Influences of certain growth regulators upon fruit cracking and mechanical properties of the tomato fruit skin

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Influences of certain growth regulators upon fruit cracking and mechanical properties of the tomato fruit skin

by

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INTRODUCTION

Ever since Charles Darwin initiated scientific investigations in the field of physiology of plant growth, and published his book "The Power of Movement in Plants" in 1881, a number of scientists have sought answers to fundamental questions in this field. Presently there are many experimental evidences indicating that growth substances play an important role in the physiology of plant growth and development. With the discovery of auxins, gibberellins, cytokinins and other classes of growth hormones, the progress in the study of various aspects of plant growth and development has been accelerated. Today, there are several growth regulators available for agricultural use. Although many of these chemicals have been tested in the biological laboratories, only a few have been useful in the practical sense in the horticultural field. The useful practices in horticulture aided by growth regulators are: rooting of cuttings, grafting, prevention of preharvest fruit drop, induction of seedless tomatoes, increase in fruit set, prevention of sprouting of plant parts during storage, regulation of flowering, defoliation to facilitate harvesting practices and selective weed control.

One of the problems in horticulture that has not yet been solved by growth regulator treatment is the cracking of tomato fruit. Great losses have been inflicted on the tomato producer by this problem due to reduction of market quality and the
invasion of cracked fruits by *Drosophila* fruit flies and rotting microorganisms. There have been many attempts to find a solution to this problem through altering the environmental conditions, applying different cultural practices and breeding for crack-resistant cultivars and hybrids. There is no evidence from past history that the problem of tomato fruit cracking may be affected by the physiology of growth regulating substances. In earlier unpublished work, however, the author made preliminary observations indicating a relationship between growth regulators and tomato fruit cracking may exist.

The present study, which included several greenhouse and field experimentations, was conducted in order to substantiate the effect of growth regulators on the incidences of fruit cracking. Additional information was sought concerning the relationship of mechanical properties and anatomy of the tomato fruit skin to fruit cracking. The "Instron" tensile testing machine was utilized in developing a technique to measure or detect the mechanical properties that might be directly related to fruit cracking. This information should be of value to plant scientists for their work in improving the evaluation methods for development of crack-resistant tomato cultivars. It also may be possible to use growth regulators for production of crack-free tomatoes.
REVIEW OF LITERATURE

General Background

Realization of the tomato fruit cracking dates back to the early 1920's. Extensive losses often occur in the production of tomatoes as a result of cracking the skin and fleshy tissues prior to ripening or after harvesting. Most of the research work in studying this problem has been directed towards determining types of cracking and the environmental factors that cause cracking (15, 25, 26, 27, 50, 60, 77).

Types and severity of cracking

Reynard (60) in his review of tomato fruit cracking classified the cracks into the following types.

Radial cracks Cracks which radiate from the stem scar area. Cracks of this type may extend deep into the locular area.

Concentric cracks Cracks which appear in arcs or circles at the stem end or the shoulder of the fruit. Cracks of this type may extend well into the fleshy portion of the outer carpel wall.

Side wall cracks These types of cracks are described as deep cracks not connected to the stem scar, but occurring on the sides, at the blossom end, or at random around the fruits. This type was termed as "burst" type by Young (78).

Vertebrae crack line This type appears as complete or
incomplete longitudinally arranged skin cracks which are somewhat corky.

Other types of cracks such as star, pox, radiating stem-end stripes, cracks from exserted carpels, bursting and self-peeling were described by Young (78).

Frazier (26) reported that practically all of the radial cracks are connected with the corky region of the stem end. These cracks are located mostly along the creases of the fruit which lie above or along the septae or interlocular walls. The concentric cracking of the "netted" type, Frazier explained, is more likely to occur in ripened fruits than in green ones.

Possible causes of cracking

Investigations of cracking problems in apples and cherries, have shown that fruit cracking can be associated with abnormal acceleration of fruit growth as a result of an increase in water supply to the tissues. Vemer and Blodgett (71) found that cherry fruits cracked as a result of increasing osmotic absorption of water through the fruit skin during a prolonged period of rainfall.

Verner (72) concluded that cracking of Stayman Winesap apples was promoted by the increased water supply to the fruit tissue as a result of depressed transpirational water loss under conditions of high humidity. He explains that cracking occurrence was always associated with very low rates of evaporativity regardless of the periods of rainfall.
Frazier and Bowers (25) associated water uptake with cracking because cracked fruits always were observed several hours after rain. They explained that radial cracks occurred over the interlocular septae and they postulated that such cracks were due to the anatomical weakness of the two adjacent septal walls. Young (77) and others (15, 26, 44, 45, 50, 60) also found the same correlation between the water supply to the fruit and cracking, and it seems likely that fruit cracking results from stress on the pericarp due to absorbed water.

Moore, et al. (45) found that close spacing substantially reduced tomato fruit cracking. The results of their experiments on different irrigation levels indicated that cracking was increased by the higher levels. Similar results were found by other workers (15, 25, 44, 50). Temperature and exposure of fruits to sunlight have been considered as possible causes of cracking (25, 26, 77). Wind and humidity in the atmosphere surrounding the fruit also may be contributing factors to cracking (25). Cotter and Seay (20) reported that fruit cracking was considerably increased when a stream of air was circulated in a plastic greenhouse.

Many investigators agree with the theory that cracking of the fruit is basically a physical phenomenon caused by internal stresses due to turgor pressure and differential growth between the parenchymatous tissue and the skins. Tetley (68), in an anatomical study of Bramley Seedling apple, found that both
cell division and cell enlargement continued in the hypodermal layer of cells later than in any other part of the cortex. Verner (72), in his histological studies of a more crack-susceptible apple variety, found a marked restriction of growth in the hypodermal layer late in the growing season, while the fleshy portions of the fruit were enlarging at a normal or a faster rate. Under normal conditions, he observed, this restricted growth of the hypodermal cells is supplemented by a tangential stretching. The combination of these processes of stretching and growth enables the hypodermal layer to expand and keep pace with enlargement of the main body of the fruit.

Frazier and Bowers (25) reported that radial splitting of tomato fruits is a result of internal pressure created by expanding locular contents such as the placentae and seeds. They further speculated that cracking of any type may be due to changes in growth rates and fluctuation of fruit size when maturity is reached.

It has been noted that cracking of tomatoes varies from year to year, among different cultivars, among adjacent vines of the same cultivar, among fruits of the same physiological age and even among fruits on the same cluster (27).

Measurements and rating systems used

Most investigators used the simple conventional method of evaluating cracking incidences. Reynard (60) used a classification method where numbers were assigned to categories to
indicate severity; 20 for severely cracked, 80 for short cracks and 100 for fruits with no visible radial cracks. Prashar and Lambeth (56), Iverson (36), and Brown and Price (15) used a numerical rating system such as 0-3 or 1-5 with the lowest number indicating freedom from cracks and the highest number indicating the most severely cracked. White and Whatley (76) contributed an easy method of measuring the length of cracks with a map measure. More recently, Armstrong and Thompson (2) developed a rating system which combined the counting of the number of cracks and measurement with the map measure in order to place each fruit in its proper category.

**Detection of mechanical properties**

A number of investigators have suggested the use of mechanical properties of the fruit skin as a measure in detecting or determining the fruit's resistance to cracking (26, 37, 61, 73, 74). The first test used for mechanical strength of the skin was the puncture test, using a simple mechanical puncturing device (26, 37, 61). Voisey, et al. (74) introduced a skin piercing apparatus to test the mechanical strength of the tomato skin. They explained that the data obtained from this test would depend on a combination of several mechanical factors such as tensile and shear strength and elasticity of the skin and flesh of the fruit. Voisey and Lyall (73), in their comparison of the tensile test, the puncture test, and the bursting of the skin test concluded that the puncture test
was the most suitable one for determining the susceptibility of tomatoes to radial cracking. However, the importance of the position of the point of puncture in relation to the stem scar and creases in the fruit must be examined further.

In designing a skin piercing apparatus, it was suggested that the following conditions must be taken into account (78):

1) The load must be applied at a constant speed to eliminate time effects, since fruits and vegetables do not react to loads in a purely elastic manner. 2) The apparatus should be robust and capable of accommodating fruits or vegetables covering a wide range of sizes and textures so that it is not restricted to one particular experiment. 3) Automation of as many operating functions as possible should be achieved to eliminate human errors but the apparatus should also be inexpensive and simple to maintain. 4) A simple calibration method should be available which is easy to perform and the resulting plots of load against chart reading should be linear so that chart readings can be converted to piercing loads by a multiplication factor.

The possibility of using the "Instron" tensile tester to determine mechanical properties of the tomato skin has been investigated (8). The study indicated that this stress-strain test can be used with a fair degree of accuracy and speed to determine the extensibility and strength of the skin. Modulus of elasticity, which determines the stiffness of materials (42), was not closely correlated with degrees of fruit cracking (8).

The "Instron" tensile testing instrument is an American tester which uses the bonded-wire type of strain gage for detecting and recording the load applied to the sample under test (11). The instrument is described as utilizing electronic
principles both for weighing the forces on the sample and for controlling its extension (34). It was designed with the idea of controlling these time functions and to prevent any physical interferences which may affect the accuracy of the testing results. Booth (11) pointed out that "in order to accommodate a wide variety of specimens several interchangeable load cells containing the strain gages are used. The load cell is located centrally in the fixed crosshead. The upper jaw is suspended from the cell through a universal coupling. The lower jaw is mounted on the traversing crosshead which is driven upwards or downwards by screwed rods on each side. A range gear changer enables the speed of the crosshead to be varied in steps from 0.05 cm to 125 cm/min. The load cell output is fed by cable to the control cabinet which houses the various electronic circuits and pen recording equipment. The main controls for load range selection, calibration, etc., are mounted on the front panel below the recording chart."

As described by Marin (42), stress (S) is generally expressed in p.s.i. and the strain (C) in in./in. Occasionally the stress and strain are designated as unit stress and unit strain to denote that these quantities are the internal forces of a material for a unit area and the internal deformation per unit length. Marin explains, that an examination of most tensile stress-strain diagrams (curves) shows two ranges in the diagram representing different behaviors of materials. The initial or elastic range includes the region of the diagram
where the sample will regain its original dimensions upon removal of the load. Beyond this range is the plastic range where permanent deformations take place. Sometimes the initial elastic range is essentially a straight line and the stress-strain relation is expressed by Hooke's Law or $S = \varepsilon E$ or $E = S/\varepsilon$, where $E$ is called the modulus of elasticity or Young's modulus.

**Measures of control for cracking**

Several investigators studied the feasibility of applying different cultural practices to prevent or reduce cracking of tomato fruits (14, 15, 22, 36, 78). Brown and Price (15) and others (50) reported that shading was beneficial in reducing the severity of cracking. Young (78) suggested the practice of allowing the tomato plants to grow freely on the ground without pruning in order to minimize fruit cracking, while Brooks (14) indicated a reduction of cracking by means of modified pruning. Young observed that supplemental irrigation to avoid drought for tomatoes growing in fertile soil may minimize cracking.

Potassium permanganate treatment of soils in which tomato transplants were grown reduced percentage of cracking (36). Balanced fertilization of the soils was reported to be helpful in reducing cracks (78). Dickinson and McCollum (22) investigated the effect of calcium, sodium and potassium salts on cracking of tomatoes. Cracking was induced by the Illinois vacuum-immersion method. They found that calcium chloride
treatment in the infiltrating solution prevented cracking in fruits of a crack-resistant cultivar, and reduced the severity of cracking in a susceptible cultivar.

