RESPONSE OF TOMATO FRUITS TO CERTAIN GROWTH
REGULATORS WITH EMPHASIS ON PECTOLYTIC
ENZYMES, CELLULASE, AND ETHYLENE

By
CRAIG H. LAMPE

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1970

UNIVERSITY OF FLORIDA

3 1262 08552 3040

DEDICATION

To Molly and Richard

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. C. B. Hall, Professor of Vegetable Crops, for his guidance and assistance during the course of this investigation.

The assistance of Dr. H. H. Bryan at the Sub-Tropical Experiment Station, Homestead, Florida, is appreciated. To Dr. V. F. Nettles, Dr. B. D. Thompson, Dr. D. D. Gull, Dr. H. Biggs and Dr. T. Humphrey, I extend my appreciation for their advice and assistance.

Deep appreciation is extended to all members of the Vegetable Crops Department for their interest and cooperation. For the necessary financial support, I extend appreciation to the Department of Vegetable Crops and NDEA.

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Abstract of Dissertation Presented to the Graduate Council
in Partial Fulfillment of the Requirements for the Degree of
Doctor of Philosophy

RESPONSE OF TOMATO FRUITS TO CERTAIN GROWTH REGULATORS
WITH EMPHASIS ON PECTOLYTIC ENZYMES, CELLULASE, AND ETHYLENE

By

Craig H. Lampe

December, 1970

Chairman: Dr. C. B. Hall

Major Department: Vegetable Crops

The effect of various growth regulators on certain ripening processes in tomato fruits was studied. Fruits treated at the mature green stage were sampled at various days following incipient coloring for pectolytic enzymes, cellulolytic enzymes, firmness and color.

Gibberellic acid (GA) solutions as low as 50 ppm, applied as a 30 minute dip to mature green fruit, were effective in inhibiting polygalacturonase (PG) and polymethylgalacturonase (PMG) in fruits as they ripened. Generally, cellulase activity and firmness were not significantly different from the control. Indoleacetic acid (IAA) and naphthaleneacetic acid (NAA) dips were effective in the early stages of ripening in inhibiting PG and PMG, but this inhibition disappeared in the latter stages as the fruits continued to ripen. Kinetin and N⁶-benzyladenine (N⁶BA) had no effect on the enzyme activities or firmness. Ethrel treated fruits tended to respond like auxin treatments in the early stages of ripening.

Ethrel treated fruits were slower to develop color than the control after incipient color was initiated; however, after 6 days past color-break full color was attained. Gibberellic acid treated fruits were slower to

develop red color, but by the sixth day past incipient color the color values of treated fruits approached those of the control.

Fruits treated with Ethrel soon began evolving above threshold levels of ethylene. Ethrel and GA treated fruits evolved peak amounts of ethylene consistently greater than the control in all harvests.

Tissue slices of tomato pericarp incubated with concentrations of Ethrel ranging from 25 to 2400 ppm evolved higher rates of ethylene than the controls. Higher concentrations of Ethrel resulted in increases of ethylene less than the 2400 ppm concentration. Gibberellic acid at $10^{-2}M$ suppressed ethylene production; however, at 10^{-3} and $10^{-4}M$ concentrations the tissue evolved only 10% less than the control.

All fruits treated at the mature green stage with Ethrel showed incipient color within 4 days. None of the other treatments differed from the control which took 4 to 7 days for 50% of the fruits to show incipient color.

INTRODUCTION

Much of the current horticultural research with tomatoes is concerned with improving fruit color, flavor and firmness. Color and flavor are quite important from the consumer's standpoint, while firmness of tomato fruits is essential for handling, marketing, shipping and mechanical harvesting (El Sayed et al., 1966, McColloch, 1963, Thompson et al., 1962, Garrett et al., 1960).

The loss of firmness of the flesh is an important change during ripening, and the most striking chemical change related to firmness is the conversion of pectins from insoluble to soluble form (Biale, 1960). Rosa (1925) surmised the softening process leading to breakdown and decay in fruit was due to the increase in soluble solids and the conversion of calcium pectate of the middle lamella to "pectic acid". He observed the process to be much slower in tomato fruits harvested green and artificially ripened either in air or ethylene. Changes in enzyme activity would be expected when subcellular structures undergo physical changes (Spencer, 1965), and this possibility must be considered in any explanation of fruit changes during ripening and senescence. Several enzymes associated with softening in tomato fruits have been reported.

The role that hormones play in the ripening of fruit has been reviewed by Dilley (1969) and Hansen (1966). One growth regulator, ethylene, has been studied a great deal in fruit ripening and its role has been thoroughly reviewed by Pratt and Goeschl (1968, 1969) and Burg and

Burg (1962, 1965, 1969). The role of other regulating substances has not been as thoroughly exploited although their importance in the post-harvest physiology of the fruit may be preeminent (Pharr et al., 1970).

Since the tomato fruit is capable of prolonged existence even after removal from the parent plant, it offers desirable material for investigation at three levels of organization: the intact organ, the intact cell in the form of a tissue slice, and subcellular fractions, i.e. mitochondrial systems (Biale, 1964).

Based on recent observations by Abdel-Kader et al. (1966), Russo et al. (1968) and Dostal and Leopold (1967), this problem was undertaken to observe changes wrought by exogenously applied growth regulators on cell separating enzymes and ethylene evolution in ripening tomato fruits.

LITERATURE REVIEW

Many changes take place in fleshy fruits as they ripen and finally senesce. Among these changes are abscission of the fruit from the plant, changes in the rate of ethylene production, changes in tissue and organelle permeability, changes in color including destruction of chlorophyll and synthesis of new pigments, changes in carbohydrate composition, changes in pectic composition, changes in protein composition and enzyme activity, synthesis and release of flavor volatiles, development of wax on the skin, changes in organic acid composition, and the well known climacteric rise in respiration (Pratt and Goeschl, 1968). The role of growth regulators (i.e. cytokinins, auxins and gibberellins) appears to be one of great importance in these processes (Pharr et al., 1970).

The alcohol insoluble solids fraction includes starch, protein and cell wall constituents such as pectin, hemicellulose and cellulose. The decrease in this fraction as fruits ripen may be attributed to a conversion of insoluble to soluble material (Woodmansee et al., 1959). Pectic substances have been correlated with whole fruit firmness (El Sayed et al., 1966). In the process of abscission the enzymatic digestion of pectic materials in the middle lamella and other portions of the cell wall represents either (1) movement of preformed protein into the separation layer, (2) the unmasking of active sites of existing enzymes, or (3) the biogenesis of the appropriate enzymes (Pollard, 1969).

Several enzymes have been implicated in the softening process which occurs as the tomato fruit ripens. Pectinesterase (PE) activity and firmness have been studied by Hamson (1952), and an inference was made to a correlation existing between the two; however, Hall and Dennison (1960) showed that no correlation existed. The PE activity is associated with the removal of methylester groups from the pectin molecule producing pectic acid (Kertesz, 1951).

Tomato polygalacturonase (PG), which hydrolyzes α -1,4 glycosidic linkage between deesterified galacturonic acid residues, has been a widely studied enzyme linked with softening (Dickinson, 1962, Hobson, 1964, 1965, 1967). Foda (1957) showed that PG activity was present in fruits at the time of incipient coloring. Polygalacturonase activity increased to a maximum at 12 days past incipient color in the cultivar Stokesdale and at 15 days in the cultivar Y-13. McCollloch et al. (1952) found 65 percent of the PG activity in the outer pericarp of ripe tomatoes and 35 percent in the radial walls and core. Hobson (1964) observed a positive association in the cultivars Potentate and Immuna between firmness and PG activity. The cultivar Potentate has been modified by the introduction of the dominant allele (Nr) at the "never ripe" locus (Hobson, 1967). Fruit from the resulting plants ripen and soften much more slowly than control fruit. This is associated with a much reduced solubilization of the pectic substances and a considerable alteration in the activity of PG.

The enzyme polymethylgalacturonase (PMG) which has only recently been observed in tomato (Hall, 1968) is capable of cleaving the esterified α -1,4 glycosidic chain (Demain and Phaff, 1957). Polymethylgalacturonase had not been observed in higher plants until its recent observation by Hall.

Cellulolytic activity has been observed in tomato fruit. Hall (1963, 1964) and Dickinson (1962) made the interesting observation that cellulase activity increased in tomato fruit as they ripened. No correlation has been established between this enzyme and firmness. Hobson (1968) concludes that cellulase is not a major factor controlling the softening of tomato fruit. Hall and Mullins (1965) observed that sterile tomato tissue (no microbial contamination) contained cellulase activity, indicating that the enzyme does not stem from bacteria in the fruit.

In an interesting experiment in 1959, Reeves considered that cell wall substances other than pectins may be related to the difference in firmness of freestone and clingstone peaches when fully ripe.

There is need for more information on the activation or synthesis of pectic enzymes during the climacteric, the nature of the energy relations involved and the senescent changes associated with loss in ripening capacity and undesirable texture in some fruits when ripened after prolonged periods of storage (Hansen, 1966).

The mode of action of growth regulators appears to be centered on the control of the mechanism by which enzymes are made in cells (Galston and Davies, 1969, Key, 1969). In fruit ripening this subject has had a great deal of its study devoted to the activity and biosynthesis of ethylene. This was evidenced in the last 10 years by several reviews and papers on ethylene and fruit ripening (Burg and Burg, 1962, 1965a, 1965b, 1969, Hansen, 1967, Lyons and Pratt, 1964, Pratt and Workman, 1962, Pratt and Goeschl, 1968, 1969, Dilley, 1969 and Crane, 1969). Burg and Burg (1969) state that since natural ripening and that induced artificially

by applied gas are indistinguishable, it follows that either directly or indirectly ethylene alone suffices to induce all of the new enzymes associated with the ripening process.

The role of ethylene in the induction of tomato fruit ripening is not new as evidenced by work 20 to 40 years ago (Work, 1928, Rosa, 1925, Harvey, 1928, Heinze and Craft, 1953).

In 1967 a chemical, 2-chloroethylphosphonic acid (Ethrel), was released by Amchem Products, Inc. A breakdown product of the compound had properties and characteristics very similar to ethylene gas. Warner and Leopold (1968) exhibited evidence that Ethrel breaks down in the presence of a base to form ethylene. This product was initially shown to exert its effect by liberation of ethylene within plants (Warner and Leopold, 1967). The use of this chemical has become widespread in applied areas of agriculture, and its use on tomato fruits at the pre- and post-harvest stages has been well documented (Gull, 1968, Robinson et al., 1968, Rabinowitch et al., 1970, Warner and Leopold, 1967, Dennis et al., 1970, and Iwahori and Lyons, 1970). Ethrel has been found to induce mature green tomatoes as well as immature green fruits to color whether applied as a pre-harvest spray or as a post-harvest dip or spray. The compound appears to break down to ethylene in tomato fruits over a period of hours thus initiating a natural ethylene burst by the fruit prior to the climacteric (Dennis et al., 1970). In post-harvest usage Rabinowitch et al. (1970) observed that dips greater than 5000 ppm resulted in discolored fruit at the full-ripe stage.

