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Early Application of Ethrel Extends Tomato Fruit Cell Division and Increases Fruit Size and Yield with Ripening Delay

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Abstract. Flowers of tomato (*Lycopersicon esculentum* Mill.) plants cv. Castle Rock were sprayed with 100 ppm of ethrel, 0.5 mM aminooxyacetic acid (AOA), or water (control) 2 days after anthesis. The fruit period of cell division was extended up to 16–18 days after anthesis with the application of ethrel but reduced from 10–12 days (control) down to only 6–8 days with the application of AOA. In a trend opposite to AOA application, fruits that received ethrel treatment were of higher ethylene and 1-aminocyclopropane-1-carboxylic acid (ACC) levels than control. This was noticed not only during the first 2 weeks after anthesis but also during the fruit climacteric phase. Mesocarp cells of ethrel-treated fruits were greater in number/mm^2 but smaller in size than control; an opposite trend was obtained with the application of AOA. This was observed for a period of 18 days after anthesis, but by that time or at earlier ages, fruits of AOA treatment were larger in size and heavier in weight than control, and both were larger and heavier than ethrel-treated ones. At 5 weeks after anthesis and thereafter, the fruit response to all treatments was totally reversed because early ethrel-treated fruits became significantly larger in size and heavier in weight with a ripening delay of about 10 and 15 days compared with those of control and AOA-treated ones, respectively. When the same treatments were applied to the whole plant, similar results were obtained because the early application of ethrel increased the fruit yield by about 15% over control with a pronounced ripening delay; an opposite trend was obtained with the application of AOA. No significant differences were found among all treatments in terms of flower or fruit abscission or fruit number/plant. The data suggest that ethylene regulates tomato fruit transmission from cell division to cell enlargement. In addition, fruit cell division is terminated only when endogenous ethylene decreases to its basal level, allowing cell enlargement to dominate and proceed as in the case of the early application of AOA. The ripening delay of ethrel-treated fruits may be caused by the longer time required for the increased cell number to reach maturation. A low level of ethrel application at the tomato early fruiting stage may be used for increasing fruit yield by increasing fruit size and consequently its quality.

Key Words. Tomato—Ethrel—AOA—Cell division—Cell enlargement—Ethylene—ACC—Ripening

During its ontogeny, tomato fruit undergoes certain developmental stages. First and directly after set, the fruit passes through a short period of growth characterized by its slow rate. This slow rate of growth during this very early period is caused entirely by the dominant process of cell division (CD) (Davies and Cooking 1985, Gillaspy et al. 1993). The fruit then passes through a long period of rapid growth as fruit cells move to the stage of CE (Iwahori 1967, Nitsch and Nitsch 1961). The length of this rapid growth period is strongly controlled by either the tomato cultivar or environmental factors (Lacheene 1990). The third developmental stage then occurs and lasts for about 2 additional weeks of slow growth, by which the fruit gains a little more weight and reaches maturation (Abdel-Rahman 1977). Two or 3 days later, fruit ripening is initiated (Atta-Aly et al. 1992). These facts indicate strongly that the tomato fruit growth pattern follows a single sigmoid growth curve (Rhodes 1980). This also suggests that the ultimate size

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Abbreviations: CD, cell division; CE, cell enlargement; ACC, 1-aminocyclopropane-1-carboxylic acid; AOA, aminooxyacetic acid; TCA, trichloroacetic acid; LSD, least significant differences.

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of tomato fruit is determined during its very early stages of development (Houghtaling 1935). The extent and relative importance of CD and expansion may therefore play pivotal roles as key factors in controlling not only tomato fruit size and shape but also total fruit yield.