Breeding for crack-resistant tomatoes

The wide variation in cracking of fruits was realized by many investigators (1, 25, 46, 51, 56, 59, 66, 76). Prashar and Lambeth (56) investigated the genetics of radial cracking. They concluded that crack susceptibility was a dominant character, but dominance was incomplete. It was assumed by them and others (76) that there are two strong and two weak genes for cracking with an interallelic interaction present. Armstrong and Thompson (1) found that lines which are the most resistant to cracking were the best combiners for resistance. It was suggested by many researchers (1, 56, 60) that it is possible to maintain the resistant lines and transfer the levels of crack resistance to the progenies. Armstrong and Thompson (1) indicated, however, that maintenance of levels of resistance in the progeny of crosses can be possible only if adequate selection is practiced.

Anatomical Considerations

In all types of tomato fruits, the skin consists of an epidermal layer within which are three, occasionally four, well-defined layers of collenchymatous tissue (29, 32). Groth (29) described the epidermal cell as all being polyhedral in shape
and rarely showing curved boundary lines on a surface view. The epidermal cells are covered by a thin cuticle, a heavy cuticular layer, and an inner noncuticularized layer of cutin. The cuticular layer always covers the outer surface completely and extends into the radial and inner tangential walls of the epidermal cells to a varying degree (29, 32). All tomato skins studied by Groth contained single epidermal cells or patches of cells, usually smaller than the average, which appear brown and show some distortion. According to Haberlandt (31) and Esau (23) the outermost lamella of the epidermal wall contains the greatest amount of cutin and constitutes the cuticle which forms a continuous pellicle over the entire epidermal surface. The thickness of the outer wall has a two-fold significance. In the first place it serves to diminish the rate of transpiration, and secondly, it has the effect of increasing the mechanical strength of the epidermis. Cotner, et al. (19) reported a distinct variation in the anatomy of the fruit epidermis among different cultivars. Fruits from a crack-resistant line possessed flattened epidermal cells, while fruits from a susceptible line had rounded epidermal cells.

Cell wall structure

The greater area of the wall consists of a mesh of microfibrils of the order of 100 Å in width, somewhat randomly disposed but with a tendency towards transverse orientation,
lying in a highly hydrated matrix of incrusting substances (57). Setterfield and Bayley (64) asserts that microfibrils of the wall are relatively inert and rigid and their number and organization are largely responsible for the structural characteristics of the wall. Matrix materials, however, usually are more reactive than microfibrils and have been assumed to control wall rigidity, thereby influencing cell elongation. Preston and Cronshaw (57) reported that the matrix material consists of pectic substances and hemicelluloses that are "associated with anions (e.g. Ca++, Mg++) and cations (e.g. HPO$_4$$^{2-}$), which, presumably, have an effect on the rigidity and mechanical behavior of the cell wall.

Growth Regulators

The auxin effect on fruit growth

There is a good deal of experimental evidence that auxin is one of the main growth regulators in fruit development. Gustafson (30) found that tomato extracts, especially of the seeds and the tissue surrounding them, contained high concentrations of auxin. Nitsch (48) demonstrated the direct effect of achenes of the strawberry on the receptacle development and morphology. Only fertilized achenes were active and the application of synthetic auxins was able to replace the effect of fertilized achenes. Sastry and Muir (63) showed that acropetal movement of auxin resulted from a saturation of the transport
system in the pedicel with auxins formed in the ovary following pollination and fertilization. They also reported a greater rate of growth when exogenous indoleacetic acid was injected into the tomato ovaries. The relationship of auxins and developing ovaries were further substantiated by Luckwill (41). In his investigations he found that the natural stimulus in the tomato ovary due to indoleacetic acid may be entirely replaced by a single application of any one of a wide range of synthetic auxins. Auxin extracted from apple seeds also was effective in bringing about the normal response.

**Auxin-gibberellin interactions**

Sastry and Muir (63) found that diffusible auxin was not present in tomato flowers at anthesis, but significant amounts were obtained after the plants received gibberellin treatment. Similarly Nitsch (49) found that sumac shoot tips treated with gibberellic acid had greater amounts of extractable auxin than untreated shoots. Kuraishi and Muir (39) also reported an increase in diffusible auxins from gibberellin-treated Alaska peas and sunflower plants.

Brian and Hemming (13) found that several synthetic auxin treatments were effective in increasing the length of green peastem sections and in eliciting a gibberellic acid response. Internodes from plants pretreated with gibberellic acid extended appreciably faster than those of untreated plants, but only if an auxin was supplied in the induction medium.
Phillips, et al. (55) supported the conclusions of Brian and Hemming in their explanation of a complementary action of gibberellins and auxins, adding that the gibberellic acid applied to the plant may exert a synergistic effects upon the endogenous growth substances or auxins, thereby yielding growth promotion far in excess of what one would expect from gibberel- lin or from auxin alone. Their experimental results also seemed to strengthen Brian and Hemming's suggestion that giberellic acid combines with an endogenous auxin to form a complex which has both auxin and gibberellin-like physiological activity.

**Auxin effect on cell wall extensibility**

In his review of cell elongation, Heyn (33) indicated three possible mechanisms of wall enlargement. One of these require active wall synthesis to provide the driving force in the process. The other two required turgor pressure to cause either an elastic or a plastic extension of the wall. If elastic extension occurred, wall synthesis would be required to strengthen its extended position. Assuming these to be the only possibilities, there are several sites upon which auxins could act to stimulate enlargement. The first is that auxins may play a role in regulating wall synthesis. Secondly, they may be involved in altering the structure of the wall by making it more or less plastic. Thirdly, they may have an effect on the turgor pressure within the cell either by alter-
ing the permeability of the membrane or by changing the osmotic pressure of the cell sap.

Preston and Hepton (58) reported that the cell sap obtained from an auxin-treated tissue had a lower osmotic pressure than that from auxin-free control tissue. They explained that auxin-induced water uptake can be explained neither in terms of salt accumulation nor in terms of starch hydrolysis. It is, therefore, becoming generally assumed that the major growth regulating effect of auxin is exercised on the cell wall.

The possible effects of auxins on the synthesis of cell wall components have also been studied. Baldovinos (6) stated that "cell enlargement would seem to be limited by enzymatic reactions dependent upon the presence of auxins. We may postulate that these reactions involve the lengthening of the cellulose micelles of the cell wall in such a way as to allow expansion of the cell by hydrostatic pressure." While working with potato tissues, Buffel and Carlier (16) found that auxins caused a change in cell wall composition, with pectins increasing relative to cellulose. They proposed that through the hydration of pectins the wall becomes more extensible. Bentley (10) concluded from his studies of the effect of auxin on the cell wall that the optimum auxin concentration for growth increased the content of pectic substances in the wall relative to cellulose; inhibitory concentrations enhanced the cellulose synthesis, leading to a more rigid wall. When oat coleoptile sections elongated in an optimum osmotic concentra-
tion of sugar solutions, the presence of indoleacetic acid induced an increase in cellulose synthesis with sucrose being used as a substrate (14) or with galactose being used as a substrate (57). Galston and Purves (28) cited evidence in their review of auxin action mechanisms that "auxin can produce its effects on plasticization and on elongation at low temperatures (2 to 4°C) at which no increase in the weight of cell wall material occurs. This leads to the conclusion that auxin acts on some protoplasmic system, this action leading to an altered arrangement of cell wall component, this in turn leading to a greater extensibility."

Heyn (33) in his experiments was able to measure elastic and plastic components of oat coleoptiles by bending them with a weight. This procedure allowed him to test the effects of applied auxin on each of these components and he found that auxin increased the plasticity of the cell wall. Cleland (18) proposed that extension of cell wall requires an increase in plasticity, intussusception of new cell wall material, osmoregulation, and water for expansion. Of these, however, "only the loosening of the cell wall is auxin dependent."

From their experiments of studying mechanical properties of cell walls, Preston and Hepton (58) found that indoleacetic acid treatments increased the extensibility of cell walls. Tagawa and Bonner (67) presented evidence for a decrease in both elasticity and plasticity when oat coleoptiles were treated with calcium or magnesium solutions, but this was
reversed when the coleoptile sections were treated with potassium or indoleacetic acid solutions. They explained that addition of potassium ions to the indoleacetic acid augments the softening and plasticizing effects of the indoleacetic acid on the cell wall. Calcium ions, however, repress the plasticizing effect on cell walls by making them more rigid. Ordin and Bonner (52) have shown that esterification of pectic acid by methyl-derived carbon is an auxin-controlled process. It is suggested that methylation of carboxyl groups on adjacent pectic molecules, under the auxin control, involves the splitting of anhydride or calcium bridges which contribute to the mechanical properties of the wall (58).

**Effects on fruit cracking**

To the knowledge of the author, there is no literature available concerning the effects of growth regulators on cracking of tomato fruits. An unpublished thesis by Batal (?) presented experimental evidence that indoleacetic acid, gibberellic acid, naphthaleneacetic acid, kinetin and their combinations effectively reduced tomato fruit cracking when applied directly to the fruit with several applications starting from the time of anthesis until fruit maturity.

There has been a limited amount of work in studying the effect of naphthaleneacetic acid on fruit cracking of apricots and cherries. Bullock (17) reported that cracking of cherries was effectively reduced when fruits and foliage were sprayed
with sodium salts of α-naphthaleneacetic acid at 0.1 to 1.0 mg/l solution (17). Crane (21) showed that apricot fruits, when sprayed with 100 ppm of α-naphthaleneacetic acid at the beginning of pit hardening, cracked considerably less than the control fruits.
GENERAL MATERIALS AND METHODS

Plant Materials

In order to evaluate cracking of the fruit and cracking response to certain growth regulator treatments, the following cultivars were selected with varying degrees of susceptibility to cracking:

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Susceptibility to Cracking</th>
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<tr>
<td>Heinz 1350</td>
<td>Moderately resistant</td>
</tr>
<tr>
<td>Scarlet Beauty</td>
<td>Moderately resistant</td>
</tr>
<tr>
<td>Caravelle</td>
<td>Moderately susceptible</td>
</tr>
<tr>
<td>Spring Giant</td>
<td>Moderately susceptible</td>
</tr>
<tr>
<td>Sun Up</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Marglobe</td>
<td>Susceptible</td>
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</table>

Growth Regulator Treatments

For each of the growth regulator experiments, the preparation of chemical solutions and applications were similar. The anhydrous forms of indole-3-acetic acid (IAA), α-naphthalene-acetic acid (NAA), 75% potassium salt form of gibberellic acid (GA), kinetin (K) and benzyladenine (BA) were dissolved in small amounts of 50% (v/v) ethanol and diluted to 25% ethanol to make the desired concentrations. All stock solutions were made to have a double concentration so that the proper concentrations can be achieved when two or more chemicals are combined.

The solutions were applied directly to the fruits by a small DeVilbiss-No. 15 atomizer for all greenhouse experiments. The fine mist of the solutions was directed at the fruit until the entire surface of the fruit was wet. The other
fruits on the same cluster were shielded with a plastic cover. In all experiments the first application was made immediately after anthesis or when the corolla was in the separating stage. At this stage, the pistils were approximately 5 mm in diameter and were showing signs of expansion and increase in size. Subsequent applications were made at intervals of 7 days for a total of 5 treatments. In all cases treated fruits were tagged for identification and proper timing of the treatments.

For the field experiment, the chemicals were applied to the entire cluster of fruits with a "Jet-Pack" sprayer. No attempt was made to protect the other fruits or clusters on the same plant. The frequency of application was every 3 days in order to treat newly developed fruits on each cluster.

Mechanical Properties

Stress-strain testing procedures

Stress-strain tensile testing of the tomato fruit skins was accomplished with using the "Instron" tensile testing instrument, Model TT-BM, equipped with load cell type A and No. 61-2A fiber clamps.