No specific ripening process, i.e. a series of defined enzymatic reactions, has been attributed to ethylene in ripening fruits. Whether

ethylene produces a general effect influencing all the processes involved in ripening, acts only initially as a triggering mechanism, or is required throughout the ripening process is not known (Hansen, 1966). Ethylene has been reported in other systems (Abeles and Holm, 1967) to promote nucleic acid and protein synthesis which leads to the synthesis of degradative enzymes (Abeles, 1969, Osborne, 1968).

Although the role and activity of ethylene in fruit ripening is far from being settled, the involvement of other plant growth substances has remained even more obscure. Three reviews (Dilley, 1969, Crane, 1969, Galston and Davies, 1969) and a few papers (Dostal and Leopold, 1967, Russo et al., 1968, Scott and Leopold, 1967, Pharr et al., 1970, Iwahori, 1967, Emmert and Southwick, 1954) have concentrated on processes directly involved in post-harvest fruit physiology.

In immature tomato fruit (11 mm diameter) the effect of the auxin naphthaleneacetic acid (NAA) has been attributed to its stimulation of ethylene evolution (Abeles and Rubenstein, 1964). This role of auxin has been further extended to the fig tree where Maxie and Crane (1967) found increased ethylene production in the fruit following treatment with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). The role of auxins in mature green tomato development has been followed by Iwahori (1967); however, in fruit older than 30 days past anthesis no indoleacetic acid (IAA) activity was detected. Observations on the effect of post-harvest dipping of 2,4-dichlorophenoxyacetic acid or NAA (Mitchell and Marth, 1944, Emmert and Southwick, 1954, Hartman, 1959) have revealed little or no change on mature green tomato fruit. Abdel-Kader et al. (1966) observed a delay in the onset of incipient coloring after dipping fruit for 30 minutes in IAA or the ethyl ester of IAA. The effect of auxin may

be masked or destroyed by the fruit's endogenous production of IAA oxidase during ripening (Oyer, 1970). Gortner (1969) observed marked preservative properties in pineapples with 1 ppm 2,4,5-T or 100 ppm NAA applied as a dip. The effect of these regulators was not one leading to abnormal metabolism. The ripening processes are delayed, but apparently not otherwise altered.

In other plant organs IAA has been directly or indirectly, through ethylene stimulation, shown to induce degradative enzymes. Fan and MacLachlan (1966) observed the auxin induced elongation in Alaska pea seedlings to be accompanied by cellulase production. Abeles and Rubenstein (1964) and Burg and Burg (1968) entertained the possibility of auxin stimulated ethylene being the primary step in this process; however, Ridge and Osborne (1969) showed auxin induced cellulase was independent of any ethylene effect. Abeles (1969) has concluded that ethylene is responsible for cellulase production in the cell separation process involved in abscission. Dakto and MacLachlan (1968) observed increases in glucanases and pectic enzymes in response to ethylene; however, these enzymes appeared only slowly in intact pea epicotyls, far longer than the 10 minute time required for auxin to affect growth (Galston and Davies, 1969). The observation has been made that applications of β -1,3 glucanase promoted pea stem elongation to an extent similar to auxin within 30 minutes, but cellulase and PE did not have this effect (Wada et al., 1968).

Inhibition of enzyme synthesis by IAA has been reported for enzymes such as 3'-nucleotidase in wheat embryo, an insoluble invertase in artichoke tuber tissue, two peroxidase isoenzymes in tobacco pith and peroxidase in sugar cane (Glasziou, 1969).

In 1966 Abdel-Kader et al. observed not only a delay in the ripening of mature green tomato fruit treated with gibberellic acid (GA), but an increased shelf life of up to 9 days. The optimum concentration of potassium gibberellate was a 100 ppm dip for 30 minutes. The shelf life was terminated by both decay and softening. This and the succeeding work by Dostal and Leopold (1967) with banana tissue indicated an effect of GA on maintaining the firmness of the tissue. Russo et al. (1968) present data with GA treated bananas that implicate a retardation of chlorophyll degradation and loss of firmness. In one experiment with GA treated bananas, Ethrel was applied, and it was found to overcome the GA effect as indexed by respiration and chlorophyll degradation (Russo et al., 1968). Abdel-Kader et al. (1966) observed some abnormalities associated with color development in GA treated tomato fruit; however, this effect was more thoroughly researched by Dostal and Leopold (1967). Gibberellic acid treated fruits developed half-red color in approximately 14 days in contrast to the water treated controls which reached half-red color in approximately 8 days. Gibberellic acid treated fruit, subsequently treated with ethylene, behaved like the control. Gibberellic acid can delay the progress of some processes associated with the ripening of the fruit, preventing some of the changes triggered by ethylene.

Gibberellic acid has not been reported in mature tomato fruits although it is hypothesized to be present in all developing fruit (Dilley, 1969).

It is important to recognize the difficulty of determining a tomato fruit's age or its physiological status. This is especially true for fruit at the so-called mature green stage. McCollum (1956) states

that maturity cannot be determined accurately by age, size or apparent characteristics; nevertheless, the time of incipient coloring is a good reckoning point for maturity. Seasonal variability should be recognized. Hall (1966) points out the necessity for dealing with fruits of the same physiological age when making comparisons based on separate harvests.

MATERIALS AND METHODS

I. Harvest and Handling

Experiments were conducted with two cultivars of tomato, Homestead 24 and Walter, in the winter and spring of 1970. Preliminary screening of growth regulating substances was begun using fruits of the Homestead 24 cultivar. Harvests were made on 1/28, 2/27 and 3/23. The fruits were harvested at the mature green stage and transported to Gainesville, Florida, for treatment. Upon arrival the fruits were stored at 20°C in the dark except during observation periods. Only fruits that were physically free from bruises and defects were used. A replicated experiment was conducted with fruit of the Homestead 24 cultivar from a 4/22 harvest. A harvest was also made on 4/30 for ethylene analysis. Weather conditions were mild on the days that fruit were harvested.

Four harvests of the Walter cultivar were made from Bradenton, Florida. The harvest dates were 5/30, 6/6, 6/16 and 6/25. The last harvest was made under adverse weather conditions when the temperatures in the field were above 90°F. Fruits from the 6/25 harvest remained in the field several hours before being transported to Gainesville. All fruits were harvested at the mature green stage.

II. Growth Regulator Treatments on Whole Fruits

A. 3/23 Harvest

In this preliminary experiment several growth regulating compounds were screened, i.e. 10 and 100 ppm Kinetin, 10 and 100 ppm N⁶-benzyladenine (N⁶BA), 100 and 1000 ppm GA and 10 and 100 ppm IAA. Injections of Ethrel, as well as 1000 ppm dips, were employed to observe the effect of the chemical. All dips were 30 minutes in duration (Dostal and Leopold, 1968, Abdel-Kader et al., 1966). In the injection treatments 10 microliters of Ethrel or water were injected into the core at a depth of about 40 mm. The 40 mm depth was approximately the center of the fruit.

In all cases except the Ethrel treatments a period of 48 hours was allowed to transpire before the treated fruits were selected for the ripening study. Those fruits that underwent incipient coloring within the 48 hours after dipping were discarded. This offers the assurance that the fruits selected for analysis were mature green and not at the breaker stage on the treatment date. A period of 24 hours was allowed for fruits treated with Ethrel because of the rapid induction to ripen resulting from this treatment. It was not uncommon for 50 to 75 percent of the fruit to undergo incipient coloring 24 hours after being treated with Ethrel. When the treated fruits began turning color, they were set aside and allowed to ripen for a predetermined number of days. Daily observations were made on the fruits as they began to turn color and ripen. In this harvest, seven lots of 5 fruits each at incipient color were randomly selected from each treatment and allowed to ripen for 1, 2, 3, 4, 5, 6 or 7 days. After firmness measurements the 5 fruits were combined and the tissue was frozen for future analysis.

B. 4/22 Harvest

Fruits of the Homestead 24 cultivar were selected at the mature green stage and dipped for 30 minutes in either water, 1000 ppm Ethrel, 100 ppm IAA, 100 ppm NAA or 100 ppm GA. The fruits that began turning color within 24 hours were discarded. Five lots of 5 fruits, each at the incipient color stage, were selected from each treatment and allowed to ripen for 1, 2, 3, 4 or 5 days. The 5 fruits were not combined as in the 3/23 harvest but were analyzed individually. A daily record was made on the fruits as they turned and ripened.

In a separate experiment involving observations of ethylene evolution from treated fruits, three lots of 5 fruits each of the Homestead 24 cultivar were selected at the mature green stage and dipped for 30 minutes in either water, 100 ppm GA or 1000 ppm Ethrel. Using the method of Iwahori and Lyons (1970), the fruits were weighed and placed in quart jars equipped with a serum cap suitable for needle injections. Each day a record was made of the fruit's maturity status based on the scheme of Workman et al. (1957). At the end of the sampling period (7 days past incipient color), the fruits were weighed and weight loss determined. The jars were left open between sampling periods. Prior to sampling, the fruits' containers were sealed for 5 hours. At the end of this period 1 ml of the air above the fruit was removed with a syringe and injected into a HY-FY Aerograph Model 600-D gas chromatograph equipped with a 9 foot by 1/8 inch column packed with activated alumina. A column temperature of 100°C and injector temperature of 150°C was maintained at all times. Known concentrations of reference gas were used for calibration at each time of measurement. Results were expressed in

microliters ethylene/kg-hr. Ethylene evolution was followed for 3 days prior to the turning stage until 7 days past this stage.

C. 4/30 Harvest

Homestead 24 fruits were dipped for 30 minutes in 100 ppm NAA, 100 ppm GA, 1000 ppm Ethrel or water. The fruits were set up for ethylene analysis as described in the 4/22 harvest. In this experiment 7 replicates were employed for each treatment. The stage of ripeness was determined daily by visual observation.

D. 5/30 Harvest

Fruits of the Walter cultivar were dipped at the mature green stage for 30 minutes in either water or 100 ppm GA. As in the 4/22 harvest, fruits were analyzed individually. Four lots of three to five fruits were selected from each treatment as the fruits underwent incipient coloring, and they were allowed to ripen for 1, 4, 7 or 10 days. In the GA treated lot, fruits which began to ripen in the first 24 hours after dipping were not discarded but set aside and used as a treatment.

To determine the amount of GA solution absorbed during dipping, fruits were wiped clean, weighed to the nearest mg, dipped for 30 minutes, allowed to dry and weighed again. The fruits were numbered such that all fruits that were later analyzed had a known amount of GA solution absorbed.

Ethylene evolution from fruits treated with 30 minute dips of 100 ppm GA, 1000 ppm Ethrel or water was followed. Ten replicates of each treatment were made and a 30 minute sealing time was employed prior to sampling for ethylene.

E. 6/6 Harvest

Fruits of the Walter cultivar were selected at the mature green stage and dipped for 30 minutes in either water, 100 ppm GA, 100 ppm IAA or 100 ppm IAA + 100 ppm catechol. One lot of fruits was selected at the breaker stage and dipped in 100 ppm GA. The lots of fruit were ripened for 1, 4, 7 or 10 days before sampling.

F. 6/16 Harvest

Ethylene evolution from Walter fruits dipped for 30 minutes in 100 ppm GA, 1000 or 5000 ppm Ethrel and water was followed. Eight replicates of each treatment were made.

