 1964. Regulation of ethylene evolution and leaf abscission by auxin. *Plant Physiology* 39: 963-969.

14. Aharoni, Y. 1968. Respiration of oranges and grapefruits harvested at different stages of development. *Plant Physiology* 43: 99-102.

15. Aharoni, Y., F. S. Lattar, and S. P. Monselise. 1969. Post-harvest response of oranges to ethylene. *Plant Physiology* 44: 1473-1474.

16. Archer, S. A. and E. C. Hislop. 1975. Ethylene in host-pathogen relationships. *Annals of Applied Biology* 81: 121-126.

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

18. Barmore, C. R. and R.H. Biggs. 1972. Ethylene diffusion through citrus leaf and fruit tissue. *Journal of the American Society for Horticultural Science* 97: 24-27.

19. Basiouny, F. M. and R. H. Biggs. 1976. Pectin-polygalacturonase in *Citrus*. *Planta* 128: 271-273.

20. Baur, A. H. and S. F. Yang. 1969. Precursors of ethylene. *Plant Physiology* 44: 1347-1349.

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

22. Baur, A. H., S. F. Yang, H. K. Pratt, and J. B. Biale. 1971. Ethylene biosynthesis in fruit tissues. *Plant Physiology* 47: 696-699.

23. Ben-Yehoshua, S. and R. H. Biggs. 1970. Effects of iron and copper ions in promotion of selective abscission and ethylene production by citrus fruit and the inactivation of indoleacetic acid. *Plant Physiology* 45: 604-607.

24. Beyer, E. M. 1976. A potent inhibitor of ethylene action in plants. *Plant Physiology* 58: 268-271.

25. Beyer, E. M. and P. W. Morgan. 1971. Abscission: the role of ethylene modification of auxin transport. *Plant Physiology* 48: 208-212.

26. Biale, J. B., R. E. Young, and A. J. Olmstead. 1954. Fruit respiration and ethylene production. *Plant Physiology* 29: 168-174.

27. Biggs, R. H. 1971. Citrus abscission. *HortScience* 6: 388-392.

28. Biggs, R. H. and A. C. Leopold. 1957. Factors influencing abscission. *Plant Physiology* 32: 626-632.

29. Biggs, R. H. and S. K. Murphy. 1978. Fruit abscission and chemical aids to harvest. *Proceedings of the International Citrus Congress*. In Press.

30. Burdett, A. N. 1972. Ethylene synthesis in lettuce seeds: its physiological significance. *Plant Physiology* 50: 719-722.

31. Burg, S. P. 1962. The physiology of ethylene formation. *Annual Review of Plant Physiology* 13: 265-302.

32. Burg, S. P. and E. A. Burg. 1962. Role of ethylene in fruit ripening. *Plant Physiology* 37: 179-189.

33. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. *Science* 148: 1190-1196.

34. Burg, S. P. and E. A. Burg. 1965. Relationship between ethylene production and ripening in bananas. *Botanical Gazette* 126: 200-204.

35. Burg, S. P. and E. A. Burg. 1966. Auxin-induced ethylene formation: its relation to flowering in the pineapple. *Science* 152: 1269.

36. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. *Plant Physiology* 42: 144-152.

37. Burg, S. P. and E. A. Burg. 1968. Ethylene formation in pea seedlings; its relation to the inhibition of bud growth caused by indole-3-acetic acid. *Plant Physiology* 43: 1069-1074.

38. Burg, S. P. and C. O. Clagett. 1967. Conversion of methionine to ethylene in vegetative tissue and fruits. *Biochemical and Biophysical Research Communications* 27: 125-130.

39. Burg, S. P. and K. V. Thimann. 1959. The physiology of ethylene formation in apples. *Proceedings of the National Academy of Science* 45: 335-344.

40. Burg, S. P. and K. V. Thimann. 1960. Studies on the ethylene production of apple tissue. *Plant Physiology* 35: 24-35.