Skin samples were prepared for testing by cutting out transverse strips of skin from the shoulder or median portions of the fruit. A strip of the fruit pericarp, 0.5 cm wide and about 3 cm long, was first cut out with a double-bladed knife
(Figures 1, 2, 3). The strip was then removed from the fruit and was placed skin side down on a moist paper towel and the flesh was separated from the skin with the aid of a scalpel (Figure 4). The skin strip then was dipped in water to prevent dehydration and was immediately fixed in the Instron clamps (Figure 5). The length of the strip being tested was determined by the distance set between the jaws of the two clamps, which was 2.0 cm.

To determine the extensibility of the fruit skin and the force required to break the skin, the stress was applied on the sample by automatically moving the cross-head downward, thereby stretching the sample longitudinally. The strain rate was 0.2 cm per minute, and the stress force was recorded at the 20 X (200 gram maximum load) scale during the initial stages of each test. When loading approached 200 grams without failure of the skin tissue, the stress scale was changed to 50 X (500 grams maximum load) as illustrated in (Figure 6). The loading was continued until the tissue failed to resist the extension force and broke at some point.

The stress strain data were recorded continuously on the recorder chart with a speed of 10 cm per minute. The resulting curves were used for computations in determining the desired mechanical properties of the stressed samples. The following properties were calculated:

(1) Breaking Elongation - based on previous observations, two categories were considered in this study:
Fig. 1. Double-bladed knife positioned at the shoulder area of a tomato sample.

Fig. 2. Cutting out a portion of the pericarp.
Fig. 3. Removal of pericarp portion from the shoulder area of the fruit with the aid of a scalpel.

Fig. 4. Separation of the flesh from the skin with the aid of a scalpel.
Fig. 5. Skin sample fixed in the Instron upper clamp.
Fig. 6. A typical stress-strain curve obtained from an Instron test of a tomato skin.
a) Extension of skin samples at the initial stress of 10 Kg/cm², which constituted about 50 per cent of the ultimate force to failure for most samples. It was expressed as:

\[
\text{Elongation at 10 Kg/cm}^2 = \frac{\text{Sample length at 10 Kg/cm}^2}{\text{Initial sample length}} \times 100
\]

b) Total elongation of skin samples at failure which was expressed as:

\[
\text{Maximum Elongation} = \frac{\text{Sample length at failure}}{\text{Initial sample length}} \times 100
\]

(2) Ultimate Force - In preliminary investigations, a mechanical property was found to be of some value relation to cracking of the tomato fruits. It is defined as the maximum stress or ultimate force required to break the skin sample. This was expressed as:

\[
\text{Ultimate Force} = \frac{\text{Stress force (Kg)}}{} \frac{\text{Cross sectional area of the sample (cm}^2)}{}
\]

In all experiments, the fruit samples were collected for evaluation and tested when 90 per cent or more of the fruit surface was red.

Thickness of skin samples for each cultivar was determined by a micrometer caliper. Measurements were made at five points of the skin strips from 20 fruits of each cultivar.
EXPERIMENT I

This experiment consists primarily of those growth regulator treatments which were reported by Batal (7) to influence carpel wall thickness and crack resistance of tomato fruit. The purpose of repeating these treatments was to see if similar results could be obtained with a different cultivar and in a different environment.

Materials and Methods

Marglobe, a crack susceptible cultivar, was used for the growth regulator treatments, and one untreated plot of Heinz 1350, a crack resistant cultivar, was included in the trial. Plants for this experiment were grown by seeding directly in a 1:1:1 ratio of loam, peat, and gravel mixture in a greenhouse bench. All plants were pruned to a single stem which was supported upright by a string. Four successive flower clusters were allowed to develop. Other clusters were clipped off at an early stage in development. Each of the four developed clusters set 5-8 fruits. Flowers developing beyond the 8th fruit of a cluster also were clipped off.

The treatments consisted of control (no growth regulator treatment), 15 ppm IAA, 15 ppm GA, and the following combinations: 30 ppm NAA + 8 ppm K, 15 ppm IAA + 8 ppm K, and 15 ppm IAA + 15 ppm GA. Each fruit was treated individually. Treated fruits from the second and fourth clusters were collected at intervals of five days, ten days, and fifteen days after the
first application of growth regulators. Thus, various samples of fruits with different stages of development and different levels of treatments were obtained. Part of these samples were frozen and part were preserved in alcohol-formol-acetic (FAA) solution for histological studies. Free-hand transverse sections of the outer portions of the pericarp were made and stained with safranin and fast green.

Fruits of the first and third clusters were used to evaluate their cracking and morphological differences. Each fruit was picked when ripe, weighed and the number of cracks were counted. Then the fruits were sliced transversely at the center point, and the outer and inner carpel walls were measured with a caliper. The measurements were taken at two places for each wall of each locule.

A simple randomized block design was used for this experiment. It consisted of 7 treatments including a crack-resistant control, and untreated control, and 5 different growth regulator preparations. The treatments were randomly distributed in each of 3 blocks. The data were statistically analyzed using the following model:

\[ X_{ij} = \mu + T_i + B_j + e_{ij}, \text{ with limits, } \mu = 0, \]
\[ i(\text{treatments}) = 1 \ldots 7, \ j(\text{blocks}) = 1 \ldots 3, \]
\[ e_{ij} \overset{i.i.d.}{\sim} (0, \sigma^2). \]

The analysis of variance (ANOVA) was as follows:
### Results

The effect of various treatments on fruit weight, percentage of cracked fruits, number of cracks per fruit and thickness of carpel walls are summarized in Table 1. All growth regulator treatments were effective (P<0.01) in reducing percentage of cracked fruits compared to untreated control. The resistant cultivar had a significantly lower percentage of cracked fruits. The NAA + K treatment resulted in a significant reduction in percentage of cracked fruits when compared to the other growth regulator treatments, but not when compared to the crack resistant cultivar. There was no significant difference in percentage of cracked fruits treated with the other four growth regulators.

The growth regulator treatments and the crack resistant cultivar were significantly lower in number of cracks per fruit when compared to the control. NAA + K and IAA treatments significantly reduced the number of cracks per fruit compared to the other treatments and the control. No significant difference was evident in number of cracks per fruit among IAA + K, IAA + GA and GA treatments.

The effect of chemical treatments on thickness of outer
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average wt/fruit(g)</th>
<th>Percentage of cracked fruit</th>
<th>Average number of cracks per fruit</th>
<th>Average thickness of carpel walls (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Outer walls</td>
<td>Inner walls</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>126.47</td>
<td>73.8</td>
<td>2.1</td>
<td>5.1</td>
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<tr>
<td>Resistant Cultivar</td>
<td>119.36</td>
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<td>0.5</td>
<td>4.6</td>
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<tr>
<td>NAA(30ppm)+K(8ppm)</td>
<td>130.21</td>
<td>10.0</td>
<td>0.2</td>
<td>6.3</td>
</tr>
<tr>
<td>IAA(15ppm)+K(8ppm)</td>
<td>131.25</td>
<td>23.5</td>
<td>1.6</td>
<td>5.6</td>
</tr>
<tr>
<td>IAA(15ppm)+GA(15ppm)</td>
<td>123.77</td>
<td>22.5</td>
<td>1.3</td>
<td>6.7</td>
</tr>
<tr>
<td>IAA(15ppm)</td>
<td>133.18</td>
<td>21.7</td>
<td>0.5</td>
<td>6.1</td>
</tr>
<tr>
<td>GA(15ppm)</td>
<td>147.35</td>
<td>27.2</td>
<td>1.4</td>
<td>6.3</td>
</tr>
<tr>
<td>L.S.D., 5%</td>
<td>N.S.</td>
<td>11.4</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>L.S.D., 1%</td>
<td>N.S.</td>
<td>15.9</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

* Data were averages of 30 fruits per treatment.*
and inner carpel walls were observed (Table 1). All growth regulator treatments significantly increased the thickness of outer and inner carpel walls compared to those of the control. IAA and NAA + K treatments significantly increased the thickness of inner carpel walls compared to the other treatments. IAA + GA, NAA + K, GA and IAA treatments significantly increased the thickness of outer carpel walls compared to IAA + K treatment. Fruits from the control appeared to have thicker outer carpel walls than inner walls. Fruits of the crack resistant cultivar, on the other hand, had thinner outer walls than inner walls.

**Anatomical**

Comparative anatomy of the pericarps of the treated fruits 15 days after the first treatment is presented in Figures 7a-15b. Figure 7a shows the untreated fruit with rounded or raised epidermal cells. Several depressed areas were observed on the surface of the epidermis. Several developing trichomes also may be seen. The cuticle layer covering the epidermal surface was very thin or absent in some areas. Very thin and elongated subepidermal layers were observed, but the parenchymatous cells of the mesocarp were relatively large and oblong in shape.

Figure 8a shows the thicker skin developed by the crack-resistant cultivar. It had flattened epidermal cells with a thick layer of cuticle. Figure 7b and 8b show a higher magnification of the skin of the crack-susceptible and the crack-
Fig. 7a. Cross section of pericarp of untreated control, epidermal layer showing the depressed area (DA) and region of trichoma development (tr), subepidermal layer (SE) and mesocarp tissue (MC). X 139.

Fig. 7b. Enlargement of epidermis and subepidermal layers (arrow) indicated in Fig. 7a. X 347.

Fig. 8a. Cross section of pericarp of resistant control. Epidermis and subepidermal layer (long arrow), parenchymatous cells (short arrow). X 139.

Fig. 8b. Enlargement of the upper portion of the pericarp indicated in Fig. 8a. Cuticular layer (upper arrow) and subepidermal layers (lower arrow). X 347.

Fig. 9a. Cross section of pericarp treated with NAA + K. Epidermis and subepidermal layer (arrow). X 139.

Fig. 9b. Enlargement of the upper portion of pericarp indicated in Fig. 9a. Cuticular layer (upper arrow), parenchyma cells (lower arrow). X 347.
resistant controls respectively. An abrupt change in the size of cells was observed going from the subepidermal layer to the parenchymatous mesocarp in the susceptible cultivar, while this change was gradual in the resistant cultivar.

Figure 9a shows the change in structure of the susceptible control due to the NAA + K treatment. It appeared that the epidermal cells were flattened and with even surface. The subepidermal layers were thickened and the parenchymatous cells were somewhat oblong. Figure 9b shows the epidermal cells covered with a thick layer of cuticle and gradual change in size of the subepidermal cells towards the parenchymatous mesocarp tissue.

Figure 10 is a cross-sectional view of the pericarp of the crack-susceptible cultivar. Figure 11 is a cross-sectional view of the pericarp of the crack-resistant cultivar. Both figures show the epidermal cells, the subepidermal layers and the area immediately below the subepidermal layers. The latter in the susceptible cultivar were relatively thin, elongated and somewhat tapered at their ends, but in the resistant cultivar, they were thicker, somewhat shorter and less tapered. The parenchyma cells of the susceptible cultivar appeared to be elliptical in shape with thick cell walls. The parenchyma cells of the resistant cultivar, however, appeared oblong in shape with relatively thin cell walls.

Figure 12a shows the effect of IAA on the structure of the pericarp. The epidermal cells appeared much smaller than
Fig. 10. Cross section of pericarp of untreated control. Rounded epidermal cells and cuticle (CI), subepidermal cells with tapered ends (SE), and thick-walled parenchymatous cells (PC). X 735.

Fig. 11. Cross section of pericarp of resistant control. Cuticle (CI), larger subepidermal cells (SE), and relatively thin-walled parenchymatous cells (PC). X 735.
those of the control, but the subepidermal cells were larger. There was some indication of a gradual change from shorter to longer cells from the subepidermal layers and to the mesocarp. The increase in thickness of cell wall both of the subepidermal layers and the mesocarp, is shown in Figure 12b. It also shows the depressed area of the epidermis like the ones observed in the susceptible control.