G. 6/25 Harvest

To determine the minimum effective concentration of GA on pectolytic enzymes, fruits of the Walter cultivar were dipped for 30 minutes in water or 10, 50 or 100 ppm GA. The fruits that turned in the first 24 hours were discarded. After 24 hours the fruits were allowed to ripen for 2, 4 or 7 days.

III. Determination of Firmness

A. 3/23, 4/22, 5/30 and 6/6 Harvests

Following growth regulator treatments in which the predetermined stage of ripeness had been reached (Section II, A, B, D, E), the fruits were cut into quarters and the locular material and inner pericarp were carefully removed and discarded. Each quarter section was separately measured for firmness with an Allo-Kramer Shear Press equipped with a 250 or 2500 pound ring. The shear forces, expressed in pounds, from the quarter sections were combined and a mean calculated. All shear force readings that are reported for each fruit are the means of the 4 quarters.

IV. Determination of Color

A. 3/23 Harvest

After firmness had been determined, the fruits (from Section II-A) were frozen. The pericarps from the 5 fruits were thawed to room temperature and blended for 1 minute in a Waring blender. Following blending the tissue was degassed in a vacuum oven. Upon removing entrapped and dissolved gas each sample was poured into a cuvette and an a/b value was calculated from readings observed on the Hunter Color Difference Meter. The meter was calibrated against a standard with a $R_d=7.0$, an $\underline{a}=33.1$ and a $\underline{b}=16.2$.

B. 4/22 Harvest

Because of the use of single fruits as replicated samples, it was not possible to determine a/b values on the blended pericarp. There was not enough tissue from 1 fruit to get a reading; therefore, to measure color in response to an applied growth regulator, a separate experiment was conducted. Lots of 100 fruits each were dipped at the mature green stage in 100 ppm GA, 100 ppm IAA, 100 ppm NAA, 1000 ppm Ethrel or water. Three lots of five fruits each were selected at the incipient coloring stage and allowed to ripen for 3 or 6 days. At each sampling date the outer pericarp from the 5 fruits of each lot was combined, blended for 1 minute and deaerated. The color of each sample was evaluated with the Hunter Color Difference Meter.

V. Polygalacturonase, PMG and Cellulase Analysis

A. 3/23, 4/22, 5/30, 6/6 and 6/25 Harvests

Following thawing, blending and deaeration of the pericarp tissue, the enzymes were extracted according to the method of Hall (1963, 1964)

and subjected to enzymatic analysis according to the method of Bell et al. (1950). Three enzymes were followed as fruits ripened: cellulase, polygalacturonase and polymethylgalacturonase. Five ml of either 1.2% carboxymethylcellulose (pH=5.0), 1.2% sodium polypectate (pH=5.0) or 1.2% citrus pectin (pH=4.0) were used as substrate, and each was pipetted into viscometer tubes and allowed to equilibrate to 30°C. The initial drain time was measured immediately following addition and mixing of the enzyme preparation. A standard time interval of 15, 30, 45 and 60 minutes was utilized between drain time measurements of the enzyme-substrate preparation. Change in drain time of the solution was measured and results reported using the following equation:

$$\frac{D_f}{D_o} \cdot 100 = \% \text{ decrease in drain time}$$

D_f = Final drain time

D_o = Initial drain time

The tissue was analyzed according to the scheduled ripening period as described in Section II of the materials and methods for each harvest date.

VI. Determination of Pectinesterase Activity

A. 5/30 and 6/6 Harvests

Pectinesterase activity from these two harvests was determined from the same pericarp tissue as used to determine PG, PMG and cellulase. The PE activity was made according to the method of Rouse and Adkins (1955) with a slight modification in pH after Hall and Dennison (1960). Results are expressed in pectinesterase units per gram fresh weight of tissue.

VII. Response of Tissue Slices of Tomato Pericarp to Growth Regulators

To determine the effect of GA and Ethrel on the evolution of ethylene from early breaking tissue, an experiment was designed to observe the response.

Tomato fruits at the early breaker stage were selected and the outer pericarp was separated from the inner pericarp and locule. The early breaker stage refers to fruits that were mature green but showed slight coloration at the styler end. When sliced in half, these early breakers showed light yellow to pink color in the locule and core. The outer pericarp which was still green was then carefully sliced with a razor blade into cubes approximately 0.5 cm on a side. Ten to fifteen fruits were handled in this manner and the cubes were then weighed and added to a buffered media (10 ml) previously pipetted into 50 ml Erlenmeyer flasks. The media was prepared according to the method of Lieberman and Kunishi (1969). Gibberellic acid and Ethrel were added to the media prior to the addition of the tomato cubes. The flasks were sealed with a sleeve-type rubber septum and gas samples were removed after the desired incubation period by means of a Hamilton airtight syringe. Tissue used came from harvests of Homestead 24 tomato fruits during the month of March, 1970. The gas chromatograph was equipped as previously described. Differences rendered by a specific treatment were expressed as percent increase or decrease over a given control. The control constituted duplicated flasks with Ethrel + media and tomato tissue + media. When incubating tissue with GA, the control was a flask with tomato tissue + media. Where applicable water was used in place of a given weight of tissue.

VIII. Analysis of Differences in Enzyme Activity and Firmness of Fruits Treated with Growth Regulators

A. 4/22, 5/30 and 6/6 Harvests

On each day after incipient color the PG, PMG, cellulase and firmness measurements were subjected to analysis of variance. If the F proved significant, the means were ranked and tested for differences by use of The Duncan' New Multiple Range Test.

RESULTS

I. Effect of Growth Regulators on Enzyme Activity, Firmness and Fruit Color as Determined by Hunter Color Readings

A. 3/23 Harvest

Fruits harvested on 3/23 revealed marked differences in enzyme activity based on stage of ripeness and growth regulator treatments. As fruits ripened, PG and PMG activity increased in all treatments except GA. In Ethrel treated fruits an appearance of enzyme activity was not noted until the third and fourth day past incipient color (Tables 1 and 2). The fruits treated with GA do not exhibit PG and PMG activity to any substantial degree (Tables 1 and 2). The PMG activity is greater than the PG activity. It is interesting to observe the high activity associated with the growth regulators other than GA. This is most apparent with PMG activity of IAA treated fruits where diluting the enzyme extract 50:1 still results in activity twice that of the GA treatment (Table 2). The cellulolytic activity of the treated fruits does not respond like PG and PMG (Table 3). In all cases the activity is low at the day of incipient color and one day after; however, it increases rapidly and stays high through the seventh day. The cellulase activity does not appear to be altered by GA treatments. Kinetin and N⁶BA treated fruits did not reveal any reduced activity of the enzymes when compared to the control. Extracts from fruits treated with the two

Table 1.--Polygalacturonase activity¹ of sodium chloride extracts of Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 3/23.

Treatment ²	Days past incipient color						
	0	1	2	3	4	5	7
Water	0.0	0.0	28.0	37.0	29.0(5:1)	53.8(5:1)	46.0(5:1) 23.0(5:1)
Ethrel (1000 ppm)	0.0	0.0	0.0	23.4	24.6(5:1)	38.2(5:1)	50.8(5:1) 50.0(5:1)
IAA (100 ppm)	0.0	-	-	-	54.1	-	- 61.6
GA (100 ppm)	0.0	-	-	-	2.7	-	- 1.3
Kinetin (10 ppm)	0.0	-	-	-	30.8(10:1)	-	- 29.3(10:1)
⁶ N ⁶ BA (10 ppm)	0.0	-	-	-	18.2(10:1)	-	- 37.1(10:1)

¹pg activity was measured as percent decrease in drain time of 1.2% sodium polypectate (pH = 5.0). Incubation time was 30 minutes. One ml of extract (25 g of pericarp concentrated to 5 ml), diluted as indicated, was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of various growth regulators.

Table 2.--Polymethylgalacturonase activity¹ of sodium chloride extracts of Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 3/23.

Treatment ²	Days past incipient color							
	0	1	2	3	4	5	6	7
Water	0.0	0.0	68.1	41.3(5:1)	69.5(5:1)	77.8(5:1)	76.5(5:1)	65.3(5:1)
Ethrel (1000 ppm)	0.0	0.0	10.1	62.0	68.3(5:1)	73.9(5:1)	76.6(5:1)	77.6(5:1)
IAA (100 ppm)	4.8	-	-	-	15.4(50:1)	-	-	36.4(50:1)
GA (100 ppm)	2.5	-	-	-	8.8	-	-	10.0
Kinetin (10 ppm)	-	-	-	-	70.3(10:1)	-	-	67.1(10:1)
N ⁶ BA (10 ppm)	-	-	-	-	55.6(10:1)	-	-	71.6(10:1)

¹PMG activity was measured as percent decrease in drain time of 1.2% pectin (pH = 4.0). Incubation time was 30 minutes. One ml of extract (25 g of pericarp concentrated to 5 ml), diluted as indicated, was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of various growth regulators.

Table 3.--Cellulolytic activity¹ of sodium chloride extracts of Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 3/23.

Treatment ²	Days past incipient color							
	0	1	2	3	4	5	6	7
Water	0.0	2.6	16.0	16.5	11.6	13.2	17.1	12.8
Ethrel (1000 ppm)	4.4	2.6	11.5	20.8	15.1	17.5	10.7	12.0
IAA (100 ppm)	6.7	-	-	-	14.1	-	-	11.6
GA (100 ppm)	2.4	-	-	-	15.2	-	-	10.7
Kinetin (10 ppm)	-	-	-	-	12.7	-	-	9.5
N ⁶ BA (10 ppm)	-	-	-	-	18.3	-	-	13.3

¹Cellulolytic activity was measured as percent decrease in drain time of 1.2% carboxy-methyl-cellulose (pH = 5.0). Incubation time was 30 minutes. One ml of extract (25 g of pericarp concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of various growth regulators.

cytokinins, when diluted twice that of the control, showed activity equal to or greater than the control at 4 and 7 days past incipient color (Tables 1 and 2).

The softening rate of treated fruits was measured by an Allo-Kramer Shear Press. The firmness of the fruits decreased as the number of days past incipient color increased (Table 4). This trend in the Ethrel and water treated fruits is well illustrated. The other treatments at 4 and 7 days past incipient color are quite similar to the control. The GA treated fruits were only 2 pounds greater than the control at 4 and 7 days past incipient color (Table 4). In the Ethrel and water treated fruits, the greatest amount of softening took place in the first two days following incipient color.

The color measurements which were made from the 3/23 harvest are included (Table 5) to illustrate the relative maturity of the fruits based on the Hunter color values. Gibberellic acid treated fruits had the lowest a/b values of all treatments, but these fruits developed considerable color by the fourth and seventh days. Ethrel treatments triggered an initiation of color, but there was not a resulting color development by the fruits comparable to the control until the fourth day.

B. 4/22 Harvest

The preliminary observations were followed by a replicated experiment involving 4 growth regulators and a control. Polygalacturonase activity, PMG activity and cellulase were followed from 1 through 5 days past incipient color. There was a marked reduction in the PG and PMG activity of GA treated fruits when compared on a daily basis with the control and other treatments.

Table 4.--Shear press measurements¹ of Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 3/23. Units are in pounds.