41. Caprio, J. M. 1956. An analysis of the relation between regreening of Valencia oranges and mean monthly temperatures in southern California. *Proceedings of the American Society for Horticultural Science* 67: 222-235.

42. Carns, H. R., F. T. Addicott, K. C. Baker, and R. K. Wilson. 1961. Acceleration and retardation of abscission by gibberellic acid. In: Plant Growth Regulation, R. M. Klein, Ed. Iowa State University Press. p. 559-565.

43. Carr, D. J. and W. J. Burrows. 1967. Studies in leaflet abscission of blue lupine leaves. I. Interaction of leaf age, kinetin and light. *Planta* 73: 357-368.

44. Chatterjee, S. K. And A. C. Leopold. 1965. Changes in abscission processes with aging. *Plant Physiology* 40: 96-101.

45. Chaudhri, S. A. 1957. Some anatomical aspects of fruit drop in citrus. Ph.D. Dissertation, University of Florida.

46. Clark, R. K. and W. C. Wilson. 1975. The effect of several adjuvants on the abscission activity of Release with 'Valencia' orange. *Proceedings of the Florida State Horticultural Society* 88: 100-103.

47. Coggins, C. W. and L. N. Lewis. 1962. Regreening of 'Valencia' orange as influenced by potassium gibberellate. *Plant Physiology* 37: 625-627.

48. Collins, R. P. and R. P. Scheffer. 1958. Respiratory responses and systemic effects in *Fusarium*-infected tomato plants. *Phytopathology* 48: 349-355.

49. Coombe, B. G. 1973. The regulation of set and development of the grape berry. *Acta Horticulturae* 34: 261-273.

50. Cooper, W. C. and W. H. Henry. 1968. Effect of growth regulators on the coloring and abscission of citrus fruit. *Israel Journal of Agricultural Research* 18: 161-174.

51. Cooper, W. C. and W. H. Henry. 1968. Field trials with potential abscission chemicals as an aid to mechanical harvesting of citrus in Florida. *Proceedings of the Florida State Horticultural Society* 81: 62-68.

52. Cooper, W. C. and W. H. Henry. 1971. Abscission chemicals in relation to citrus fruit harvest. *Journal of Agricultural and Food Chemistry* 19: 559-563.

53. Cooper, W. C. and W. H. Henry. 172. Effect of growth regulators on the response of citrus fruit to cycloheximide-induced abscission. *Proceedings of the Florida State Horticultural Society* 85: 29-32.

54. Cooper, W. C. and W. H. Henry. 1973. Chemical control of fruit abscission. In: Shedding of Plant Parts, T. T. Kozlowski, Ed. Academic Press. p. 476-524.

55. Cooper, W. C., W. H. Henry, and C. J. Hearn. 1970. Ethylene production by the 'Valencia' orange tree as related to the use of abscission chemicals. *Proceedings of the Florida State Horticultural Society* 83: 89-92.

56. Cooper, W. C. and G. Horanic. 1973. Induction of abscission at hypobaric pressures. *Plant Physiology* 51: 1002-1004.

57. Cooper, W. C., G. K. Rasmussen, B. J. Rogers, P. C. Reece, and W. H. Henry. 1968. Control of abscission in agricultural crops and its physiological basis. *Plant Physiology* 1560-1576.

58. Cooper, W. C., G. K. Rasmussen, and E. S. Waldon. 1969. Ethylene evolution stimulated by chilling in *Citrus* and *Persea* Sp. *Plant Physiology* 44: 1194-1196.

59. Cooper, W. C. and P. C. Reece. 1969. Effect of chilling on ethylene production, senescence, and abscission in leaves of evergreen and deciduous fruit trees. *Proceedings of the Florida State Horticultural Society* 82: 270-274.

60. Craker, L. E. 1971. Postharvest color promotion in cranberry with ethylene. *HortScience* 6: 137-139.

61. Craker, L. E. and F. B. Abeles. 1969. Abscission: role of abscisic acid. *Plant Physiology* 44: 1144-1149.

62. Craker, L. E., A. V. Chadwick, and G. R. Leather. 1970. Abscission: movement and conjugation of auxin. *Plant Physiology* 45: 790-793.