IAA + K treatment appeared to decrease the thickness of the skin, and to increase the size of the parenchymatous cells of the mesocarp (Figure 13a). Figure 13b shows the rounded shape of the epidermal cells and the decreased number of the subepidermal layers. Figure 14a shows the effect of IAA + GA on the shape of the epidermal cells. These cells were of a papillose shape and covered with a thick layer of cuticle. The subepidermal layers were thicker and greater in number compared to those of IAA + K treatment. The gradual increase in size of the subepidermal cells towards the mesocarp also was observed. Figure 14b shows the saw-toothed appearance of the papillose epidermal cells and the thick layer of cuticle which covers them. It also shows the thick-walled parenchymatous cells of the mesocarp.

GA treatment also affected the amount of cuticular layer which covered the epidermis (Figure 15a). There was an increase in amount of cuticle present compared to the control but the size of the epidermis appeared to be smaller. The parenchymatous cells also were much smaller than those of the con-
Fig. 12a. Cross section of pericarp treated with IAA. Depressed area (arrow), thick-walled subepidermal cells. X 139.

Fig. 12b. Enlargement of upper portion of pericarp indicated in Fig. 12a. Epidermal and subepidermal layer (upper arrow), thick-walled parenchyma cells (lower arrow). X 347.

Fig. 13a. Cross section of pericarp treated with IAA + K. Thin subepidermal layers (upper arrow), large parenchyma cells (lower arrow). X 139.

Fig. 13b. Enlargement of upper portion of pericarp indicated in Fig. 13a. Rounded epidermal cells and cuticle (upper arrow), very thin subepidermal layer (lower arrow). X 347.

Fig. 14a. Cross section of pericarp treated with IAA + GA. Gradually enlarging subepidermal cells (arrow). X 139.

Fig. 14b. Enlargement of portion of pericarp indicated in Fig. 14a. Papillose epidermal cells with thick cuticle (upper arrowhead), thick-walled subepidermal cells (lower arrowhead). X 347.

Fig. 15a. Cross section of pericarp treated with GA. Subepidermal layer (arrow). X 139.

Fig. 15b. Enlargement of portion of pericarp indicated in Fig. 15a. Thick cuticle (top arrowhead), thick subepidermal layer (arrow). X 347.
but the subepidermal layers were considerably thicker and more numerous (Figure 15b).

Discussion

Results of this experiment indicated that growth regulator treatments were effective in reducing the severity of cracking in tomato fruit. NAA + K treatment was most effective in reducing the percentage of cracked fruits and number of cracks per fruit, but IAA treatment was nearly as effective in the latter case. It appears that the addition of kinetin or gibberellin to IAA altered the effectiveness of IAA in reducing the number of cracks per fruit. Therefore, it seems that the auxins were more effective than GA or K in reducing cracking. The observed differences in the percentage of cracked fruits between the two auxins might be caused by, either the differences in concentrations used, or the chemical differences in the two forms of the auxins.

The increase in thickness of outer carpel walls, which occurred as a result of all treatments except IAA + K, appeared to be associated with an increase in size and number of cells of the pericarp tissue. It was evident from the anatomical studies that NAA + K increased the size and the number of the subepidermal layers. The IAA and IAA + GA treatment increased the size of cells of the subepidermal layers and the mesocarp but the epidermal cells were smaller. NAA + K and IAA treatments, which reduced the number of cracks most effectively,
changed the structure of the pericarp comparable to that of
the crack-resistant cultivar. The gradual increase in size of
the cells from the epidermis towards the endocarp, seems to be
an important factor in the resistance of a tomato fruit tissue
to cracking. It might be possible that this type of cellular
arrangement, which is induced by NAA treatments, has the advan-
tage of resisting any stress that may result from possible
changes in the cellular contents brought about by the fluctu-
ating environmental and physiological conditions. The fruits
of the susceptible cultivar, on the other hand, had a consid-
erable difference in cell size of the mesocarp tissue and the
peripheral skin tissue. Based on this fact, it would be pos-
sible to theorize that any mechanical stress resulted from a
change in turgor pressure of the large parenchymatous cells of
the mesocarp, undoubtedly will severely affect the mechanical
behavior of the adjacent small subepidermal and epidermal cells.
This, in turn, would affect cracking of the fruit.

The greater increase in thickness of inner carpel walls
than of the outer carpel wall due to NAA + K and IAA might be
explained by the fact that auxin transport is affected by its
concentration in the tissue. Soon after anthesis and the be-
ginning stages of seed development, the endogenous auxin would
diffuse into the tissues adjacent to the seeds and eventually
would reach the outer carpel wall. But, if the concentration
of auxin in this region is raised by the addition of NAA, it
might slow down the supply of endogenous auxin by diffusion and
possibly would reverse the direction of movement inward, which could cause the eventual increase of auxin supply for the inner walls. It was evident from the observations in this experiment that GA or IAA + GA did not produce this type of differential rate of growth in the outer and inner carpel walls. This seems to agree with the idea that the transport of auxins through plant tissues is increased at first by the concentration, but as the transport system becomes saturated, the movement of auxins is affected (63). It was evident from the anatomical studies that the IAA + GA treatment increased the thickness and number of subepidermal layers. This might be the reason why the outer walls of the pericarp treated with IAA + GA were slightly thicker than those of the inner cross walls.

According to the data obtained from this experiment, it is likely that physiological interactions exist between auxin and gibberellin and between auxin and kinetin in their effects on cracking and anatomy of the fruit. Since this experiment was not completely factorial in its nature, it was difficult to make further inferences on the possible interactions among the four growth substances involved.
plants which were to receive the 0 ppm level of all chemicals were treated with the carrier solution (25% ethanol). Each treatment was applied to 2 fruits on each of 3 clusters per plant. All treatments were replicated in 2 randomized blocks.

Fruits were harvested when ripe, weighed, and the cracks on each fruit were counted and measured. Only one fruit of each of the first and second clusters were used to determine the mechanical properties of their skins. Skin samples were taken from two locations, the shoulder and the median, of each fruit and tested with Instron as described in the General Materials and Methods.

Three separate statistical analyses were carried out on the data. Analyses 1 and 2 apply only to the 2 fruits per plant from which skin samples were taken. Analysis 1 is restricted further to the shoulder samples, but analysis 2 compares the shoulder samples with the median samples. Analysis 3 encompasses all 6 fruits per plant that were treated but does not include skin characteristics.

The statistical methods for the 3 analyses were as follows:

(1) Analysis of the data for the evaluation of the mechanical properties of skin samples taken from the shoulder areas only. The following model and analysis of variance were used:

Model \[ Y_1, Y_2, T Y_1 = A(i) + B(j) + C(k) + D(l) + BC(jk) + BD(jl) + CD(kl) + BCD(jkl) + AB(ij) + \]
EXPERIMENT II

The purpose of this experiment was to investigate the main effects of three growth substances and their interactions on cracking and mechanical properties of the tomato skin. The auxin NAA was selected for this experiment on the basis of its performance in Experiment I. GA was included in this test with the purpose of examining its possible interaction with NAA in their combined effect on fruit cracking. BA was used in place of kinetin in order to examine the effects of a different kind of cytokinin on fruit cracking. To intensify their effects, higher concentrations of GA and BA were used.

Materials and Methods

Basically the same cultural procedures for raising tomato plants as in Experiment I were used for Experiment II. Spring Giant, a hybrid which is moderately susceptible to cracking, was used in this experiment. Only three successive flower clusters were allowed to develop on each plant. All developing fruits beyond the second fruit on each cluster were clipped off. A factorial in a randomized complete block design was selected for this experiment. It consisted of 3 chemicals at 2 levels with all possible combinations. The upper level of the growth regulators consisted of 30 ppm (30 mg/l) NAA, 30 ppm (30 mg/l) 75% K salt of GA and 50 ppm (50 mg/l) BA. These were prepared in all possible combinations in 25% ethanol. The second level for each chemical was 0 ppm. Fruits on
AC(ik) + AD(il) + ABC(ijk) + ABD(ijl) + ACD(ikl) + 
ABCD(ijkl) + B_3X_3, B_4X_4, B_5X_5 + E(ijklm)

Limits  I (blocks) = 2, j (levels of NAA) = 2,
K (levels of GA) = 2, l (levels of BA) = 2, m
(fruits) = 2, total number of observations = 32

Y_1 = Number of cracks per fruit
T_{y_1} = \sqrt{\text{No. of cracks} + 0.5} \text{ transformation}
Y_2 = Average length of cracks
X_3 = Extension of skins at 10 Kg/cm
X_4 = Total extension of skin
X_5 = Ultimate force

ANOVA

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<th>Factors</th>
<th>d.f.</th>
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</thead>
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</tr>
<tr>
<td>GA</td>
<td>1</td>
</tr>
<tr>
<td>BA</td>
<td>1</td>
</tr>
<tr>
<td>NAA + GA</td>
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</tr>
<tr>
<td>NAA + BA</td>
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</tr>
<tr>
<td>GA + BA</td>
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</tr>
<tr>
<td>NAA + GA + BA</td>
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<td>Error (blocks)</td>
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<td>Error</td>
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</tr>
<tr>
<td>Total</td>
<td>31</td>
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</tbody>
</table>

(2) Analysis of data for the evaluation of mechanical
properties of skin samples taken from the fruit.
The model and analysis of variance for the data were
as follows:

Model  Y_3, Y_4, Y_5 = A(i) + B(j) + C(k) + D(l) +
BC(jk) + ED(jl) + CD(kl) + BCD(ikl) + AB(ij) +
AC(ik) + AD(il) + ABC(ijk) + ABD(ijl) + ACD(ikl) +
ABCD(ijkl) + F(m) + AF(im) + BF(jm) + CF(km) +
DF(ilm) + ABF(ijm) + ACF(ikm) + ADF(ilm) + BCF(jkm) +
BDF(jlm) + CDF(klm) + ABCF(iklm) + ABDF(ilm) +
ACDF(iklm) + BCDF(jklm) + ABCDF(ijklm) + G(n) +
BG(jn) + CG(kn) + DG(ln) + E(ijklmn).

Limits  i = 2, j = 2, k = 2, l = 2, m = 2, n
(location of skin sample) = 2, total number of
observations + 64

Y_3 = extension of skin at 10 KG/cm^2
Y_4 = total extension of skin
Y_5 = ultimate force

ANOVA

<table>
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<tr>
<td>F (fruits)</td>
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<tr>
<td>Error (treated)</td>
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</tr>
<tr>
<td>BG (NAA X location)</td>
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</tr>
<tr>
<td>CG (GA X location)</td>
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</tr>
<tr>
<td>DG (BA X location)</td>
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</tr>
<tr>
<td>Error</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
</tr>
</tbody>
</table>

(3) Analysis of data for the over-all experimental
sampling in order to evaluate the effects of treat­
ments on fruit weight and cracking variations at
different cluster levels. The model and analysis of
of variance used for the data were:

Model  Y_1, Y_2, Y_3, Y_4, Ty_2 = A(i) + B(j) +
C(k) + D(l) + BC(jk) + BD(jl) + CD(kl) + BCD(jkl) +
AB(ij) + AC(ik) + AD(il) + ABC(ijk) + ABD(ijl) +
ACD(ikl) + ABCD(ijkl) + F(m) + BF(jm) + CF(km) + DF(lm) + E(ijklmn)

Limits  \( i = 2, j = 2, k = 2, l = 2, M \text{ (clusters)} = 3, n \text{ (fruits)} = 2, \) total number of observations = 96

\( Y_1 \) = average fruit weight
\( Y_2 \) = number of cracks

\( T_{Y_2} = \sqrt{\text{No. of cracks} + 0.5} \) transformation
\( Y_3 \) = total length of cracks per fruit
\( Y_4 \) = average length of cracks

**ANOVA**

<table>
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<th>Factors</th>
<th>d.f.</th>
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<tr>
<td>Blocks</td>
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<tr>
<td>NAA</td>
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<tr>
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<td>NAA X GA</td>
<td>1</td>
</tr>
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<td>GA X Clusters</td>
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<td>BA X Clusters</td>
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<tr>
<td>Error</td>
<td>72</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>95</td>
</tr>
</tbody>
</table>

**Results**

**Analysis 1**

Only those portions of the data which were statistically significant will be presented here. The complete data can be found in the Appendix Tables A-1 and A-2.