Treatment ²	Days past incipient color						
	0	1	2	3	4	5	6
Water	106	63	43	32	33	27	28
Ethrel (1000 ppm)	90	97	42	36	30	32	22
IAA (100 ppm)	96	-	-	-	26	-	-
GA (100 ppm)	119	-	-	-	35	-	-
Kinetin (10 ppm)	101	-	-	-	36	-	-
N ⁶ BA (10 ppm)	119	-	-	-	32	-	-

¹Each number represents the average of 4 readings each from 5 fruits.

²Treatments consisted of 30 minute dips of various growth regulators.

Table 5.--Hunter a/b values¹ for Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 3/23.

Treatment ²	Days past incipient color								
	0	1	2	3	4	5	6	7	
Water	0.0	0.0	0.77	1.49	1.82	2.12	2.36	2.27	
Ethrel (1000 ppm)	0.0	0.0	0.29	0.98	1.78	2.02	2.31	2.31	
IAA (100 ppm)	0.0	-	-	-	1.89	-	-	2.54	
GA (100 ppm)	0.0	-	-	-	1.36	-	-	1.91	
Kinetin (10 ppm)	0.0	-	-	-	2.05	-	-	2.39	
N ⁶ BA (10 ppm)	0.0	-	-	-	1.80	-	-	2.32	

¹The higher the value the more red the fruit. Each value is based on the compilation of 5 fruits.

²Treatments consisted of various growth regulators used as 30 minute dips.

The PG activity increased markedly in the control between 1 and 2 days, and it continued to rise through the fifth day (Table 6). The activity in fruits of the four growth regulator treatments were quite low at 1 and 2 days, and Ethrel and NAA treated fruits did not reach the level of the control until the third day. The fruits treated with GA had very little activity at any day. Ethrel, IAA and GA treated fruits were significantly lower in activity at 2 days from the control. On the fourth day GA treated fruits were significantly lower in PG activity than the water, NAA, IAA and Ethrel treated fruits. The PG activity in Ethrel as well as GA treated fruits was significantly lower on the fifth day.

Polymethylgalacturonase activity (Table 7) was much higher than PG. The fruits treated with Ethrel or NAA did not approximate the level of the control until the third day. Indoleacetic acid treated fruits were significantly lower than the control until the fourth day. The GA treated fruits showed negligible activity on the first 4 days and only slight activity on the fifth. All GA treated fruits were significantly lower than the control from day 2 through day 5. As with PG activity, the PMG activity of Ethrel treated fruits was significantly lower than the control on the fifth day.

Cellulolytic activity increased rapidly in the control between 1 and 2 days past incipient color and remained high (Table 8). No treatments were significantly different from the control until the fourth day when the GA treated fruits were lower. Ethrel and GA treatments showed significantly less activity on the fifth day. Gibberellic acid

Table 6.--Polygalacturonase activity¹ of sodium chloride extracts of Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 4/22.

Treatment ²	Reps	Days past incipient color				
		1	2	3	4	5
Water	A	0.0 ^x	25.5	21.8	41.1	-
	B	4.1	43.1	31.6	58.8	59.6
	C	0.0	10.0	21.9	52.7	47.2
	D	-	-	44.4	50.0	58.9
	E	-	-	25.0	-	-
Avg.		1.4 a	26.2 a	28.9 a	50.7 a	55.2 a
Ethrel (1000 ppm)	A	0.0	0.0	32.7	37.0	-
	B	0.0	1.7	16.4	55.1	50.8
	C	1.9	1.8	25.7	51.9	40.0
	D	-	-	20.4	38.9	34.5
	E	-	-	32.8	-	-
Avg.		0.6 a	1.2 b	25.6 a	45.7 a	41.8 b
IAA (100 ppm)	A	0.0	1.6	9.1	47.3	-
	B	1.7	7.8	8.9	35.7	50.9
	C	0.0	0.0	1.9	48.2	50.9
	D	-	-	10.8	33.3	53.6
	E	-	-	11.1	-	-
Avg.		0.6 a	3.1 b	8.4 b	41.1 a	51.8 ab
NAA (100 ppm)	A	1.7	3.5	28.6	43.8	-
	B	0.0	1.5	33.3	57.7	57.1
	C	1.8	32.0	7.3	55.0	57.4
	D	-	-	15.5	48.3	50.8
	E	-	-	21.0	-	-
Avg.		1.2 a	12.3 ab	21.1 a	51.2 a	55.1 a
GA (100 ppm)	A	0.0	0.0	1.7	0.0	0.0
	B	0.0	0.0	0.0	0.0	0.0
	C	0.0	0.0	0.0	0.0	0.0
	D	-	-	0.0	7.7	-
	E	-	-	0.0	-	-
Avg.		0.0 a	0.0 b	0.3 b	1.9 b	0.0 c

¹PG activity was measured as percent decrease in drain time of 1.2% sodium polypectate (pH = 5.0). Incubation time was 30 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of mature green fruits.

^xTreatment means with the same letter on each ripeness date are not significant at the .05 level.

Table 7.--Polymethylgalacturonase activity¹ of sodium chloride extracts of Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 4/22.

Treatment ²	Reps	Days past incipient color				
		1	2	3	4	5
Water	A	1.7 ^x	62.3	19.3 ³	39.3 ³	-
	B	7.5	67.9	28.1	49.2	58.3 ³
	C	0.0	39.3	21.1	31.0	45.3
	D	-	-	39.3	9.1	58.5
	E	-	-	21.3	-	-
	Avg.	3.1 a	56.8 a	25.8 a	32.2 a	54.0 a
Ethrel (1000 ppm)	A	1.5	6.1	27.8 ³	33.9 ³	40.3 ³
	B	0.0	7.4	12.3	43.3	48.3
	C	0.0	8.2	22.2	13.6	41.9
	D	-	-	20.3	7.5	-
	E	-	-	32.1	-	-
	Avg.	0.6 a	7.2 b	22.9 a	24.6 a	43.5 b
IAA (100 ppm)	A	0.0	16.1	8.5 ³	33.9 ³	39.3 ³
	B	0.0	14.1	3.3	26.8	45.8
	C	0.0	5.2	0.0	23.4	54.5
	D	-	-	10.9	5.4	-
	E	-	-	11.5	-	-
	Avg.	0.0 a	11.8 b	6.8 b	22.4 a	46.5 ab
NAA (100 ppm)	A	0.0	8.5	22.7 ³	40.3 ³	-
	B	0.0	7.8	30.4	47.5	52.5 ³
	C	0.0	62.3	7.9	13.8	55.9
	D	-	-	17.9	11.5	46.6
	E	-	-	25.6	-	-
	Avg.	0.0 a	26.2 b	20.8 a	28.3 a	51.7 ab
GA (100 ppm)	A	0.0	1.7	0.0	6.1	8.8
	B	0.0	0.0	4.3	0.0	6.0
	C	0.0	1.5	0.0	0.0	3.2
	D	-	-	0.0	0.0	-
	E	-	-	3.2	-	-
	Avg.	0.0 a	1.1 b	1.5 b	1.5 b	6.0 c

¹PMG activity was measured as percent decrease in drain time of 1.2% pectin (pH = 4.0). Incubation time was 30 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of mature green fruits in various growth regulators.

³All treatments except GA were diluted 10:1 at 3, 4, and 5 days past incipient color.

^xTreatment means with the same letter on each ripeness date are not significant at the .05 level.

Table 8.--Cellulolytic activity¹ of sodium chloride extracts of Homestead 24 tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 4/22.

Treatment ²	Reps	Days past incipient color				
		1	2	3	4	5
Water	A	5.5 ^x	14.4	16.5	14.3	19.5
	B	10.4	13.4	26.5	18.9	24.8
	C	6.5	9.6	16.4	14.7	23.2
	D	-	-	11.4	25.3	15.5
	E	-	-	20.8	-	-
	Avg.	7.5 a	12.5 a	18.3 a	18.3 a	20.8 a
Ethrel (1000 ppm)	A	2.7	8.3	14.5	20.1	18.5
	B	5.9	13.6	10.7	19.7	13.5
	C	3.1	8.5	17.5	17.1	18.8
	D	-	-	17.1	15.9	8.3
	E	-	-	11.6	-	-
	Avg.	3.9 a	10.1 a	14.3 a	18.2 a	14.8 b _c
IAA (100 ppm)	A	6.4	10.2	11.0	23.1	25.2
	B	8.1	14.2	17.5	21.1	22.4
	C	11.1	6.4	16.0	16.6	18.4
	D	-	-	10.4	19.8	20.0
	E	-	-	19.5	-	-
	Avg.	8.5 a	10.3 a	14.9 a	20.2 a	24.5 a
NAA (100 ppm)	A	6.6	10.3	25.6	27.1	21.2
	B	1.6	7.9	18.1	23.4	18.6
	C	2.4	14.7	23.3	29.6	19.8
	D	-	-	23.4	17.5	16.8
	E	-	-	12.1	-	-
	Avg.	3.5 a	11.0 a	20.5 a	24.4 a	19.1 a _b
GA (100 ppm)	A	9.6	11.7	19.1	7.8	12.3
	B	13.1	10.8	15.0	15.0	12.9
	C	3.3	4.8	15.3	5.9	14.1
	D	-	-	11.2	8.1	9.2
	E	-	-	12.7	-	-
	Avg.	8.7 a	9.1 a	14.7 a	9.2 b	12.1 c

¹Cellulolytic activity was measured as percent decrease in drain time of 1.2% carboxy-methyl-cellulose (pH = 5.0). Incubation time was 60 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml substrate.

²Treatments are 30 minute dips of mature green tomato (Homestead).

^xTreatment means with the same letter on each ripeness date are not significant at the .05 level.

treated fruits were consistently lower than the control, but the difference was not nearly so marked as in the case of PG and PMG (Tables 6-7).

The firmness of the pericarp revealed significant differences on all but the fourth day (Table 9). On the first day no treatments were different from the control although Ethrel treatments resulted in firmer fruits than either GA or IAA. On days 2 and 3 the Ethrel treated fruits were significantly firmer than the water and GA. On the fifth day GA treated fruits were significantly firmer than the fruits dipped in IAA or water. The softening in the GA treated fruits plateaus after 3 days. The marked observation in this experiment is the extremely low PG activity in GA treatments; yet, softening proceeds normally (Tables 6 and 9). The cellulolytic activity is the only enzyme of those measured that could account for this loss of firmness in GA treated fruits (Table 8).

To determine the effect of treatment on Hunter color values, five lots of 100 fruits each were treated and sampled at 3 and 6 days past incipient color (Table 10). At the 3 day stage a/b ratios of Ethrel and GA treated fruits were less than the water and IAA treatments; however, at 6 days this difference was not nearly as marked. As with firmness, the color ratios cannot be used to predict PG or PMG activity in the GA treated fruits. This particular tendency was noted throughout the course of this investigation.

The color of GA treated fruits at 3 and 6 days is less than the control primarily because of a high b value while the Ethrel treated fruits at 3 days have a low a value (Table 10). The overall color

Table 9.--Shear press measurements¹ of Homestead 24 tomatoes treated with certain chemical dips for 30 minutes and ripened for 1 to 5 days in the dark at 20°C. Fruits were harvested on 4/22.