63. Crocker, W., A. E. Hitchcock, and P. W. Zimmerman. 1935. Similarities in the effects of ethylene and the plant auxins. *Contributions from the Boyce Thompson Institute* 7: 231-248.

64. Cronshaw, K. K. and G. F. Pegg. 1976. Ethylene as a toxin synergist in *Verticillium* wilt of tomato. *Physiological Plant Pathology* 9: 33-44.

65. Davies, F. S., W. C. Cooper, and F. E. Galena. 1975. A comparison of four abscission compounds for use on 'Hamlin', 'Pineapple', and 'Valencia' oranges. *Proceedings of the Florida State Horticultural Society* 88: 107-113.

66. Doughty, C. C., M. E. Patterson, and A. Y. Shawa. 1967. Storage longevity of the 'McFarlin' cranberry as influenced by certain growth retardants and stage of maturity. *Proceedings of the American Society for Horticultural Science* 91: 192-204.

67. Eaks, I. L. 1970. Respiratory response, ethylene production, and response to ethylene of citrus fruit during ontogeny. *Plant Physiology* 45: 334-338.

68. Edgarton, L. J. and A. H. Hatch. 1969. Promoting abscission of cherries and apples for mechanical harvesting. *Proceedings of the New York State Horticultural Society* 114: 109-113.

69. Elstner, E. F., J. R. Konze, B. R. Selman, and C. Stoffer. 1976. Ethylene formation in sugar beet leaves. *Plant Physiology* 58: 163-168.

70. Erickson, L. C. 1960. Color development in 'Valencia' oranges. *Proceedings of the American Society for Horticultural Science* 75: 257-261.

71. Esashi, Y., Y. Ohhara, K. Kotaki, and K. Watanabe. 1976. Two C_2H_4 producing systems in cocklebur seeds. *Planta* 129: 23-26.

72. Esau, K. 1960. Anatomy of Seed Plants. John Wiley and Sons.

73. Fuchs, Y. and M. Lieberman. 1968. Effects of kinetin, IAA, and gibberellin on ethylene production, and their interactions in growth of seedlings. *Plant Physiology* 43: 2029-2036.

74. dela Fuente, R. K. and A. C. Leopold. 1968. Senescence processes in leaf abscission. *Plant Physiology* 43: 1496-1502.

75. Gaffney, J. J. 1971. Reflectance properties of citrus fruits. Paper number 71-811, presented at Annual Meeting, *American Society of Agricultural Engineers*.

76. Gamborg, O. L. and T. A. G. LaRue. 1971. Ethylene production by plant cell cultures. *Plant Physiology* 48: 399-401.

77. Gertman, E. and Y. Fuchs. 1972. Effect of abscisic acid and its interactions with other plant hormones on ethylene production in two plant systems. *Plant Physiology* 50: 194-195.

78. Goeschl, J. D., H. K. Pratt, and B. A. Bonner. 1967. An effect of light on the production of ethylene and the growth of the plumular portion of etiolated pea seedlings. *Plant Physiology* 42: 1077-1080.

79. Goren, R. and E. E. Goldschmidt. 1970. Regulative systems in the developing citrus fruit. I. The hormonal balance in orange fruit tissues. *Physiologia Plantarum* 23: 937-947.

80. Greenberg, J., R. Goren, and J. Riov. 1975. The role of cellulase and polygalacturonase in abscission of young and mature 'Shamouti' orange fruits. *Physiologia Plantarum* 34: 1-7.

81. Grierson, W. and W. F. Newhall. 1960. Degreening of Florida citrus fruits. *University of Florida Agricultural Experiment Station Bulletin #620*.

82. Hall, W. C. 1951. Studies on the origin of ethylene from plant tissues. *Botanical Gazette* 113: 55-65.

83. Hansen, E. 1942. Quantitive study of ethylene production in relation to respiration of pears. *Botanical Gazette* 103: 543-558.