**Effect on extensibility of the skin**

Analysis of
variance of the data for the effect of growth regulators on the mechanical properties of the skin, indicated that GA significantly (P<0.05) reduced the percentage extension of skin at a stress force of 10 Kg/cm² (Table 2). NAA treatment seemed to have some effect (P<0.10) on increasing percentage of skin extension. BA treatment did not show any significant effect on extensibility.

**Effect on ultimate force to breakage**  
Table 3 shows that GA treatment significantly (P<0.01) increases the ultimate force required to break the extended tomato skin. NAA and BA treatments did not appear to affect the amount of force required to break the skin.

**Effect on number of cracks per fruit**  
Statistical analysis of the transformed data indicated that NAA interacts with BA and GA in their effects on number of cracks per fruit. NAA X BA interaction was significant (P<0.05), as illustrated in Figure 16. NAA and BA reduced the number of cracks when applied separately, but, when both were present, the reduction in number of cracks was not significantly affected. The t-test for the interaction were significant (P<0.05).

NAA X GA interaction showed some significance (P<0.10) (Figure 17). It appears that GA treatment reduced the number of cracks when applied with the absence of NAA, but the GA treatment apparently had no effect in reducing the number of cracks when NAA was present. The t-test for the NAA X GA interaction was significant (P<0.05). The over-all effects of
Table 2. Main effects of growth regulators on skin extensibility

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(-)^a</th>
<th>(+)^a</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA (30 ppm)</td>
<td>3.01</td>
<td>3.77</td>
<td>3.72δ</td>
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<tr>
<td>GA (30 ppm)</td>
<td>3.88</td>
<td>2.91</td>
<td>6.11*</td>
</tr>
<tr>
<td>BA (50 ppm)</td>
<td>3.22</td>
<td>3.56</td>
<td>N.S.</td>
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</table>

^ Treatment level
δ Statistically significant at 10% level
* Statistically significant at 5% level

Table 3. Main effects of growth regulators on ultimate force to breakage

<table>
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<tr>
<th>Treatment</th>
<th>(-)^a</th>
<th>(+)^a</th>
<th>F-test</th>
</tr>
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<td>NAA (30 ppm)</td>
<td>26.80</td>
<td>27.68</td>
<td>N.S.</td>
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<tr>
<td>GA (30 ppm)</td>
<td>21.90</td>
<td>32.62</td>
<td>17.63**</td>
</tr>
<tr>
<td>BA (50 ppm)</td>
<td>29.11</td>
<td>25.41</td>
<td>N.S.</td>
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</table>

^ Treatment level
**Statistically significant at 1% level
Fig. 16. Number of cracks per fruit as a response to NAA X BA interaction.

Fig. 17. Number of cracks per fruit as a response to NAA X GA interaction.
growth regulator treatments on cracking of tomato fruits are shown in Figures 18, 19 and in the Appendix Tables A-1 and A-2.

The stress-strain curves resulted from stretching the skin samples of different treatments were variable. The overall shape of the curve was determined by the degree of steepness of the vertical line and the area under the curve as shown in Figure 20. The differences in these curves due to various treatments can be shown in terms of different value of the ultimate force and the percentage of total extension for each treatment (Table A-1). In most cases, the curve lines of the control showed a uniform change in slope giving rise to a smooth arc starting from approximately 20% of the ultimate stress. NAA and BA treatments produced somewhat similar curves. The most prominent feature of their curves was that the portion of the elastic range, located between the initial stress and approximately 50% of the ultimate stress, was either a straight line or slightly relaxed, but beyond this elastic range it was the same as in the control. GA treatment, on the other hand, produced very steep curves which were straight from the initial load up to the point of skin breakage.

Correlations Regression and analysis of covariance was applied in order to calculate the correlation coefficients for the different variates and covariates. There was a significant (P < 0.01) negative correlation between number of cracks and
Fig. 18. Reduction of cracking incidence as a result of NAA + K treatment.

Fig. 19. Cracking variations as a result of growth regulator treatments.
Fig. 20. Typical stress-strain curves of tomato skins treated with different growth regulators. Curves were recorded at 20X scale.
percentages of skin extension at 10 Kg/cm² (Table 4). A negative correlation between number of cracks and total percentage of extension also was significant (P<0.05). A positive correlation was indicated between the number of cracks and ultimate force, but it was not statistically significant.

Table 4. Correlations between number of cracks and mechanical properties of the fruit skin

<table>
<thead>
<tr>
<th>Correlation Coefficients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
</tr>
<tr>
<td>Number of cracks&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> No. + 0.5 transformation  
* Significant at 5%  
** Significant at 1%

Analysis 2

Effect of sampling location  
Analysis of variance of the measurements taken at different areas of the fruit indicated that there were significant differences in measurements between the shoulder and the median areas of the fruit. Table 5 shows that extension of skin at 10 Kg and total extension was significantly (P<0.01) lower at the shoulder area compared to the median portions of the fruit. There were no significant differences in the "Ultimate Force" measurements taken at different locations.
Analysis 3

Effect on fruit weight
Examination of the analysis of variance indicated that there was a slight variation in weights of the treated fruit. Fruits treated with Benzyladenine weighed less than those which did not receive this treatment. The F-test was significant only at 10% level (Table 6).

Effect on number of cracks per fruit on different clusters
The number of cracks per fruit was significantly affected, (P< 0.05) by treatment X cluster interactions. Figure 21 illustrates that the greatest reduction in the number of cracks as a result of NAA treatment was accomplished in the fruits of the first cluster. The t-test for this interaction was significant at 5% level. Figure 22 illustrates the significant interactions between clusters and GA. It was evident that the greatest reduction in the number of cracks per fruit by GA treatment occurred in the fruits of the third cluster. The t-test for this interaction was significant at 5% level.

Effects on total length of cracks per fruit on different clusters
Total length of cracks per cracked fruit was reduced due to NAA and BA treatments in different magnitudes on different clusters (Figures 23, 24). The t-tests for the interactions were significant at 5% level. The NAA treatment apparently is more effective in reducing the total length of cracks in fruits developed on the first cluster. BA treatment, however, seemed more effective in reducing length of cracks in fruits developed on the first and the third cluster.
Table 5. Main effects of sampling location on the mechanical properties of tomato skin

<table>
<thead>
<tr>
<th>Sampling location</th>
<th>Extension of skins at 10 Kg/cm²(%)</th>
<th>Total skin extension (%)</th>
<th>Ultimate Force (Kg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoulder</td>
<td>3.39</td>
<td>9.11</td>
<td>27.26</td>
</tr>
<tr>
<td>Median</td>
<td>3.83</td>
<td>11.26</td>
<td>27.18</td>
</tr>
<tr>
<td>(F(1,28))</td>
<td>77.57**</td>
<td>17.68**</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

** Statistically significant at 1% level

Table 6. Main effects of growth regulators on tomato fruit weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of Fruit (g) (-)(^a)</th>
<th>(+)(^a)</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>279.2</td>
<td>287.7</td>
<td>N.S.</td>
</tr>
<tr>
<td>GA</td>
<td>285.5</td>
<td>281.3</td>
<td>N.S.</td>
</tr>
<tr>
<td>BA</td>
<td>294.1</td>
<td>272.8</td>
<td>(\delta)</td>
</tr>
</tbody>
</table>

\(^a\) Treatment level
\(\delta\) Statistically significant at 10% level
Fig. 21. Number of cracks per fruit as a response to NAA X Cluster interaction.

Fig. 22. Number of cracks per fruit as a response to GA X Cluster interaction.
Fig. 23. Total length of cracks per fruit as a response to NAA X Cluster interaction.

Fig. 24. Total length of cracks per fruit as a response to BA X Cluster interaction.
Discussion

The results of this experiment seem to confirm the importance of growth regulators in influencing the cracking incidence in tomato fruits. It is possible that NAA, BA and GA exert their influence on fruit cracking by directly influencing the mechanical behavior of the skin. The NAA treatment alone increased skin extensibility, while the GA treatment decreased it, and the BA treatment had no statistically significant effect (Table 2). Also it was observed that the effect of these substances on skin extensibility was accentuated at the shoulder area of the fruit (Table 5). Since the tissues of the shoulder are physiologically active longer than the other parts of the fruit, it is possible that the treatments are more effective in increasing extensibility at the shoulder than at the median.

The effect of NAA in increasing the extensibility of the tomato skin tissue is in agreement with the idea that auxins affected the extensibility of parenchyma cells of Avena coleoptiles (58), potato tuber tissue (24), elastic and plastic components (33), and increased deformability of oat coleoptiles (53). It is possible that the effect of NAA on the mechanical properties of the skin is a result of plasticization of the cell wall materials. Conceivably, NAA might have an influence on the synthesis of celluloses or pectins in the cell walls of the skin tissue. It has been suggested by Boroughs and Bonner (12) that IAA induces cellulose synthesis in oat coleoptile sections
when sucrose was used as a substrate. Similar induction of cellulose synthesis was reported by Ordin and Bonner (52), when galactose was used as a substrate. Kerr (38), however, suggested that it is the pectins and related substances which strengthen the cell wall in the longitudinal direction rather than the cellulose. Furthermore, the relationship of the auxin action and plasticizing effects have been considered to explain the auxin-controlled process of cell extensibility. It was explained by Tagawa and Bonner (67) that potassium ions and IAA induces softening and plasticization of the cell wall. It has been suggested that methylation of carboxyl groups on adjacent pectic molecules, under auxin control, involves the splitting of the calcium bridges which contribute to the mechanical properties of the wall (58). Therefore, it is likely that the addition of NAA to the developing tomato fruit, augments the softening process of the cell wall structure, thereby increasing extensibility of the skin.

The GA treatment induced a large increase in the ultimate force required to break the skin, while the NAA and BA treatments had no statistically significant effect on this characteristic (Table 3). Combining this effect of GA with the reduction of extensibility caused by GA indicates that GA plays an important physiological role by either interfering with the normal action of auxins or by affecting some unknown metabolic process which may, directly or indirectly, influence the mechanical properties of the skin. It might be possible that GA
acts upon one or more metabolic pathways involving the synthesis of celluloses or pectic substances by directly influencing the synthesis of certain enzymes or their activities in controlling these processes, especially in the formation of calcium or magnesium cross-linkages (58). It has been shown by Tagawa and Bonner (67) that calcium or magnesium ions are in fact responsible for decreasing both the elasticity and plasticity of the oat coleoptile tissue. They concluded that calcium ions repress the plasticizing effect of potassium ions on the cell walls.

In terms of the mechanical behavior of polymers or cellulosic materials, it is known that the degree of stiffness of these materials can be detected by the steepness of the stress-strain curves or by the calculated ultimate forces required to stress a tissue until breakage occurs (42, 47, 58). The stress-strain curve gives a visual presentation of the reaction of the tomato skin to mechanical stress. As seen in Figure 20, GA treated skin produced a much steeper curve than the untreated, indicating a reduction in elasticity and plasticity of the skin as a result of the treatment. The NAA and BA curves, however, had a gentler slope than the control curve, indicating that these treatments increased the elasticity and plasticity of the skin. The appearance of the NAA and BA curves show that the major difference between them and the control curve is found in the first half of the curves. This is an agreement with the fact that only in extensibility at 10 Kg/cm\(^2\), and not in total extensibility, significant differences were found.
between treated and untreated fruits. Since this portion of the curve has been called the elastic range (5, 42, 43, 47, 58), it seems that NAA and BA affected the elasticity of the skin rather than the plasticity as might be suggested by other work (28, 33, 58, 67). The steepness of the GA curve indicates less elasticity, and perhaps plasticity, for the GA treated skin as compared with the untreated skin.