Treatment	Reps	Days past incipient color				
		1	2	3	4	5
Pounds						
Water	A	177 ^x	81	79	55	39
	B	130	76	83	86	44
	C	134	142	68	51	59
	D	161	99	99	62	42
	E	-	93	79	52	-
	Avg.		150 ab	98 b	81 b	61 a
Ethrel (1000 ppm)	A	160	117	100	66	72
	B	237	207	123	116	60
	C	172	123	121	64	85
	D	217	161	96	72	89
	E	-	133	97	70	-
	Avg.		197 a	148 a	107 a	78 a
IAA (100 ppm)	A	141	92	110	66	58
	B	126	135	89	62	39
	C	123	144	126	99	48
	D	126	108	89	78	63
	E	-	91	83	104	-
	Avg.		129 b	114 ab	99 ab	82 a
NAA (100 ppm)	A	146	151	92	74	88
	B	220	153	123	87	68
	C	196	80	100	81	67
	D	167	154	76	92	58
	E	-	123	122	68	-
	Avg.		182 a	132 ab	109 a	80 a
GA (100 ppm)	A	116	101	93	75	115
	B	115	99	90	94	87
	C	167	112	92	72	74
	D	190	97	53	53	49
	E	-	107	57	88	-
	Avg.		147 b	103 b	77 b	76 a

¹ Each reading represents the average of four separate slices of pericarp from each fruit.

^x Treatment means with the same letter on each ripeness date are not significant at the .05 level.

Table 10.--Hunter color readings of Homestead 24 tomato pericarp treated with certain growth regulators for 30 minutes and ripened in the dark for 3 or 6 days past incipient color. Fruits were harvested on 4/22.

Treatment	Days past incipient color	Reps	R_d	Hunter Color Values		
				a	b	a/b ¹
Water	3	1	12.6	22.2	16.7	1.33
		2	12.6	23.4	16.0	1.46
		3	11.8	30.0	16.3	1.84
		Avg.	12.3	25.2	16.3	1.54
Water	6	1	8.6	36.5	14.2	2.57
		2	8.5	36.3	14.1	2.57
		3	10.4	38.5	14.8	2.60
		Avg.	9.2	37.1	14.3	2.58
Ethrel (1000 ppm)	3	1	11.0	7.4	16.8	0.44
		2	10.4	4.8	17.3	0.28
		3	10.8	8.0	16.5	0.48
		Avg.	10.7	6.7	16.9	0.40
Ethrel (1000 ppm)	6	1	9.4	32.8	15.3	2.14
		2	9.2	33.4	15.3	2.18
		3	9.4	31.6	14.1	2.24
		Avg.	9.3	32.6	14.9	2.19
IAA (100 ppm)	3	1	11.7	25.5	15.5	1.65
		2	12.2	18.6	16.5	1.13
		3	11.4	21.8	15.5	1.41
		4	12.0	30.2	16.2	1.86
		Avg.	11.8	24.0	15.9	1.51
IAA (100 ppm)	6	1	9.9	36.7	15.6	2.35
		2	9.2	36.0	15.0	2.40
		3	9.4	37.0	14.5	2.55
		Avg.	9.5	36.6	15.0	2.43
GA (100 ppm)	3	1	14.6	14.6	20.0	0.73
		2	14.7	18.5	19.2	0.96
		Avg.	14.7	16.6	19.6	0.85
GA (100 ppm)	6	1	9.0	33.7	16.0	2.11
		2	9.8	34.2	16.2	2.11
		3	10.6	34.8	16.0	2.18
		Avg.	9.8	34.2	16.1	2.13

¹Each reading represents the blended, deaerated pericarp of 5 fruits.

development in GA treated fruits varied from that of the control. Often the yellow pigments developed before the red; however, the red pigment eventually would develop. The response by Ethrel treatments was different in that the fruits after the onset of incipient color were slower to develop any red or yellow color to the extent of the control or other treatments.

C. 5/30 Harvest

Fruits of the Walter cultivar were treated with GA at the mature green stage, and sampled for enzymes at 1, 4, 7 and 10 days past incipient color. Fruits treated with GA that turned color within the first 24 hours following treatment were also sampled (Tables 11-13). In these early breaking fruits a much reduced PG and PMG activity was observed (Tables 11-12). An interesting observation made in this harvest was that a greater activity of PG than PMG was present in GA treated fruits. This activity was not reflected in the water treatment. On 1, 4 and 7 days past incipient color the two GA treatments were significantly lower in PG than the control (Table 11). At day 10 the data were not analyzed statistically; however, the undiluted PG activity of the GA treatments was much lower than the diluted water treatment. The PMG activity (Table 12) is significantly lower in the treatments at 1, 4, 7 and 10 days. The control was diluted 10:1 at days 4, 7 and 10 while the GA remained concentrated; yet, there were still significant differences. Cellulase measurements showed a significant difference only on the fourth day (Table 13). The GA treated fruits were significantly lower in cellulase than the control and early breaker fruits treated with GA only on the fourth day.

Table 11.--Polygalacturonase activity¹ of sodium chloride extracts of Walter tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 5/30.

Treatment ²	Reps	Days past incipient color			
		1	4	7	10
Water	1	2.5 ^x	61.2	62.4	22.2 ³
	2	36.6	60.3	63.2	29.0
	3	42.5	53.7	53.7	27.3
	Avg.	27.2 a	59.7 a	59.8 a	26.2
GA (100 ppm)	1	0.7	6.7	11.6	-
	2	1.6	15.6	46.3	4.9
	3	0.0	1.3	3.7	-
	4	-	-	-	6.7
	5	-	-	-	5.1
	Avg.	0.8 b	7.9 b	20.5 b	5.6
GA (Early breaker)	1	1.4	10.1	4.8	38.2
	2	0.8	6.7	3.5	4.8
	3	1.7	13.0	8.4	11.5
	Avg.	1.3 b	9.9 b	5.6 b	18.2

¹Polygalacturonase activity was measured as percent decrease in drain time of 1.2% sodium polypectate (pH = 5.0). Incubation time was 30 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments are 30 minute dips of mature green Walter tomatoes.

³Diluted 10:1.

^xTreatment means with the same letter on each date are not significant at the .05 level.

Table 12.--Polymethylgalacturonase activity¹ of sodium chloride extracts of Walter tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 5/30.

Treatment ²	Reps	Days past incipient color			
		1	4	7	10
Water	1	3.2 ^x	39.1 ³	54.4 ³	50.4 ³
	2	40.7	40.0	47.4	55.2
	3	50.0	36.5	45.0	50.0
	Avg.	31.3 a	38.5 a	48.9 a	51.9 a
GA (100 ppm)	1	1.6	4.2	4.6	47.2
	2	1.8	11.3	42.1	9.2
	3	0.0	0.0	0.0	1.7
	4	-	-	-	7.5
	5	-	-	-	4.5
	Avg.	1.1 b	5.2 b	15.6 b	14.0 c
GA Early breaker	1	0.0	4.3	2.4	52.6
	2	0.9	5.3	2.5	3.1
	3	0.0	0.0	4.4	21.2
	Avg.	0.3 b	3.2 b	3.1 b	25.6 b

¹ Polymethylgalacturonase activity was measured as percent decrease in viscosity of 1.2% pectin (pH = 4.0). Incubation time was 30 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

² Treatments are 30 minute dips of mature green tomatoes.

³ Dilution of enzyme extract, 10:1.

^x Treatment means with the same letter on each date are not significant at the .05 level.

Table 13.--Cellulolytic activity¹ of sodium chloride extracts of Walter tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 5/30.

Treatment ²	Reps	Days past incipient color			
		1	4	7	10
Water	1	3.6 ^x	9.0	8.0	4.6
	2	9.4	11.2	9.9	5.7
	3	6.3	12.4	11.7	3.4
	Avg.	6.4 a	10.9 a	9.9 a	4.6 a
GA (100 ppm)	1	7.2	6.1	5.1	6.8
	2	5.6	5.8	9.3	5.4
	3	7.8	3.4	3.8	8.4
	4	-	-	-	-
	5	-	-	-	-
	Avg.	6.9 a	5.1 b	6.1 a	6.9 a
GA (Early breaker)	1	5.7	7.8	5.9	4.3
	2	4.3	7.8	6.7	6.9
	3	5.0	10.1	7.2	5.8
	Avg.	5.0 a	8.6 a	6.6 a	5.7 a

¹Cellulolytic activity was measured as percent decrease in drain time of 1.2% carboxy-methyl-cellulose (pH = 5.0). Incubation time was 60 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments are 30 minute dips of mature green tomatoes.

^xTreatment means with the same letter on each date are not significant at the .05 level.

This experiment also attempted to illustrate the very small amount of solution that is absorbed by the fruits upon dipping. Variable amounts of material (Table 14) were absorbed per fruit; nevertheless, the enzyme activity (Tables 11-13) was altered even in the lowest absorbing fruits. With only a few exceptions, high PG and PMG activities in GA treated fruits were associated with very low uptake of solution.

Pectinesterase activity (Table 14) was followed in the ripening fruits treated with water or GA. The activity was not in any way uniform between replicates or between sampling dates. The GA treated fruits appeared to be very much like the control at all stages insofar as activity of this enzyme was concerned.

Shear force readings (Table 14) on water and GA treated fruits at 1, 4, 7 and 10 days past incipient color showed that the GA treated fruits were not significantly different from the control.

D. 6/6 Harvest

Walter tomato fruits were treated at the mature green stage with IAA, IAA + catechol and GA. Enzyme activity of GA treated fruits which were at incipient color on the treatment date was also determined (Tables 15-17). The fruits treated at incipient color had significantly higher PG and PMG activity than those treated at the mature green stage. At 10 days past incipient color the fruits treated at incipient color with GA were significantly lower in PG and PMG activity than the control, but significantly higher than the fruits treated with GA at the mature green stage. At 4, 7 and 10 days fruits treated with GA at the mature green stage were significantly lower in PG than the control.

Table 14.--Shear press measurements, pectinesterase activity and amount of Gibberellic acid solution absorbed per fruit. Fruits were harvested on 5/30.

Treatment	Reps	Days past incipient color							
		1	4	7	10	1	4	7	10
				Pounds		(PE.u.)g x 10 ³			
Water	1	225	77 ^x	71	54	53.8	99.0	89.5	65.0
	2	83	75	84	55	81.2	88.3	66.6	94.2
	3	109	78	49	35	50.8	55.4	57.8	51.1
	Avg.	139	77 a	68 a	48 a	61.9	80.9	71.3	70.1
GA (100 ppm)	1	160	68	85	44	68.0	69.0	71.1	100.0
	2	220	76	101	59	66.0	93.0	82.2	94.0
	3	123	105	76	52	79.6	44.4	71.1	-
	Avg.	168	83 a	87 a	52 a	71.2	68.8	74.8	97.0
GA (Early breaker)	1	-	93	63	83				
	2	-	77	60	43				
	3	-	88	63	79				
	Avg.		86 a	62 a	68 a				
Grams of 100 ppm Dip Absorbed per Fruit									
GA (100 ppm)	1	.383	.539	.304	.268				
	2	.000	.000	.229	.655				
	3	.260	1.065	1.129	.000				
	4				.097				
	5				.000				
GA (Early breaker)	1	.703	.298	.496	.108				
	2	.968	.233	1.059	.244				
	3	.236	.260	.832	.802				

^xTreatment means with the same letter on each date are not significant at the .05 level.

Table 15.--Polygalacturonase activity¹ of sodium chloride extracts of Walter tomato fruit pericarp at various stages of maturity. Fruits were harvested on 6/6.