84. Hanson, A. D. and H. Kende. 1976. Biosynthesis of wound ethylene in morning-glory flower tissue. *Plant Physiology* 57: 538-541.

85. Hodgson, R. W. 1918. An account of the mode of foliar abscission in citrus. *University of California Publication (Bot.)* 6: 417-428.

86. Holm, R. E. and F. B. Abeles. 1967. Abscission: the role of RNA synthesis. *Plant Physiology* 42: 1094-1102.

87. Holm, R. E. and W. C. Wilson. 1976. Loss in the capacity of 'Valencia' oranges treated with abscission chemicals to produce ethylene and fruit loosening during the regreening period. *Proceedings of the Florida State Horticultural Society* 89: 35-38.

88. Horton, R. F. and D. J. Osborne. 1967. Senescence, abscission and cellulase activity in *Phaseolus vulgaris*. *Nature* 214: 1086-1088.

89. Hyodo, H. 1977. Ethylene production and respiration of satsuma mandarin (*Citrus unshiu* Marc.) fruit harvested at different stages of development. *Journal of the Japanese Society for Horticultural Science* 45: 427-432.

90. Hyodo, H. 1977. Ethylene production by albedo tissue of satsuma mandarin (*Citrus unshiu* Marc.) fruit. *Plant Physiology* 59: 111-113.

91. Imaseki, H., I. Uritani, and M. A. Stahmann. 1968. Production of ethylene by injured sweet potato root tissue. *Plant and Cell Physiology* 9: 757-768.

92. Jackson, M. B. and D. J. Osborne. 1970. Ethylene, the natural regulator of leaf abscission. *Nature* 225: 1019-1022.

93. Jones, W. W. and T. W. Embleton. 1959. The visual effect of nitrogen nutrition on fruit quality of 'Valencia' oranges. *Proceedings of the American Society for Horticultural Science* 73: 234-236.

94. Kang, B. G., C. S. Yocum, S. P. Burg, and P. M. Pay. 1967. Ethylene and carbon dioxide: mediation of a hypocotyl hook-opening response. *Science* 156: 958-959.

95. Kende, H. and A. D. Hanson. 1976. Relationship between ethylene evolution and senescence in morning-glory flower tissues. *Plant Physiology* 57: 523-527.

96. Kenney, D. S., R. K. Clark, and W. C. Wilson. 1974. ABG-3030: an abscission chemical for processing oranges: biological activity. *Proceedings of the Florida State Horticultural Society* 87: 34-36.

97. Ketring, D. L. and P. W. Morgan. 1970. Physiology of oil seeds. I. Regulation of dormancy in Virginia-type peanut seeds. *Plant Physiology* 45: 268-273.

98. Lau, O. L. and S. F. Yang. 1973. Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production. *Plant Physiology* 51: 1011-1014.

99. Lau, O. L. and S. F. Yang. 1976. Stimulation of ethylene production in the mung bean hypocotyls by cupric ion, calcium ion, and kinetin. *Plant Physiology* 57: 88-92.

100. Leinweber, C. L. and W. C. Hall. 1958. Foliar abscission in cotton. I. Effect of age and defoliants on the respiratory rate of blade, petiole, and tissues of the abscission zone. *Botanical Gazette* 120: 144-151.

101. Levy, D. and S. Marco. 1976. Involvement of ethylene in epinasty of CMV-infected cucumber cotyledons which exhibit increased resistanct to gaseous diffusion. *Physiological Plant Pathology* 9: 121-126.

102. Lewis, L. N. and J. C. Bakshi. 1968. Interactions of indole-acetic acid and gibberellic acid in leaf abscission control. *Plant Physiology* 43: 351-358.

103. Lewis, L. N., R. L. Palmer, and H. Z. Hield. 1968. Interaction of auxins, abscission accelerators and ethylene in the abscission of citrus fruit. In: Biochemistry and Physiology of Plant Growth Substances, Wightman and Setterfield, Eds. Runge Press. p. 1303-1313.