The number of cracks per fruit also was affected by the different growth regulator treatments. Although NAA, BA and GA reduced the number of cracks when each was applied separately, they showed different responses in their effects on cracking when applied as NAA-BA or NAA-GA combinations (Figures 16, 17). As to the relationship between auxins and GA, it has been shown by Brian and Hemming (13) that a complementary action of these is evident. Although it has been suggested that GA alone induces no extension of pea internodes without the presence of an auxin, the present experiment indicates that the GA effect on cracking was negated by the presence of NAA. It can be speculated that the GA effect was at or near its optimal level with complementary action by the endogenous auxins in the fruit, but when the auxin content was increased by the addition of NAA, this action may have been either blocked or inhibited. It is conceivable, however, that the interactions of NAA and GA are inconsistent in their effects on growth of the fruit as has been reported also by Brian and Hemming.
The inconsistency of auxin action is indicated by the interactions between certain treatments and the location of fruits in different clusters (Figures 21, 23). The increased crack reduction by NAA on fruits of the first cluster may have been due to the greater amounts of auxins available to the fruits at this level. Conceivably, the endogenous auxins being produced in the apical meristem could have been distributed to the different flower clusters with a larger amount being available for the first one. The GA treatment, however, exerted its greatest effect in crack reduction on fruits of the third cluster (Figure 22). This, presumably, corresponds with the results of NAA X GA interaction in their effect on cracking. Whenever the concentration of NAA, and possibly other auxins, is built up, the GA effect on crack reduction is decreased or nullified. It might be possible then, that the amount of NAA present in the fruits decreased going from the first cluster to the third one, while GA may have been the same in all clusters. This seems to be in agreement with the previous reports which showed that GA does not translocate as readily as IAA does (63).

Total length of cracks also was affected differently by the treatments at different cluster levels. The NAA X cluster interaction followed about the same way as it did with NAA effect on the number of cracks. The BA treatment, however, had its greatest effect on reduction of the total length of cracks on fruits of the first and the third clusters. From the exper-
imental results observed in this study, it can be speculated that BA has a similar physiological effect on fruit cracking as does NAA. The possibility that BA caused a reduction in fruit size also may have been an important factor in the lower amount of cracking found in the BA treatment as compared to the control.

The relationship between tomato fruit cracking and the mechanical properties of the fruit skin is shown by the negative correlation between number of cracks per fruit and skin extensibility. The reduction in fruit cracking and the increase in skin extensibility which resulted from the NAA treatment fit into this same relationship. The GA treatment, however, decreased skin extensibility, increased the ultimate force necessary to break the skin, and reduced fruit cracking. This indicates that the reduction in cracking for this treatment is related to increased skin strength rather than increased skin extensibility.
EXPERIMENT III

This experiment was designed to study the cracking response to NAA and GA treatments under field conditions.

Materials and Methods

Caravelle tomato was chosen for this experiment because of its consistency in radial cracks. The plants were started in the greenhouse and transplanted to the field. They were pruned to two stems and these were supported upright by tying to metal stakes. The number of flower clusters developed on each stem was not restricted, but only the first 4 clusters of each stem of each plant were treated. The plants were grown under severe weather conditions of high wind velocities, fluctuating humidity and rainfall, dusty air and excessive exposure of fruits to full sunlight.

The treatments consisted of untreated control, 50 ppm NAA and 50 ppm GA. NAA solution was prepared in 25% ethanol, while GA was prepared by diluting a concentrated commercial preparation\(^1\) with distilled water. Since this experiment was carried out under field conditions, higher concentrations of NAA and GA were used. The substances were applied directly to the developing fruit on the cluster without shielding the other fruits or flowers on the same cluster.

Skin samples for testing with the "Instron" were obtained

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\(^1\)Gib-Sol (2.13% GA), Elanco Products Company, Indianapolis, Indiana
from the shoulder areas of the fruits in the manner described in the General Materials and Methods.

Completely randomized plot design was used for this experiment and sampling of fruits for evaluation of fruit cracking and testing for mechanical properties, was divided into two categories:

1. A total of 9 fruits from each plant were sampled for the purpose of analyzing for the effect of growth regulator treatments on weight and cracking of the fruit. A simple model and analysis of variance were used for this part as shown below:

   Model: \( Y_{ij} = \mu + t_i + e_{ij} \)

   Limits, \( \mu = 0, t_i \) (treatments) = 3, \( j \) (replication) = 5, \( e_{ij} \) (experimental error) NID \((0, \sigma^2_e)\). Total number of observations = 15.

   ANOVA

   \[
   \begin{array}{ll}
   \text{Factors} & \text{d.f.} \\
   \text{Treatments} & 2 \\
   \text{Error} & 12 \\
   \text{Total} & 14 \\
   \end{array}
   \]

2. One fruit from each of the first and second clusters of each of the two stems of each plant were sampled for the purpose of analyzing for the effect of growth regulator treatments on fruit cracking and mechanical properties of the fruit skin. In addition to the
analysis of variance, analysis of variance-covariates was used in order to calculate the correlation coefficients using the number of cracks and average length of cracks as covariates and the three mechanical properties as variates. The model and analysis of variance were as follows:

**Model**

\[ Y_2, Y_4, Y_5, Y_6 = A(i) + B(ij) + C(k) + AC(ik) + B_1X_1, B_3X_3 + E(ijk) \]

Limits, \( i \) (treatments) = 3, \( j \) (plants) = 5, \( k \) (fruits = 4, total number of observations = 60

**Variates**

- \( Y_2 = \) Total length of cracks per fruit
- \( Y_4 = \) Extension of skin at 10 Kg/cm²
- \( Y_5 = \) Total extension
- \( Y_6 = \) Ultimate force
- \( X_1 = \) Number of cracks per fruit
- \( X_3 = \) Average length of cracks per fruit

The analysis of covariance was computed by using \( X_1, X_3 \) as covariates and \( Y \)'s as variates.

**ANOVA**

<table>
<thead>
<tr>
<th>Factors</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>2</td>
</tr>
<tr>
<td>Experimental Error</td>
<td>12</td>
</tr>
<tr>
<td>Fruits</td>
<td>3</td>
</tr>
<tr>
<td>Treatment X Fruits</td>
<td>6</td>
</tr>
<tr>
<td>Error</td>
<td>36</td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
</tr>
</tbody>
</table>
Results

Analysis 1

Table 7 summarizes the data analyzed for weight and cracking of fruits. It appeared that GA treatment may have increased the average weight of fruits, but not significantly.

Number of cracks per fruit were significantly decreased \( (P<0.01) \) by the NAA treatments as compared with the other treatments while there was no significant difference in number of cracks between GA treatment and the control.

GA treatment significantly \( (P<0.01) \) increased the total length of cracks per fruit as compared to NAA treatment and the control. The average length of cracks was decreased significantly \( (P<0.05) \) by NAA as compared to control, while the difference in average length of cracks between GA and control was not significant.

Analysis 2

Table 8 summarizes the effects of treatments on number of cracks, length of cracks and mechanical properties of the skin. As in analysis 1, NAA treatment significantly \( (P<0.01) \) reduced the number of cracks compared to GA and control. There was no significant difference in number of cracks between GA treatment and the control.

NAA reduced the total length of cracks \( (P<0.01) \) as compared to GA and the control. There was no significant difference in total length of cracks between GA and the control. GA, how-
Table 7. Effect of GA and NAA on cracking and size of tomato fruit

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit weight (g)</th>
<th>Cracks/fruit (No.)</th>
<th>Total length of cracks/fruit (mm)</th>
<th>Length of cracks/fruit (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA (50 ppm)</td>
<td>186.4</td>
<td>2.5</td>
<td>47.4</td>
<td>18.9</td>
</tr>
<tr>
<td>GA (50 ppm)</td>
<td>216.0</td>
<td>5.1</td>
<td>130.3</td>
<td>25.8</td>
</tr>
<tr>
<td>Control</td>
<td>191.1</td>
<td>4.7</td>
<td>107.0</td>
<td>23.0</td>
</tr>
</tbody>
</table>

L.S.D., 5% N.S. 1.5 15.3 2.8

L.S.D., 1% N.S. 2.2 22.3 N.S.

Data are an average of 45 fruits sampled from 9 plants per treatment
Table 8. Effect of GA and NAA on fruit cracking and mechanical properties of tomato fruit skin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cracks/fruit (No.)</th>
<th>Total length of cracks (mm)</th>
<th>Avg. length of crack (mm)</th>
<th>Extension of Fruit Skin At 10 Kg/cm² (%)</th>
<th>Total (%)</th>
<th>Ultimate force (Kg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA (50 ppm)</td>
<td>2.3</td>
<td>30.2</td>
<td>9.7</td>
<td>10.1</td>
<td>15.1</td>
<td>13.2</td>
</tr>
<tr>
<td>GA (50 ppm)</td>
<td>4.6</td>
<td>101.0</td>
<td>20.9</td>
<td>5.5</td>
<td>13.2</td>
<td>24.0</td>
</tr>
<tr>
<td>Control</td>
<td>4.9</td>
<td>76.0</td>
<td>14.0</td>
<td>8.1</td>
<td>12.8</td>
<td>15.4</td>
</tr>
</tbody>
</table>

L.S.D., 5%   1.6  32.1  4.6  1.3  N.S.  2.5
L.S.D., 1%   2.1  42.6  6.1  1.8  N.S.  3.3

Data are averages of 20 fruits/treatment
ever, increased the average length of cracks compared to the control (P<0.01), but there was no significant difference in average length of cracks between NAA and the control.

Extension of the skin at the 10 Kg stress force was significantly increased by NAA treatment (P<0.01) compared to the control, while it was significantly decreased by the GA treatment (P<0.01). Total extension, however, was not significantly affected by the treatments.

Ultimate force required to break the skin was increased due to GA treatment (P<0.01) compared to NAA and the control. There was no significant difference in ultimate forces between NAA treatment and the control.

Table 9 shows that some correlations existed between the mechanical properties and number or length of cracks in the fruits. The extension of skin at 10 Kg/cm² and total extension were negatively correlated (P<0.01) with number of cracks. Although the correlation between both extension measurements and the average length of cracks was negative, it was not significant. The ultimate force, on the other hand, was positively correlated (P<0.05) with the average length of cracks. There was a nonsignificant positive correlation between ultimate force and the number of cracks per fruit.

Discussion

It was evident that the NAA treatments consistently reduced cracking incidence in tomato fruits regardless of the
Table 9. Correlation coefficients for factors involved in responses to growth regulator treatments

<table>
<thead>
<tr>
<th>Factors</th>
<th>No. of cracks/fruit</th>
<th>Avg. length of crack/fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extension at 10 Kg/cm²</td>
<td>-0.677**</td>
<td>-0.239</td>
</tr>
<tr>
<td>Total Extension</td>
<td>-0.568**</td>
<td>-0.280</td>
</tr>
<tr>
<td>Ultimate Force</td>
<td>+0.155</td>
<td>+0.388*</td>
</tr>
</tbody>
</table>

** Statistically significant at 1% level
* Statistically significant at 5% level
cultivar used or the environment under which the plants were grown. It has been demonstrated that a direct relationship existed between cracking and the mechanical properties of the skin. In this experiment as well as in the previous one, strong negative correlations between the number of cracks and the extensibility of skin at a force of 10 Kg/cm² were evident. It may be possible that a major factor in cracking resistance of a particular cultivar or even a particular fruit would be the degree of extensibility of its skin. Since these factors are directly influenced by NAA treatments it would seem logical to suggest that NAA or a related endogenous auxin is responsible for increasing the skin extensibility, thereby rendering the fruit less susceptible to cracking. It also seems reasonable to think that the addition of NAA to fruits known to be susceptible to cracking would limit the degree of cracking or reduce the severity of cracking. This is in agreement with the findings of Bullock (17), who found a reduction of cracking of cherry fruits by NAA treatments, and of Crane (21), who showed that apricot fruits also cracked less as a result of NAA treatments.