Treatment ²	Reps	Days past incipient color			
		1	4	7	10
Water	1	5.4 ^x	57.1	63.0	60.3
	2	24.1	57.1	62.7	62.7
	3	0.8	59.3	45.3	57.1
	Avg.	10.1 a	57.8 a	57.0 a	60.0 a
GA (100 ppm)	1	0.0	1.5	3.8	0.0
	2	0.0	5.7	0.8	0.0
	3	0.0	0.0	1.5	4.8
	Avg.	0.0 a	2.4 b	2.0 b	1.6 c
IAA (100 ppm)	1	0.0	46.5	62.1	-
	2	4.0	53.2	59.2	-
	3	0.0	57.7	61.6	-
	Avg.	1.3 a	52.5 a	61.3 a	-
IAA + Catechol (100 ppm)	1	0.9	54.3	61.3	-
	2	1.8	17.0	61.1	-
	3	3.3	57.7	52.9	-
	Avg.	2.0 a	43.0 a	58.4 a	-
GA (Treated at breaker stage) (100 ppm)	1	-	51.9	-	22.9
	2	-	44.3	-	45.8
	3	-	50.0	-	16.5
	Avg.	--	48.7 a	-	28.4 b

¹ Polygalacturonase activity was measured as percent decrease in drain time of 1.2% sodium polypectate (pH = 5.0). Incubation time was 30 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

² Treatments are 30 minute dips of mature green Walter tomatoes.

^x Treatment means with the same letter on each ripeness date are not significant at the .05 level.

Table 16.--Polymethylgalacturonase activity¹ of sodium chloride extracts of Walter tomato fruit pericarp at various stages of maturity. Fruits were harvested on 6/6.

Treatment ²	Reps	Days past incipient color			
		1	4	7	10
Water	1	8.5 ^x	39.3 ³	45.8 ³	54.1 ³
	2	51.6	41.5	50.4	57.1
	3	0.0	46.7	46.4	61.7
	Avg.	20.0 a	42.5 a	47.5 a	57.6 a
GA (100 ppm)	1	1.4	0.9	7.7	0.0
	2	1.6	17.9	2.7	0.9
	3	0.0	3.4	2.6	1.8
	Avg.	1.0 a	7.4 b	4.3 b	0.9 c
IAA (100 ppm)	1	1.6	23.7 ³	55.6 ³	-
	2	2.8	32.5	56.4	-
	3	0.8	42.9	56.4	-
	Avg.	1.7 a	33.0 a	56.1 a	-
IAA + Catechol (100 ppm)	1	0.7	33.3 ³	50.9 ³	-
	2	2.4	6.3	38.5	-
	3	5.5	42.3	27.7	-
	Avg.	2.9 a	27.3 a	39.0 a	-
GA (Treated at breaker stage) (100 ppm)	1	-	62.3	-	-
	2	-	53.6	-	55.6
	3	-	63.6	-	24.6
	Avg.	-	59.8	-	40.1 b

¹Polymethylgalacturonase activity was measured as percent decrease in drain time of 1.2% pectin. Incubation time was 30 minutes. One ml of extract (10 g of tissue concentrated to 5 ml and then diluted) was added to 5 ml of substrate.

²Treatments are 30 minute dips of mature green Walter tomatoes.

³The water, IAA and IAA + Catechol were diluted 10:1 at 4, 7 and 10 days. The GA readings are based on a concentrated extract.

^xTreatment means with the same letter on each ripeness date are not significant at the .05 level.

Table 17.--Cellulolytic activity¹ of sodium chloride extracts of Walter tomato fruit pericarp at various stages of maturity. Fruits were harvested on 6/6.

Treatment ²	Reps	Days past incipient color			
		1	4	7	10
Water	1	12.1 ^x	17.8	11.5	6.3
	2	-	19.1	7.8	11.4
	3	11.0	17.4	9.2	10.3
	Avg.	11.6 a	18.1 a	9.5 a	9.3 a
GA (100 ppm)	1	6.8	4.4	13.0	12.9
	2	7.5	8.8	9.8	14.0
	3	9.7	13.4	13.0	10.3
	Avg.	8.0 a	8.9 b	11.9 a	12.4 a
IAA (100 ppm)	1	7.7	20.2	-	-
	2	11.4	16.5	-	-
	3	10.4	19.1	-	-
	Avg.	9.8 a	18.6 a	-	-
IAA + Catechol (100 ppm)	1	8.5	15.8	14.8	-
	2	7.5	13.8	9.7	-
	3	11.0	18.5	9.3	-
	Avg.	9.0 a	16.0 a	12.7 a	-
GA (Treated at breaker stage) (100 ppm)	1		9.4 ³		13.3
	2		10.2		12.8
	3		12.2		16.5
	Avg.		10.6		14.2 a

¹Cellulolytic activity was measured as percent decrease in drain time of 1.2% carboxy-methyl-cellulose (pH = 5.0). Incubation time was 60 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments are 30 minute dips of mature green tomatoes.

³Incubation time was 30 minutes.

^xTreatment means with the same letter on each ripeness date are not significant at the .05 level.

Catechol (Libbert et al., 1969) has been shown to inhibit IAA oxidase, and IAA oxidase has been observed in ripening tomato fruits (Oyer, 1970); thus, IAA + catechol was included as a treatment. The results with the catechol tended to be lower than the IAA alone but not significantly (Tables 15-16).

Polymethylgalacturonase activity was higher than PG activity. Growth regulator treatments had the same effect on this enzyme as they did on PG (Table 16). Cellulase activity was variable between sampling days for this harvest (Table 17). The activity of the enzyme in GA treated mature green fruits is significantly lower than the control on the fourth day, but no difference exists at 7 and 10 days past incipient color. Fruits treated with either GA at the turning stage, IAA or IAA + catechol were not significantly different from the control at 1, 4, 7 or 10 days.

The PE activity of the GA treated and control fruits (Table 18) was very nearly the same at each sampling date.

A loss of firmness was observed in all treatments through the seventh day (Table 19). None of the treatments resulted in significant differences.

E. 6/25 Harvest

A study was made to establish a minimum, effective enzyme inhibiting concentration for GA using 10, 50 and 100 ppm GA. The 10 ppm concentration (Tables 20-21) shows an inhibiting effect on PG and PMG activity at 2 and 4 days past the breaker stage; however, at 7 days there is no readily apparent difference between this concentration and the control.

Table 18.--Pectinesterase activity of Walter tomato fruit pericarp at various stages of ripeness. Fruits were harvested on 6/6.

Treatment ¹	Reps	Days past incipient color			
		1	4	7	10
(PE.u.)g x 10 ³					
Water	1	59.1	86.8	75.0	75.7
	2	81.5	87.7	67.1	69.8
	3	61.3	96.5	69.8	83.7
	Avg.	67.3	90.3	70.6	76.4
GA (100 ppm)	1	63.1	64.8	77.3	85.0
	2	61.5	70.3	94.0	63.6
	3	67.6	89.8	92.2	93.4
	Avg.	64.1	75.0	87.8	79.2

¹Fruits were dipped for 30 minutes at the mature green stage.

Table 19.--Shear forces of Walter tomato fruit pericarp at various stages of maturity. Fruits were harvested on 6/6.

Treatment ¹	Reps	Days past incipient color			
		1	4	7	10
Pounds					
Water	1	77.5	113.3	86.0	51.9
	2	83.8	85.0	96.0	71.8
	3	183.3	118.8	79.4	56.9
	Avg.	114.9 a	105.7 a	87.1 a	60.2
GA (100 ppm)	1	127.0	113.8	76.6	76.9
	2	140.8	103.8	57.5	114.0
	3	185.0	89.5	76.3	60.6
	Avg.	150.9 a	102.4 a	70.1 a	83.8
IAA (100 ppm)	1	165.0	113.8	59.4	
	2	132.0	103.8	66.9	
	3	193.2	89.5	87.5	
	Avg.	163.3 a	102.4 a	71.3 a	
IAA + catechol (100 ppm)	1	166.3	123.8	60.4	
	2	151.3	106.3	68.1	
	3	93.8	85.0	59.8	
	Avg.	137.1 a	105.0 a	62.8 a	

¹Fruits were dipped for 30 minutes at the mature green stage.

Table 20.--Polygalacturonase activity¹ of Walter tomato fruit pericarp dipped in various concentrations of gibberellic acid and sampled at various stages of ripeness. Fruits were harvested on 6/25.

Treatment ²	Reps	Days past incipient color		
		2	4	7
GA (10 ppm)	1	1.4	18.8	47.9
	2	3.3	5.4	56.7
	3	-	25.5	55.6
	Avg.	2.4	16.6	53.4
GA (50 ppm)	1	1.6	45.5	7.0
	2	1.8	3.0	12.6
	3	1.7	6.7	3.2
	Avg.	1.7	18.4	7.6
GA (100 ppm)	1	1.6	3.6	19.7
	2	0.0	8.6	6.3
	3	-	-	-
	Avg.	0.8	6.1	13.0
Water	1	24.6	32.3	58.5
	2	37.9	52.0	17.5(10:1)
	3	-	52.6	-
	Avg.	31.3	45.6	58.5

¹Polygalacturonase activity was measured as percent decrease in drain time of 1.2% sodium polypectate (pH = 5.0). Incubation time was 15 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of mature green fruits.

Table 21.--Polymethylgalacturonase activity¹ of Walter tomato fruit pericarp dipped in various concentrations of gibberellic acid and sampled at various stages of ripeness. Fruits were harvested on 6/25.

Treatment ²	Reps	Days past incipient color		
		2	4	7
GA (10 ppm)	1	1.5	40.4	68.4 (conc)
	2	0.0	12.9	36.8 (10:1)
	3	-	45.8	44.1 (10:1)
	Avg.	0.8	33.0	40.5 (10:1)
GA (50 ppm)	1	0.0	61.9	17.4
	2	0.0	2.8	24.6
	3	1.6	15.0	1.5
	Avg.	0.5	26.6	14.5
GA (100 ppm)	1	1.5	5.1	42.4
	2	1.5	10.3	14.1
	3	-	-	-
	Avg.	1.5	7.7	28.3
Water	1	45.1	52.6 (10:1)	48.3 (10:1)
	2	56.4	70.3 (10:1)	41.8 (10:1)
	3	-	67.9 (10:1)	-
	Avg.	50.8	63.6 (10:1)	45.1 (10:1)

¹Polymethylgalacturonase activity was measured as percent decrease in drain time of 1.2% pectin (pH = 4.0). Incubation time was 15 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of mature green fruits.

Table 22.--Cellulolytic activity¹ of Walter tomato fruit pericarp dipped in various concentrations of gibberellic acid and sampled at various stages of ripeness.

Treatment ²	Reps	Days past incipient color		
		2	4	7
GA (10 ppm)	1	2.7	8.6	2.6
	2	3.8	4.9	3.8
	3	-	9.4	4.8
	Avg.	3.3	7.6	3.7
GA (50 ppm)	1	2.9	9.2	4.7
	2	2.5	5.6	3.8
	3	3.9	7.4	4.7
	Avg.	3.1	7.4	4.4
GA (100 ppm)	1	1.9	6.3	2.9
	2	4.2	4.9	6.1
	3	-	-	-
	Avg.	3.1	5.6	4.4
Water	1	4.7	7.5	2.9
	2	7.6	12.6	4.5
	3	-	8.4	-
	Avg.	6.2	9.5	3.7

¹Cellulolytic activity was measured as percent decrease in drain time of 1.2% carboxy-methyl-cellulose (ph = 5.0). Incubation time was 45 minutes. One ml of extract (10 g of tissue concentrated to 5 ml) was added to 5 ml of substrate.