104. Lewis, L. N. and J. E. Varner. 1970. Synthesis of cellulase during abscission of *Phaseolus vulgaris* leaf explants. *Plant Physiology* 46: 194-199.

105. Lieberman, M. 1975. Biosynthesis and regulatory control of ethylene in fruit ripening. *Annales de Physiologie Vegetale* 13: 489-499.

106. Lieberman, M. and A. Kunishi. 1971 Synthesis and biosynthesis of ethylene. *HortScience* 6: 355-358.

107. Lieberman, M. and A. T. Kunishi. 1975. Ethylene forming systems in etiolated pea seedlings and apple tissue. *Plant Physiology* 55: 1074-1078.

108. Lieberman, M., A. Kunishi, L. W. Mapson, and D. A. Wardale. 1966. Stimulation of ethylene production in apple tissue slices by methionine. *Plant Physiology* 41: 376-382.

109. Lieberman, M. and L. W. Mapson. 1964. Genesis and biogenesis of ethylene. *Nature* 204: 343-344.

110. Lieberman, M. and R. A. Spurr. 1955. Oxygen tension in relation to volatile production in broccoli. *Proceedings of the American Society for Horticultural Science* 65: 381-386.

111. Lyons, J. M., W. B. McGlasson, and H. K. Pratt. 1962. Ethylene production, respiration, and internal gas concentrations in cantafruits at various stages of maturity. *Plant Physiology* 37: 31-36.

112. McCoy, C. W. and L. G. Albrigo. 1975. Feeding injury to the orange caused by citrus rust mite *Phyllocoptruta oleivora* (Prostigma: Eriophyoidea). *Annals of the Entomological Society of America* 68: 289-297.

113. McGlasson, W. B. 1969. Ethylene production by slices of green banana fruit and potato tuber tissue during the development of induced respiration. *Australian Journal of Biological Sciences* 22: 489-491.

114. McGlasson, W. B., H. C. Dostal, and E. C. Tigchelaar. 1975. Comparison of propylene-induced responses of immature fruit of normal and rin mutant tomatoes. *Plant Physiology* 55: 281-222.

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

116. Mapson, L. W., J. F. March, and D. A. Wardale. 1969. Biosynthesis of ethylene 4-methylmercapto-2-oxybutyric acid: an intermediate in the formation from methionine. *Biochemical Journal* 115: 653-661.

117. Mattoo, A. K., J. E. Baker, E. Chalutz, and M. Lieberman. 1977. Effect of temperature on the ethylene-synthesizing systems in apple, tomato, and *Penicillium digitatum*. *Plant and Cell Physiology* 18: 715-719.

118. Mattoo, A. K. and M. Lieberman. 1977. Localization of the ethylene-synthesizing system in apple tissue. *Plant Physiology* 60: 794-799.

119. Maxie, E. C., P. B. Catlin, and H. T. Hartmann. 1960. Respiration and repening of olive fruits. *Proceedings of the American Society for Horticultural Science* 75: 275-291.

120. Morgan, P. W. and J. I. Durham. 1972. Abscission: potentiating action of auxin transport inhibitors. *Plany Physiology* 50: 313-318.

121. Morre, D. J. 1968. Cell wall dissolution and enzyme secretion during leaf abscission. *Plant Physiology* 43: 1545-1559.

122. Murphy, S. K., R. H. Biggs, and V. W. Winkler. 1976. Uptake and distribution of "Release" in 'Valencia' and 'Hamlin' oranges. *Proceedings of the Florida State Horticultural Society* 89: 43-45.

123. Murr, D. P. and S. F. Yang. 1975. Inhibition of *in vivo* conversion of methionine to ethylene by L-canaline and 2,4-dinitrophenol. *Plant Physiology* 55: 79-82.

124. Myers, R. M. 1940. Effect of growth substances on the absciss layer in leaves of coleus. *Botanical Gazette* 102: 323-338.