As to the effect of concentrations of the growth substances applied, it was demonstrated that an increase in concentration of NAA did not alter its effects on cracking and mechanical properties of the skin. The increase in GA concentration, however, seemed to increase the cracking severity by increasing the average length of cracks per fruit. This increase was di-
rectly related to the degree of skin stiffness and strength, which was detected by the high values of ultimate force resulting from the GA treatment.

GA does not always increase cracking severity, however, as indicated in Experiment I. In that experiment, with a greenhouse environment and a lower concentration of the chemical, GA decreased cracking incidence. The contribution of GA to skin strength, therefore, may reduce cracking under one set of conditions but may increase it if the stress is more severe.
EXPERIMENT IV

This experiment was designed to evaluate the relationship of fruit cracking to the stress-strain properties of the fruit skin of certain tomato cultivars and to ascertain if these properties were influenced by fruit size. Fruit weight was used as an easily determined approximation of fruit size.

Materials and Methods

Seeds of the cultivars Scarlet Beauty, Caravelle and Sun Up were sowed in peat pots filled with a peat-perlite growing media and grown for 6 weeks in the greenhouse. The most uniform plants then were planted in the field using a 3 foot by 6 foot spacing, 3 plants per plot and 3 replications. The plants were allowed to grow naturally without pruning or training. Supplemental irrigation was applied as needed.

The main stem of each plant was selected for the collection of fruits needed for evaluation and testing. The first, second, and third fruits of the first and the second clusters and the first and second fruit of the third cluster were tagged for collection when they became ripe (at least 90% red skin color). Immediately after collection, each sample was weighed and the number and length of cracks were determined and recorded. Data for both the radial and concentric cracks were obtained. The fruits then were used for determination of their skins' mechanical properties as described in the procedures in General Materials and Methods.
Portions of the outer pericarp and the Instron-stressed skins of each cultivar were fixed and preserved in FAA solution for anatomical studies. The skin pieces were stained with Nile blue and mounted in glycerin. The pericarp segments were transversely sectioned by hand and stained with safranin and fast green.

A randomized block design with 3 blocks and 3 cultivars was used for the statistical analysis. The statistical model was set up to utilize data from each individual fruit as follows:

\[ Y_{2}, Y_{3}, Y_{4}, Y_{5}, Y_{6}, Y_{7}, Y_{8}, Y_{9} = A(i) + B(j) + AB(ij) + BC(jk) + AC(ik) + ABC(ijk) + B_{1}X_{1} + E(ijkl). \]

Limits, I (blocks) = 3, J (cultivars) = 3, K (plants) = 3, L (fruits) = 8, total number of observations = 216.

\[ Y_{2} = \text{Number of cracks per fruit} \]
\[ Y_{3} = \text{Number of radial cracks} \]
\[ Y_{4} = \text{Average length of radial cracks} \]
\[ Y_{5} = \text{Number of concentric cracks per fruit} \]
\[ Y_{6} = \text{Average length of concentric cracks} \]
\[ Y_{7} = \text{Extension of skin at 10 Kg/cm} \]
\[ Y_{8} = \text{Total extension} \]
\[ Y_{9} = \text{Ultimate force} \]
\[ X_{1} = \text{Fruit weight} \]

The data for the number of cracks, number of radial cracks and number of concentric cracks were transformed to \( \sqrt{\text{No.} + 0.5} \).
The transformed data were used to normalize the binomial distribution and to stabilize the variances of the experimental samples. The 0.5 was added to eliminate the zeros from the data.

The analysis of variance for each of the variates had the following format:

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blocks</td>
<td>2</td>
</tr>
<tr>
<td>Cultivars</td>
<td>2</td>
</tr>
<tr>
<td>Blocks X Cultivars</td>
<td>4</td>
</tr>
<tr>
<td>Error (Blocks)</td>
<td>6</td>
</tr>
<tr>
<td>Error (Cultivars)</td>
<td>12</td>
</tr>
<tr>
<td>Error</td>
<td>189</td>
</tr>
<tr>
<td>Total</td>
<td>215</td>
</tr>
</tbody>
</table>

Correlation coefficients between fruit weight and each of the other categories were calculated.

Results

The analyses of variance indicated that significant (P<0.01) differences among cultivars existed for all variates except total extension of skin (P<0.05) and fruit weight (P<0.10) (Table 10). Caravelle had the greatest number and length of radial cracks, but Sun Up exceeded the other 2 cultivars by a much greater amount in number and length of concentric cracks. Sun Up, therefore, had the largest total number of cracks per fruit, with Caravelle having the second largest number and Scarlet Beauty having the least. This relationship among cultivars was the same for ultimate force, but was reversed for extension of the skin. Although the extension of
Table 10. Effect of cultivar on fruit cracking and mechanical properties of tomato fruit skin

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fruit weight (g)</th>
<th>Total cracks/fruit (No.)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Radial cracks (No.)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Concentric cracks (No.)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scarlet Beauty</td>
<td>179.9</td>
<td>1.2</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Caravelle</td>
<td>210.9</td>
<td>1.7</td>
<td>3.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Sun Up</td>
<td>207.5</td>
<td>2.1</td>
<td>4.5</td>
<td>1.9</td>
</tr>
</tbody>
</table>

L.S.D., 5%  N.S.  0.3  -  0.2  -  0.2  -
L.S.D., 1%  N.S.  0.4  -  0.3  -  0.3  -

<sup>a</sup> Data are averages for 24 fruits
<sup>b</sup> Actual data
<sup>c</sup> Transformed data (\(\sqrt{\text{No.} + 0.5}\))
Table 10. (Continued)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Length of radial cracks (mm)</th>
<th>Length of concentric cracks (mm)</th>
<th>Extension of Skin at 10 Kg/cm² (%)</th>
<th>Total (%)</th>
<th>Ultimate force (Kg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scarlet Beauty</td>
<td>11.9</td>
<td>8.4</td>
<td>9.7</td>
<td>16.9</td>
<td>16.6</td>
</tr>
<tr>
<td>Caravelle</td>
<td>35.8</td>
<td>20.5</td>
<td>8.7</td>
<td>16.3</td>
<td>17.9</td>
</tr>
<tr>
<td>Sun Up</td>
<td>17.6</td>
<td>97.0</td>
<td>7.3</td>
<td>14.9</td>
<td>19.6</td>
</tr>
<tr>
<td>L.S.D., 5%</td>
<td>11.6</td>
<td>18.1</td>
<td>0.8</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>L.S.D., 1%</td>
<td>15.8</td>
<td>24.7</td>
<td>1.0</td>
<td>1.9</td>
<td>2.5</td>
</tr>
</tbody>
</table>
skin for both 10 Kg/cm² and total were significantly greater for Caravelle as compared with Sun Up, only the difference in extension of skin at 10 Kg/cm² was significant when comparing Caravelle with Scarlet Beauty.

The correlation coefficients calculated from the covariance analysis indicated that only total number of cracks and number of concentric cracks were significantly (P<0.05) correlated with fruit weight (Table 11). These correlations were positive as were the non-significant correlations between fruit weight and the other cracking measurements. The correlations between fruit weight and the stress-strain measurements of the skin, however, were negative, although non-significant.

To obtain further information on the relationship of fruit weight to the other factors, the data for only one cultivar, Sun Up, were segregated according to weight classes (Table 12). These data show that, with the cultivar, there does not seem to be a relationship between fruit weight and cracking except that the 300 and over weight class seem to be more susceptible to cracking as compared with all the other classes.

The histological observations on the fruit and skin tissue revealed striking differences among the three cultivars in the anatomy of the pericarp tissues. Figure 25 shows the structure of the epidermis and the subepidermal portion of the pericarp of Scarlet Beauty. A thick layer of cuticle covers the flattened epidermal cells. Relatively small subepidermal cells are present, but the parenchymatous cells have fairly thick walls.
<table>
<thead>
<tr>
<th>Factors</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number of Cracks X Weight</td>
<td>+0.186*</td>
</tr>
<tr>
<td>Number of Radial Cracks X Weight</td>
<td>+0.099</td>
</tr>
<tr>
<td>Number of Concentric Cracks X Weight</td>
<td>+0.157*</td>
</tr>
<tr>
<td>Length of Radial Cracks X Weight</td>
<td>+0.022</td>
</tr>
<tr>
<td>Length of Concentric Cracks X Weight</td>
<td>+0.139</td>
</tr>
<tr>
<td>Extension at 10 Kg/cm² X Weight</td>
<td>-0.054</td>
</tr>
<tr>
<td>Total Extension X Weight</td>
<td>-0.128</td>
</tr>
<tr>
<td>Ultimate Force X Weight</td>
<td>-0.026</td>
</tr>
</tbody>
</table>

* Statistically significant at 5% level
Table 12. Effect of fruit size on fruit cracking and mechanical properties of fruit skin of Sun Up

<table>
<thead>
<tr>
<th>Weight class (g)</th>
<th>Total cracks (No.)</th>
<th>Radial cracks (No.)</th>
<th>Concentric cracks (No.)</th>
<th>Total length of cracks (mm)</th>
<th>Extension at 10 Kg/cm² (%)</th>
<th>Ultimate force (Kg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-125</td>
<td>5.6</td>
<td>1.2</td>
<td>4.4</td>
<td>101.4</td>
<td>7.04</td>
<td>19.50</td>
</tr>
<tr>
<td>126-150</td>
<td>4.6</td>
<td>0.3</td>
<td>4.3</td>
<td>116.5</td>
<td>7.80</td>
<td>18.87</td>
</tr>
<tr>
<td>151-175</td>
<td>2.9</td>
<td>0.4</td>
<td>2.5</td>
<td>86.5</td>
<td>7.19</td>
<td>23.60</td>
</tr>
<tr>
<td>176-200</td>
<td>3.9</td>
<td>0.4</td>
<td>3.5</td>
<td>122.4</td>
<td>7.77</td>
<td>19.24</td>
</tr>
<tr>
<td>201-225</td>
<td>4.1</td>
<td>0.6</td>
<td>3.5</td>
<td>116.1</td>
<td>6.08</td>
<td>22.00</td>
</tr>
<tr>
<td>226-250</td>
<td>5.4</td>
<td>0.6</td>
<td>4.9</td>
<td>125.7</td>
<td>6.67</td>
<td>19.63</td>
</tr>
<tr>
<td>251-275</td>
<td>4.3</td>
<td>0.0</td>
<td>4.3</td>
<td>123.8</td>
<td>8.35</td>
<td>18.63</td>
</tr>
<tr>
<td>276-300</td>
<td>3.4</td>
<td>0.2</td>
<td>3.2</td>
<td>103.6</td>
<td>6.65</td>
<td>19.68</td>
</tr>
<tr>
<td>300 and over</td>
<td>8.2</td>
<td>0.8</td>
<td>7.4</td>
<td>177.7</td>
<td>5.53</td>
<td>18.23</td>
</tr>
</tbody>
</table>
The stretched skin of this cultivar shows that few subepidermal fissures occurred as a result of the stretch (Figure 26).

The epidermal cells of Caravelle appears to be somewhat rounder and practically free of cuticle (Figure 27). Several subepidermal layers are present and some of the cells in these layers have tapered ends. Most skin samples of this cultivar had a tendency of breaking at the side of the stretched skin, as shown in Figure 28.