²Treatments consisted of 30 minute dips of mature green fruits.

The 50 ppm concentration appears to be as effective as the 100 ppm in altering the PG and PMG activity (Tables 20-21). The 50 ppm and 100 ppm treatments were considerably lower than the water at each sampling date. The variability between replicates may be due to a difference in GA uptake during dipping. Cellulase activity appears generally depressed in this harvest, and the GA treated fruits, except at 7 days, are somewhat lower than the control.

II. Effect of Growth Regulators on Ripening Rate

A. 1/28, 2/27, 3/23, 4/22 and 6/6 Harvests

Table 23 is a compilation of harvests presented to illustrate the number of days for 50% of the fruits to reach incipient color following treatment at the mature green stage. On the 1/28 harvest (Homestead 24 cultivar) 6 to 8 days were required for fruits to turn for all treatments except Ethrel. The control fruits required 7 days. On the 2/27 harvest all treated fruits except Ethrel began turning color in 5 to 6 days. The striking observation was the rate that Ethrel treated fruits turned. It took a maximum of 5 days for 100% of the treated fruits to turn following the 1/28 harvest and 4 days following the two succeeding harvests. The last harvest from Homestead required only 3 days for 100% of the Ethrel treated fruits to ripen. To illustrate the magnitude of this rate, the next fastest inducement by a treatment was the water control on the 3/23, 4/22 and 6/6 harvests which required 4 days for 50% of the fruits to turn. In these experiments no treatment suppressed the color induction mechanism of the fruits.

Table 23.--Days to incipient color of tomato fruit from 5 separate harvests in the winter and spring of 1970. Treatments were made of certain growth regulators by dipping MG fruit for 30 minutes.

Treatment	Harvest Date	Number of fruit per treatment	Days until 50% of the fruit were at incipient color
Water	1/28	30	7
	2/27	39	5
	3/23	44	4
	4/22	100	4
	6/6	50	4
Kinetin (10 ppm)	1/28	30	7
	2/27	35	4
	3/23	39	4
Kinetin (100 ppm)	1/28	30	8
IAA (100 ppm)	1/28	30	7
	1/28	30	8
	2/27	36	6
	3/23	39	4
	4/22	100	5
	6/6	50	6
IAA + Catechol (100 ppm)	6/6	50	5
GA (100 ppm)	1/28	30	8
	2/27	50	6
	3/23	59	4
	4/22	100	5
	6/6	50	5
N ⁶ BA (10 ppm)	1/28	30	6
	2/27	34	6
	3/23	39	6
Ethrel (10 u1 injection per immature fruit)	1/28	70	5 (100%)
Ethrel (10 u1 injection per mature green fruit)	1/28	25	4 (100%)
Ethrel (1000 ppm)	2/27	38	4 (100%)
	3/23	43	4 (100%)
	4/22	100	3 (100%)
NAA (100 ppm)	4/22	100	4

III. Effect of Growth Regulators on Ethylene Evolution from Whole Fruits

A. 4/22 Harvest

An experiment designed to monitor the ethylene evolution of fruits in response to growth regulators was set up from fruits harvested on 4/22. Five replicates were used and the ethylene evolution was followed from 3 days prior to turning until 7 days past. Figure 1 illustrates the ethylene evolution of GA, Ethrel and water treated fruits. Ethrel and GA treated fruits responded in a similar manner insofar as magnitude of ethylene production during ripening. In this first experiment there was a great deal of similarity between the two curves; however, Ethrel treated fruits began the ripening process much sooner than either the control or GA treatments. The level of ethylene being evolved was higher in fruits dipped in Ethrel. The water or GA dipped fruits never evolved pre-breaker ethylene levels as high as Ethrel dips. The water treated fruits reached a peak ethylene production one day before those treated with Ethrel or GA.

B. 4/30 Harvest

The results of this experiment (Figure 2) are very much like the 4/22 harvest. The NAA treatment does not have the sharp peaks characteristic of the other treatments. As was found in all experiments of this type, Ethrel treated fruits at the post-dipping stage evolved more ethylene than the control or GA treated fruits.

C. 5/30 and 6/16 Harvests

Data from observations obtained from the use of the Walter cultivar revealed that fruits from the 5/30 harvest produced twice as much ethylene as the previous two harvests. The Ethrel and GA treated

Figure 1.--Ethylene evolution from Homestead 24 tomato fruits treated at the mature green stage with 30 minute dips of 100 ppm GA, 1000 ppm Ethrel or water. Fruits were harvested on 4/22. Each day five fruits were sampled individually and a mean was calculated. B is the color-break stage of ripeness.

Figure 2.--Ethylene evolution from Homestead 24 tomato fruits treated at the mature green stage with 30 minute dips of 100 ppm GA, 100 ppm NAA, 1000 ppm Ethrel water. Fruits were harvested on 4/30. Each day 7 fruits were sampled individually and a mean was calculated. B is the color-break stage of ripeness.

fruits produced much more ethylene than the control. The control fruits peaked at 2 days past the breaker while the GA and Ethrel treated fruits peaked 4 days past this stage. It was observed (Figures 3-4) that the threshold ethylene rate for induction of ripening is less than 0.5 μl per kg-hr. The Ethrel treatments invariably resulted in 2 to 3 times this amount of ethylene coming from the fruits prior to incipient color (Figures 1-4).

In the 6/16 harvest the water and GA treated fruits did not exhibit the ethylene curve characteristic of the other harvests. Ethylene production increased to 4 days after color-break and retained this rate of production until +6 days. The 1000 ppm and 5000 ppm Ethrel treatments had similar ethylene production curves to previous harvests. The 5000 ppm Ethrel treated fruits evolved ethylene at a much increased rate over the control or other treatments. The fruits, immediately after dipping, began evolving ethylene at a rate 10 to 12 times that of the threshold level. It was also observed that ethylene evolution was lower in this harvest than the other three. The control was very low (Figure 4), ie. peak output was 4.75 $\mu\text{l/kg-hr}$; yet, the overall curve appears to plateau once the peak was obtained.

IV. Effect of Growth Regulators on Weight Loss

A. 4/22, 4/30, 5/30 and 6/16 Harvests

The weight loss of fruits as they ripened is tabulated in Table 24. On the 4/22 harvest the water dipped fruits had the greatest percentage weight loss, but this did not hold up in succeeding harvests. The only marked difference appears in the 6/16 harvest when the 5000 ppm Ethrel dip resulted in only a 1.5% weight loss.

Figure 3.--Ethylene evolution from Walter tomato fruits. Each day 10 separate fruits were sampled. B is color-break stage.

Figure 4.--Ethylene evolution from Walter tomato fruits treated at the mature green stage with 30 minute dips of 100 ppm GA, 1000 ppm Ethrel, 5000 ppm Ethrel or water. Fruits were harvested on 6/16. Each day 8 fruits were sampled individually and a mean was calculated. B is the color-break stage of ripeness.

Table 24.--Weight losses of tomato fruits treated with various growth regulators and ripened for different lengths of time.

Treatment	Harvest Date*	Reps	Percent Weight Loss	Days from Dipping
Ethrel	4/22	5	4.3%	14
GA	4/22	5	4.1%	14
Water	4/22	5	5.2%	14
Ethrel	4/30	8	4.0%	16
GA	4/30	8	4.2%	16
Water	4/30	8	4.2%	16
NAA	4/30	8	4.4%	16
Ethrel	5/30	10	3.0%	11
GA	5/30	10	2.6%	11
Water	5/30	10	2.8%	11
Ethrel (5000 ppm)	6/16	7	1.5%	11
Ethrel (1000 ppm)	6/16	7	2.3%	11
GA	6/16	7	2.2%	11
Water	6/16	7	2.2%	11

*The 4/23 and 4/30 harvests were of the Homestead cultivar; the 6/6 and 6/16 were of the Walter cultivar.

V. Effect of Growth Regulators on Ethylene Evolution from Tissue Slices

Differences in ethylene evolution were observed in response to GA and Ethrel treated slices (Table 25). Two different incubation periods are reported on the same tissue slices. The higher concentration of GA was effective in suppressing ethylene evolution. The 10^{-3} and 10^{-4} concentrations of GA were less effective. Both of the lower levels after 18 hours incubation were within 10% of the control. All experiments were replicated two to four times.

Fruit slices were exposed to Ethrel (3000 ppm) and incubated for the same period as the GA treated fruit slices (Table 25). A 15% increase in ethylene at 6 hours was observed while at 18 hours there was a 60% increase.

Based on the results of the data in Table 25, a concentration gradient of Ethrel was tested. The levels were 12, 25, 100 and 1200 ppm of Ethrel per flask. The flasks were replicated and two gas samples per flask were removed after incubation. Results of the concentration gradient experiment showed that concentrations of 12, 25, and 100 ppm were ineffective in inducing an ethylene production at 6 hours (Table 26). The 1200 ppm concentration resulted in a 30% increase after 6 hours. At the 18 hour incubation period there was a 13% increase observed in the 100 ppm treatment and a 63% increase in the 1200 ppm.

Results with additional concentrations ranging from 240 to 2400 ppm are reported (Table 27). After incubation for 18 hours an increasing percentage of ethylene evolution above the control with increasing concentrations of Ethrel was observed. The increased evolution of ethylene does not appear to be directly proportional to Ethrel concentration.

Table 25.--Percent increase or decrease in ethylene production over an appropriate control from tomato pericarp sliced into 0.5 cm cubes. The tissue was at the early breaker stage.

Treatment	Incubation Time	Percent Change
10^{-2} M GA	6 hr	33% less
10^{-3} M GA	6 hr	11% less
10^{-4} M GA	6 hr	11% less
Ethrel (3000 ppm)	6 hr	15% increase
10^{-2} M GA	18 hr	32% less
10^{-3} M GA	18 hr	9% less
10^{-4} M GA	18 hr	2% less
Ethrel (3000 ppm)	18 hr	60% increase

Table 26.--Percent increase in ethylene production by tomato pericarp sliced into 0.5 cm cubes and incubated in a buffered media with various concentrations of Ethrel.

Treatment	Incubation Time	Percent Change
12 ppm Ethrel	6 hr	0
25 ppm Ethrel	6 hr	0
100 ppm Ethrel	6 hr	0
1200 ppm Ethrel	6 hr	30% increase
12 ppm Ethrel	18 hr	0
25 ppm Ethrel	18 hr	0
100 ppm Ethrel	18 hr	13% increase
1200 ppm Ethrel	18 hr	63% increase

Table 27.--Percent increase in ethylene production by tomato pericarp sliced into 0.5 cm cubes and incubated for 18 hr in a buffered media with various concentrations of Ethrel.

Treatment	Percent Change
240 ppm Ethrel	19%
720 ppm Ethrel	25%
1200 ppm Ethrel	75%
2400 ppm Ethrel	89%

The effect of very high Ethrel concentrations (Table 28) on the capacity of the pericarp slices to evolve ethylene is presented. At both 6 and 18 hours incubation the peak ethylene evolution was observed with 2400 ppm whereas Ethrel at 4800 and 9600 was progressively more inhibiting.