125. Norman, S. and C. C. Croft. 1968. Effect of ethylene on production of volatiles by lemons. *HortScience* 6: 66-68.

126. Osborne, D. J. 1968. Hormonal mechanisms regulating senescence and abscission. In: Biochemistry and Physiology of Plant Growth Substances, Wightman and Setterfield. Eds. Runge Press. p. 815-840.

127. Osborne, D. J. and S. E. Moss. 1963. Effect of kinetin on senescence and abscission in explants of *Phaseolus vulgaris*. *Nature* 200: 1299-1301.

128. Owens, L. D., M. Lieberman, and A. Kunishi. 1971. Inhibition of ethylene production by rhizobitoxine. *Plant Physiology* 48: 1-4.

129. Pollard, J. E. and R. H. Biggs. 1970. Role of cellulase in abscission of citrus fruits. *Journal of the American Society for Horticultural Science* 95: 667-673.

130. Rasmussen, G. K. 1973. The effect of growth regulators on degreening and regreening of citrus fruit. *Acta Horticulturae* 34: 473-478.

131. Rasmussen, G. K. 1974. Cellulase activity in separation zones of citrus fruit treated with abscisic acid under normal and hypobaric atmosphers. *Journal of the American Society for Horticultural Science* 99: 229-231.

132. Rasmussen, G. K. 1975. Cellulase activity, endogenous abscisic acid, and ethylene in four citrus cultivars during maturation. *Plant Physiology* 56: 765-767.

133. Rasmussen, G. K. 1977. Loosening of oranges with Pik-Off, Release Acti-Aid and Sweep combination. *Proceedings of the Florida State Horticultural Society* 90: 4-6.

134. Rasmussen, G. K. and W. C. Cooper. 1969. Abscission of citrus fruits induced by ethylene-producing chemicals. *Journal of the American Society for Horticultural Science* 93: 191-198.

135. Rasmussen, G. K. and J. W. Jones. 1968. Abscission of calamondin fruit as influenced by humidity, ascorbic acid, and copper. *Proceedings of the Florida State Horticultural Society* 81: 36-39.

136. Ratner, A., R. Goren, and S. P. Monselise. 1969. Activity of pectin esterase and cellulase in the abscission zone of citrus leaf explants. *Plant Physiology* 44: 1717-1723.

137. Rogers, B. J. 1968. Production and translocation of ethylene in citrus fruit as related to abscission. *Proceedings of the Florida State Horticultural Society* 81: 225-229.

138. Rubenstein, B. and A. C. Leopold. 1963. Analysis of the auxin control of bean leaf abscission. *Plant Physiology* 38: 262-267.

139. Sakai, S. and H. Imaseki. 1971. Auxin-induced ethylene production by mung bean hypocotyl segments. *Plant and Cell Physiology* 12: 349-359.

140. Sakai, S. and H. Imaseki. 1972. Ethylene biosynthesis: methionine as an *in vivo* precursor of ethylene in auxin-treated mung bean hypocotyl segments. *Planta* 105: 165-173.

141. Sakai, S. and H. Imaseki. 1973. Properties of the proteinaceous inhibitor of ethylene synthesis: action on ethylene production and indoleacetylaspartate formation. *Plant and Cell Physiology* 14: 881-892.

142. Sakai, S. and H. Imaseki. 1973. A proteinaceous inhibitor of ethylene biosynthesis by etiolated mung bean hypocotyl sections. *Planta* 113: 115-128.

143. Saltveit, M. E., K. J. Bradford, and D. R. Eilley. 1977. Silver, Ag (I), inhibits ethylene synthesis and action in ripening fruits. *Plant Physiology* 59: 250 (Supplement).

144. Schwartz, A. 1975. Aftermath of a rust mite infection. *Citrus Grower and Subtropical Fruit Journal* 441: 22.

145. Sfakiotakis, E. M. and D. R. Dilley. 1973. Induction of autocatalytic ethylene production in apple fruits by propylene in relation to maturity and oxygen. *Journal of the American Society for Horticultural Science* 98: 504-508.