Sun Up generally had a very loose arrangement of epidermal cells with no visible cuticle on their surface (Figure 29). Subepidermal layers did not seem to exist in this cultivar. Thick-walled, extremely large parenchymatous cells extended to the epidermis. The stretched skin samples of the Sun Up showed numerous subepidermal fissures, particularly around the area where trichomes had been developed (Figure 30). This type of subepidermal fissure is compared in higher magnification with the side failure found in Caravelle epidermis (Figures 31, 32).

Discussion

The data of this experiment show that differences exist among the 3 cultivars in all the characteristics tested. This is in agreement with previous work by Batal, et al. (8). The present data vary from that previously reported, however, in regard to the type of cracking exhibited by Sun Up and the relationship between cracking and ultimate force. In 1969, Sun Up exhibited predominantly radial cracking, but concentric
Fig. 25. Cross section of pericarp of Scarlet Beauty: Flattened epidermal cells and thick cuticle (arrow), thick subepidermal layer (arrowhead). X 347.

Fig. 26. Surface view of stretched skin sample of Scarlet Beauty: Subepidermal fissure (arrows). X 139.

Fig. 27. Cross section of pericarp of Caravelle: Epidermis (upper arrow), subepidermal layer (lower arrow). X 347.

Fig. 28. Surface view of stretched skin of Caravelle: Breakage of skin through the epidermis (arrow). X 139.

Fig. 29. Cross section of pericarp of Sun Up: Loose, elliptical epidermal cells (arrow), large parenchyma cells adjacent to epidermal layer. X 347.

Fig. 30. Surface view of stretched skin of Sun Up: Subepidermal fissures (arrows). X 139.
Fig. 31. Enlargement of surface view of stretched skin indicated in Fig. 28. Breakage of epidermal layer (upper arrow), intracellular breakage (lower arrows). X 294.

Fig. 32. Surface view of stretched skin of Sun Up. Region of a trichome base (upper right arrow), subepidermal fissures (upper left and lower right arrows). X 294.
cracking was the type most often found in this cultivar during 1970. This indicates the difficulty of evaluating tomato cultivars for either radial or concentric cracks separately rather than for a total of all cracks. Sun Up had more radial cracks than either Scarlet Beauty or Caravelle in 1969, but these 2 cultivars had significantly greater numbers of radial cracks than Sun Up in 1970.

In regard to the difference in ultimate force results between 1969 and 1970, Sun Up shoulder skin had a significantly lower ultimate force value as compared with Scarlet Beauty in 1969. This was in agreement with Voisey, et al. (74) who had reported higher value of ultimate forces for a resistant cultivar and lower values for a susceptible one. In 1970, however, this was reversed because Scarlet Beauty had a significantly lower ultimate force values as compared with Sun Up. Since the relationship among cultivars for fruit cracking was the same in both years, it does not appear that ultimate force is a good indicator of resistance to cracking.

The relationship between fruit cracking and extensibility of the skin was the same in both years. Greater extensibility was related to a lower incidence of cracking. This is in direct agreement with the findings of Voisey, et al., and with the results of the other experiments in this study which showed that extensibility and cracking are negatively correlated.

Microscopic examination of the pericarp sections of the three cultivars revealed a noticeable difference in their
epidermal and subepidermal layers. Scarlet Beauty, which pos­sessed flattened epidermal cells and thick cuticle, was found to be highly resistant to both radial and concentric cracking. The crack resistant cultivar, Heinz 1350, in Experiment I had a similar pericarp structure. Apparently, flattened epidermal cells and thick cuticle are consistent characteristics of crack-resistant cultivars. This is in complete agreement with the results reported by Cotner, et al. (19). They speculated that the flattened epidermis might contribute to the strength and render the fruit less susceptible to cracking. In contrast to this, Voisey, et al. (74) suggested that the differences in cracking among cultivars may be a result of the deep penetration of the cutinized layer into the intercellular spaces of the epidermal layer, rather than the shape of the epidermal cells. In the present study, it was observed that a combination of factors, such as, a thick cuticular layer, flattened epidermal cells and a gradual increase in size of cells of the epidermal layer were associated with crack resistance. On the other hand, rounded or elliptical and loosely arranged epidermal cells, very thin or no subepidermal layers and extremely large parenchymatous cells adjacent to the epidermis with no distinct subepidermal layer were associated with severe crack­ing.
CONCLUSIONS AND SUMMARY

Several greenhouse and field experiments were carried out to determine the effects of growth regulators and cultivar differences on fruit cracking and mechanical properties of the tomato fruit skin. Cracking incidence and its severity were evaluated on the basis of the actual count of the cracked fruits, number of cracks and measurement of their length.

The Instron tensile tester was used to determine the breaking elongation and the ultimate force required to break the skin when subjected to a stretching stress. Skin samples for Instron testing were obtained randomly from either shoulder or median portions of the fruit as required by the particular experiment.

Several growth regulating substances were used at the initial stage of this study to establish an experimental basis for the idea that growth regulators, particularly the auxins, have an effect on cracking of tomato fruits. Naphthaleneacetic acid (NAA), indoleacetic acid (IAA), gibberellic acid (GA), kinetin (K), benzyladenine (BA) and combinations of these chemicals were applied in alcohol solutions directly to the developing fruits of different cultivars grown in the greenhouse and in the field.

The results of this study indicated that the auxins, NAA and IAA definitely reduced the incidence of cracking of tomato fruits. After the initial greenhouse experiment, NAA was selected for further investigations in the greenhouse and the
field, because of its effectiveness in reducing both the percentage of cracked fruits and the number of cracks per fruit, as compared to IAA. The NAA treatment was consistent in crack reduction throughout the various tests regardless of the environmental conditions, the cultivars on which it was applied or the concentrations used.

There were some indications that GA may have the same effect as auxins in reducing cracking, particularly when applied at 30 ppm in the greenhouse. At a 50 ppm concentration and an outdoor environment, however, GA was found to increase the severity of cracking. This indicates that the effect of GA on fruit cracking is not as predictable as that of NAA, and that the concentration used and the severity of the environmental factors are important in determining the results of GA treatment. The addition of NAA with GA was not as effective in crack reduction as was GA alone. This interaction points to further difficulties in predicting the results of GA treatment of tomatoes in relation to cracking.

Kinetin did not seem to have noticeable effect on cracking under the conditions prevailing in the first greenhouse experiment. The BA treatment which was substituted for K in the second greenhouse experiment showed some interaction with NAA. Besides reducing the number of cracks per fruit to some extent, BA also caused a reduction in fruit size which leads us to believe that the reduction in cracking due to BA treatment may have been associated with the smaller size of the fruit.
It was evident that the growth regulators, particularly NAA and GA influenced the mechanical properties of the skin. In all cases, GA increased the ultimate force required to break the skin and decreased the extensibility of the skin, while NAA substantially increased the extensibility of the skin. Since there was a strong negative correlation between extensibility of the skin and fruit cracking, it was concluded that NAA action could have been directly involved in influencing skin extensibility by its effect on plasticity and elasticity of the skin cell walls.

The difference in skin extension also was evident among the fruits of the different cultivars. The cultivar most resistant to cracking had the most extensible skin and the lowest measurement of ultimate force, but the cultivar most susceptible to cracking had the least extensible skin and measured the highest ultimate force to break the skin. This seems to indicate that the genetical differences among cultivars are influencing the physiological behavior of the fruit which is, in turn, affecting the level of endogenous auxins, and possibly the gibberellins, which seem to be involved in influencing the mechanical behavior of the fruit skin.

Distinct differences were found among fruits of the different cultivars and among different growth regulator treatments within a particular cultivar in the thickness of the cuticle and in the anatomy of the epidermal layer, the subepidermal layers and the parenchyma immediately adjacent to these layers.
The microscopic examination of the pericarp sections clearly indicated the direct effect of growth regulators on the shape and the size of cellular structure of the tissue which constituted the skin. The smooth surface of the epidermis covered with a thick cuticle and an orderly arranged subepidermal layer with gradually increasing in size of cells towards the mesocarp were associated with, either reduced cracking due to NAA or IAA, or the natural crack resistance of a particular cultivar. Based on the experimental evidence in this study we may speculate that NAA or some endogenous auxin might be responsible in determining the deposition of the cuticle and the shape and size of the pericarp tissue.


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The author certainly is proud of his wife Patricia and his children Mariam, Linda, Sami and LeaAnn who sacrificed so much and endured the hardships that usually accompany the great task of college education.
Table A-1. The effects of growth regulators on fruit cracking and mechanical properties of skin samples taken from the shoulder area

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cracks per Fruit</th>
<th>Average length of cracks (mm)</th>
<th>Breaking Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Actual (No.)</td>
<td>Transformed data (No.) b</td>
<td>Extension at 10 Kg/cm² (%)</td>
</tr>
<tr>
<td>Control (Alc.)</td>
<td>5.0</td>
<td>2.3</td>
<td>29.3</td>
</tr>
<tr>
<td>NAA (30 ppm)</td>
<td>3.4</td>
<td>2.0</td>
<td>27.1</td>
</tr>
<tr>
<td>GA (30 ppm)</td>
<td>3.3</td>
<td>1.9</td>
<td>26.8</td>
</tr>
<tr>
<td>BA (50 ppm)</td>
<td>3.5</td>
<td>1.9</td>
<td>24.3</td>
</tr>
<tr>
<td>NAA + GA</td>
<td>3.6</td>
<td>2.0</td>
<td>29.2</td>
</tr>
<tr>
<td>NAA + BA</td>
<td>4.0</td>
<td>2.1</td>
<td>28.2</td>
</tr>
<tr>
<td>GA + BA</td>
<td>3.1</td>
<td>1.8</td>
<td>25.6</td>
</tr>
<tr>
<td>NAA + GA + BA</td>
<td>4.3</td>
<td>2.2</td>
<td>31.7</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.64</td>
<td>0.18</td>
<td>14.0</td>
</tr>
</tbody>
</table>

a Data are averages of 8 fruits per treatment

b $\sqrt{\text{No.} + 0.5}$ transformation imposed
Table A-2. The effect of growth regulators on size and cracking for all fruits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fruit weight (g)</th>
<th>Cracks per Fruit</th>
<th>Transformed data (No.)</th>
<th>Total length of cracks (mm)</th>
<th>Average length of cracks (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Ale.)</td>
<td>285.6</td>
<td>4.9</td>
<td>2.3</td>
<td>118.7</td>
<td>25.8</td>
</tr>
<tr>
<td>NAA (30 ppm)</td>
<td>287.7</td>
<td>3.2</td>
<td>1.8</td>
<td>83.9</td>
<td>25.8</td>
</tr>
<tr>
<td>GA (30 ppm)</td>
<td>281.3</td>
<td>3.2</td>
<td>1.8</td>
<td>84.7</td>
<td>26.8</td>
</tr>
<tr>
<td>BA (50 ppm)</td>
<td>272.8</td>
<td>3.4</td>
<td>1.9</td>
<td>83.0</td>
<td>24.0</td>
</tr>
<tr>
<td>NAA + GA</td>
<td>288.3</td>
<td>3.4</td>
<td>1.9</td>
<td>89.9</td>
<td>27.5</td>
</tr>
<tr>
<td>NAA + BA</td>
<td>276.4</td>
<td>3.3</td>
<td>1.9</td>
<td>84.9</td>
<td>25.0</td>
</tr>
<tr>
<td>GA + BA</td>
<td>270.1</td>
<td>3.3</td>
<td>1.9</td>
<td>86.9</td>
<td>26.9</td>
</tr>
<tr>
<td>NAA + GA + BA</td>
<td>284.2</td>
<td>3.8</td>
<td>2.1</td>
<td>104.3</td>
<td>29.7</td>
</tr>
</tbody>
</table>

Standard Error      5.47  0.33  0.09  10.2  2.27

a Data are averages of 12 fruits per treatment

b $\sqrt{\text{No.} + 0.5}$ transformation imposed