VI. Effect of GA on Texture

An interesting observation was made on blended tissue previously treated with GA. The tissue remained chunky after blending and could not be pipetted because of the coagulation. Water treated tissue of the same physiological age was smooth and free to pipet. If enzyme extracted from water treated fruits was added to the blended GA treated fruit, the blendate rapidly became smooth and easy to pipet.

Table 28.--Percent increase in ethylene production by tomato pericarp sliced into 0.5 cm cubes and incubated in a buffered media with various concentrations of Ethrel.

Treatment	Incubation	Percent Change
1200 ppm Ethrel	6 hr	19% increase
2400 ppm Ethrel	6 hr	33%
4800 ppm Ethrel	6 hr	26%
9600 ppm Ethrel	6 hr	6%
1200 ppm Ethrel	18 hr	56%
2400 ppm Ethrel	18 hr	62%
4800 ppm Ethrel	18 hr	51%
9600 ppm Ethrel	18 hr	25%

DISCUSSION

Gibberellic acid treatments resulted in a significant decrease in the PG and PMG enzyme activities in the tomato pericarp of the cultivars studied. Hobson (1964) observed a correlation between PG activity and fruit softening; however, in the present study GA treated fruits were generally not significantly firmer than the control. Other ripening processes were not seriously retarded as evidenced by substantial color development and ethylene production. The activity of cellulase has been previously observed to increase during ripening (Hall, 1964); however, Hobson (1968) concluded that it was not correlated with the softening process. In this study pectinesterase and cellulase from GA treated fruits were found similar to that of the control; however, PE activity has not been correlated with softening (Hall and Dennison, 1960). There may very well be enzymes and processes associated with ripening that have not been studied. The role of cellulase is still conjectural and the present study does not rule out a role in softening since it was not observed to be significantly different from the control in GA treated fruits that softened. The suppression of PG and PMG activity by GA and the further observation of other processes in fruit ripening proceeding normally are important. The role in fruit softening of pectolytic as well as cellulolytic enzymes needs to be more carefully assessed. A well defined role for GA in processes involved in fruit ripening is not yet possible.

Dostal and Leopold (1967) concluded from GA treated fruits that ethylene production necessary for incipient color was suppressed; however, in the present study any suppressed induction of incipient color was not observed (Table 23). Furthermore, the ethylene production (Figures 1-4) by treated fruits was equal to or greater than the control during the ripening period.

Since the GA treated fruits evolve ethylene normally during ripening, it appears that ethylene is not necessary for the production of certain hydrolytic enzymes. Experiments with high concentrations of GA on early breaker stage tissue slices (Table 25) revealed up to a 33% decrease in ethylene evolution; however, the lower concentrations at the 18 hour incubation time were within 10% of the water treatment. This information with tissue slices in the case of the Ethrel agrees with the data obtained with intact fruits; however, the GA data appear inconsistent in that there is no decrease in ethylene production by whole fruits. If a whole fruit absorbs 1 g of a uniform solution of 100 ppm GA (Table 14), this is equivalent to absorbing 0.1 mg of active ingredient per fruit. Assuming a fruit weight of 100 g, this means that 10 ug of GA per 10 g of fruit exerted a pronounced effect on pectolytic enzyme inhibition. The tissue slice experiments did reveal that GA concentrations as low as 10^{-4} M were as effective in reducing ethylene evolution; however, 10^{-4} M is equivalent to 350 ug of GA per 10 g of pericarp. This is considerably higher than the conservative estimate calculated with the whole fruit; thus, the higher GA level with the tissue slices could account for the different ethylene evolution response.

As indicated, the amount of GA required to exert its action of retarding PG and PMG is extremely low. As low as 50 ppm dips were effective, while even the 10 ppm dip had a definite delaying action (Tables 20-21) on the enzyme activity. In estimating a level of GA that can exert an effect on PG or PMG activity, one can calculate from the 10 ppm concentration that less than 1 ug per 10 g of tissue can exert a delaying action on the enzymes.

Fruits that are treated at incipient color with GA, when the endogenous threshold level of ethylene necessary for ripening has already been reached, do not respond as markedly insofar as diminished PG and PMG activity is concerned (Tables 15-16). The enzyme activity of GA treated fruits that were within 12 hours of breaking when treated (Tables 11-12) shows that the activity was not significantly different from GA treated fruits at the mature green stage. This early breaker stage appears to be the upper physiological age for the fruits in order for GA to exert its maximum effect.

The effect of other growth regulators is interesting and has created some questions as to their role. In no instance did the cytokinins exert an effect on color, firmness or enzymes that was different from the water treated fruits. The auxins, IAA and NAA, did have a consistent inhibiting effect (Tables 6, 7, 15 and 16) on the PG and PMG enzymes in the early stages of ripening; however, as ripening progressed, the enzyme activity reached that of the control. Significant differences were not observed at 4 and 5 days past incipient color in IAA or NAA treated fruits. Effects of firmness, ethylene evolution and other processes were similar to previous reports for auxins (Emmert and

Southwick, 1954, Hartman, 1959, Mitchell and Marth, 1944, Abdel-Kader et al., 1966). Any long term IAA effect may be destroyed by IAA oxidase formation in ripening fruits (Oyer, 1970). Catechol inhibits IAA oxidase activity (Libbert et al., 1969), but was not effective as a treatment when used in conjunction with IAA in this study. No change in activity of cellulase in response to IAA or NAA was observed.

The action of Ethrel has been marked on the initiation of incipient color in the treated fruits. Walter and Homestead 24 tomato fruits were very responsive to Ethrel (Table 23). An above threshold evolution of ethylene from the fruits in response to treatment was always observed (Figures 1-4). The increased ethylene, as has been reported (Warner and Leopold, 1969, Russo et al., 1968, Robinson et al., 1968, Rabinowitch et al., 1970), is no doubt the ethylene gas response which characteristically initiates incipient color, the climacteric and other ripening processes.

The action of Ethrel is thought to result from its own breakdown to ethylene gas. As in the case of exogenously applied gas, the fruits are induced to produce their own ethylene which precedes the climacteric. The Ethrel treated fruits exhibited an enhanced ethylene evolution, especially at 5000 ppm concentrations (Figure 4). These results agree with the recent findings of Dennis et al. (1970). The results support a natural ethylene stimulation in response to Ethrel which then results in the onset of incipient color.

Dennis et al. (1970) concluded, based on tomato homogenate experiments, that the release of ethylene from Ethrel was nonenzymatic.

Experiments in this study with tissue slices incubated in a buffered media (Lieberman and Kunishi, 1969) add to the results of Dennis et al. (1970). Definite increases in ethylene evolution from pericarp tissue incubated with various concentrations of Ethrel were observed (Tables 25-28). After 18 hours of incubation in concentrations as low as 100 ppm Ethrel, a 13% increase was observed (Table 26). Concentrations of over 2400 ppm resulted in smaller percentage increases evolved.

The activity of PG, PMG and cellulase increased as fruits ripened in all treatments except GA. If the loss of tomato fruit firmness is enzymatically controlled, the overall results implicate cellulolytic enzymes or unknown enzymes being responsible for softening. A possibility of nonenzymatic degradation taking place as reported by Dakin (1963) for cabbage tissue is recognized.

The one consistent aspect of the ethylene evolution from those fruits treated with GA, NAA or water was that once a level between 0.25 and 0.50 $\mu\text{l/kg-hr}$ was reached the fruit would begin ripening within two days. Treatments had little effect on the shape of the ethylene curves; however, the peak ethylene production from treatments and the control varied with harvests. On the 4/22 harvest the peak ethylene production was 2 to 3 days past incipient color while three to four days was the pattern in the 4/30 harvest. Ethrel and GA treated fruits consistently evolved greater amounts of ethylene than the control (Figures 1-4). A most interesting observation was made on the 5/30 harvest of Walter tomatoes. Ethrel and GA treated fruits evolved 1/2 again as much ethylene as control fruits and reached a peak two days after the control (Figure 3). This reaction by fruits treated with the regulators was not

reflected in any daily visual observation which was made. Apparently, the production of ethylene by the fruits is variable by nature and cannot be used as a measure to predict any physiological disorders or anomalies.

SUMMARY AND CONCLUSIONS

A definite inhibition of PG and PMG activity occurred in ripening tomato fruits dipped for 30 minutes in as little as 50 ppm GA. Lower concentrations of GA as well as 100 ppm dips of IAA, 100 ppm IAA + catechol and 1000 ppm Ethrel inhibited PG and PMG activity in the early stages of ripening; however, the inhibition disappeared with time. The cellulase activity of treated fruits was not affected by any treatment. The firmness of the tissue as measured by an Allo-Karmer shear press did not appear to be modified by treatments. In control fruits the PG and PMG activity increased once incipient coloring occurred. In all treatments the firmness decreased as the fruits ripened.

The color development of GA and Ethrel treated fruits was retarded initially, but as the days past incipient color increased, the color approached that of the control.

Ethrel treatments in 1000 ppm, 30 minute dips resulted in a decrease in the time required for mature green fruits to undergo incipient color. There was no alteration in the onset of incipient color by either GA, IAA, NAA, Kinetin or N⁶BA.

Whole fruits treated with GA evolved as much or more ethylene than the control as the fruits ripened. Napthaleneacetic acid treated

fruits exhibited no differences in ethylene evolution from the control. Ethrel treated fruits produced ethylene well above the threshold for ripening and treated fruits produced higher levels throughout the ripening period than the control. Ethrel treated fruits produced ethylene in a similar pattern to the control. In three of the four harvests the ethylene curves were similar in shape to the control and other treatments.

Tissue slices treated with Ethrel evolved much more ethylene than tissue slices incubated without Ethrel. Ethylene evolution increased from tissue slices with increasing concentrations from 100 to 2400 ppm. Concentrations of Ethrel at 4800 and 9600 ppm resulted in a lower percentage increase than 2400. Gibberellic acid treatments at 10^{-2} M lowered the ethylene production by 33% when compared to the control. Decreasing the GA concentration to 10^{-3} or 10^{-4} M decreased the inhibition of ethylene production.

Changes in pectic enzymes appear to be under the control of GA. The control affected by GA on these enzymes may not be a primary one; however, if GA is applied to the mature green fruits the production of PG and PMG activity is retarded. If GA is applied to fruit at the time of incipient color, there is a reduction of enzyme activity but not nearly as great as when it is applied at the mature green stage.

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BIOGRAPHICAL SKETCH

Craig H. Lampe was born January 20, 1944, at Spencer, West Virginia. In May, 1960, he was graduated from Gassaway High School, Gassaway, West Virginia. In January, 1966, he received a Bachelor of Arts with a major in Biology from West Virginia University. In 1966, he enrolled in the Graduate School of West Virginia University. He worked as a research assistant in the Department of Horticulture until May, 1968, when he received the degree of Master of Science. From September, 1968, until the present time, he has pursued his work toward the degree of Doctor of Philosophy.

Craig H. Lampe is married to the former Molly Romig and is the father of one child. He is a member of the American Institute of Biological Science and the Florida State Horticulture Society.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1970

Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

Chairman