146. Shannon,S. and M. D. de la Guardia. 1969. Sex expression and the production of ethylene induced by auxin in the cucumber (*Cucumis sativum* L.). *Nature* 223: 186.

147. Shimokawa, K. and Z. Kasai. 1966. Biogenesis of ethylene in apple tissue. I. Formation of ethylene from glucose, acetate, pyruvate, and acetaldehyde in apple tissue. *Plant and Cell Physiology* 7: 1-9.

148. Shimokawa, K. and Z. Kasai. 1970. Ethylene formation from acrylic acid by banana pulp extract. *Agricultural and Biological Chemistry* 34: 1646-1651.

149. Suttle, J. and H. Kende. 1977. Ethylene production and flower fading in *Tradescantia hirsuticales*. *Plant Physiology* 59: 216. (Suppl.)

150. Takayanagi, K. and J. F. Harrington. 1971. Enhancement of germination rate of aged seeds by ethylene. *Plant Physiology* 47: 521-524.

151. Vendrell, M. and W. B. McGlasson. 1971. Inhibition of ethylene production in banana fruit tissue by ethylene treatment. *Australian Journal of Biological Sciences* 24: 885-895.

152. Vines, H. M., G. J. Edwards, and W. Grierson. 1965. Citrus fruit respiration. *Proceedings of the Florida State Horticultural Society* 78: 198-202.

153. Vines, H. M., W. Grierson, and G. J. Edwards. 1968. Respiration, internal atmosphere, and ethylene evolution of citrus fruits. *Journal of the American Society for Horticultural Science* 92: 227-234.

154. Watanabe, T. and N. Kondo. 1976. Ethylene evolution in marine algae and a proteinacous inhibitor of ethylene biosynthesis from red alga. *Plant and Cell Physiology* 17: 1159-1166.

155. Watanabe, T., N. Kondo, T. Fujii, and T. Hoguchi. 1977. Affinity chromatography of an ethylene-synthesizing enzyme from red alga *Porphyra tenera* on an immobilized inhibitor of ethylene evolution. *Plant and Cell Physiology* 18: 387-392.

156. Webster, B.D. 1968. Anatomical aspects of abscission. *Plant Physiology* 43: 1512-1544.

157. Webster, B. D. 1973. Anatomical and histochemical changes in leaf abscission. In: Shedding of Plant Parts, T. T. Kozlowski, Ed. Academic Press. p. 45-83.

158. Wetmore, R. H. and W. P. Jacobs. 1953. Studies on abscission: the inhibiting effect of auxin. *American Journal of Botany* 40: 272-276.

159. Williamson, C. E. 1950. Ethylene, a metabolic product of diseased or injured plants. *Phytopathology* 40: 205-208.

160. Wilson, W. C. and C. H. Hendershott. 1968. Anatomical and histochemical studies of abscission of oranges. *Proceedings of the American Society for Horticultural Science* 92: 203-210.

161. Yang, S. F. 1967. Biosynthesis of ethylene: ethylene formation from methional by horseradish peroxidase. *Archives of Biochemistry and Biophysics* 122: 481-487.

162. Yang, S. F. 1974. The biochemistry of ethylene: biogenesis and metabolism. *Recent Advances in Phytochemistry* 7: 131-164.

163. Yang, S. F. and A. H. Baur. 1969. Pathways of ethylene biosynthesis. *Qualitas Plantarum Et Materiae Vegetabiles* 19: 201-220.

164. Zauberman, G. and Y. Fuchs. 1973. Ripening processes in avocados stored in ethylene atmosphere in cold storage. *Journal of the American Society for Horticultural Science* 98: 477-480.

165. Zeroni, M., J. Galil and S. Ben-Yehoshua. 1976. Autoinhitition of ethylene formation in non-ripening stages of the fruit of sycamore fig *(Ficus sycomorus* L.). *Plant Physiology* 57: 647-650.

166. Zimmerman, P. W. and F. Wilcoxon. 1935. Several chemical growth substances which cause initiation of roots and other responses in plants. *Contributions from the Boyce Thompson Institute* 7: 209-229.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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