Ethylene, as a plant hormone, is produced by many fruits even during their early stages of growth and development (Atta-Aly 1988, Burg and Burg 1965). It was thought that the early production of ethylene is responsible for young fruit abscission. In contrast, Maxie and Crane (1968) reported that the increased level of ethylene produced by young fig fruit is the reason behind early fruit growth. Increased levels of ethylene production during early stages of fruit development were observed in many fruit species including tomato (Abdel-Rahman 1977, Atta-Aly 1988, El-Beltagy et al. 1976), sycamore fig (Maxie and Crane 1968), peach (Looney et al. 1974), and apple and cherry (Blanpied 1972). It was then believed that ethylene may have major and significant roles in fruit development during its ontogeny. In tomato, the early peak of endogenous ethylene which occurs during the very early stage of fruit development is followed shortly by a gradual decrease, reaching a stable low level until the onset of ripening, when another increase is observed with the beginning of the climacteric rise (Abdel-Rahman 1977, Atta-Aly 1988, El-Beltagy et al. 1976). The same trend was also found regarding the tomato fruit ACC content (Atta-Aly 1988) as an immediate ethylene precursor (Adams and Yang 1979).

It has been reported that the exogenous application of ethylene induces CD in potato tubers (Ilker et al. 1977), pine (Barker 1979), and aquatic plants (Metzer 1984) as well as CE in rice (Ku et al. 1970, Smith and Robertson 1971) and fig fruits (Maxie and Crane 1968). In pea apex and root, however, the exogenous application of ethylene depresses CD but increases CE (Apelbaum and Burg 1972). Increasing or inhibiting ethylene levels at a certain developmental stage may be used as a tool for magnifying or modifying the fruit growth pattern.

This present work therefore was designed to study the impact of modifying the ethylene level in tomato fruit during its very early developmental stages on fruit CD and consequently fruit final size, ripening, and yield.

Materials and Methods

Plants

Tomato (*Lycopersicon esculentum,* Mill., cv. Castle Rock) seeds were sown in foam trays filled with a mixture of peat moss and vermiculite (1:1 volume) on March 1, 1994 and 1995, for the first trial and on March 17, 1995 and 1996, for the second trial. Trays were then kept under unheated greenhouse conditions at Shalakan farm, Faculty of Agriculture, Ain Shams University, Egypt. One tray was sown 15 days ahead to serve as an indicator for monitoring seedling water need in the greenhouse as well as plant flowering dates and other fruit development stages in the field. During soil preparation, the experimental field of each trial was designed as complete randomized blocks in four replicates, each having three plots of 42 m^2 in area. Thirty-day-old seedlings were transplanted at a distance of 50 cm in 7.5-m-long rows of 80-cm width with a capacity of seven rows/plot. All agricultural management was then carried out as usually recommended for tomato production in the open field.

Treatments

When flowers of the first cluster reached their maximum blooming, they were tagged. Tagging was also carried out for the subsequent bloomed clusters continuously for a period of 2 weeks (the last 2 weeks of May and the first 2 weeks of June for the first and second trials, respectively). This was carried out to ensure that there was enough fruit during the first trial for different laboratory analyses, particularly during the early stages of fruit development or for calculating the percentage of abscised flowers during both trials. Plots of each replicate, in both trials, were then distributed randomly among the treatments.

In the first trial, tagged flowers were sprayed early in the morning with distilled water (control), 100 ppm of ethrel, or 0.5 mM AOA, until runoff. These treatments were conducted 2 days after tagging (anthesis), which is the approximate date of tomato fruit set (El-Beltagy et al. 1976). The same treatments were carried out during the second trial, but instead of the flowers, the whole plant was sprayed 1 week after the anthesis of the first cluster's flowers.

Fruits of the first trial were harvested periodically and analyzed in four replicates throughout the subsequent stages of fruit development; fruits of the second trial were left for recording flower or fruit abscission, fruit number/plant, fruit average weight, and total fruit yield.

Flower or Fruit Abscission

Fruits and the previously hung paper labels (flowers) were counted in 10 random plants/plot 3 weeks after tagging, and the percentage of abscised flowers and fruits was calculated in both trials. In the first trial, plants selected for abscission recording were left without fruit sampling.

Fruit Analyses

1. Fruit Fresh Weight. Fruits were harvested with the calyx attached throughout the whole period of fruit development starting 2 days (6 h after application) up to 55 days after anthesis. Fruits were harvested and weighed directly every other day during the first 12 days followed by sampling at intervals of 6 days during the subsequent 18 days, and then every 5 days until the fruit reached 53 days old (55 days after anthesis). Ten fruits were used in each plot for measuring the fruit average weight during the first 12 days. This number was reduced down to four fruits/plot during the subsequent stages (18–55 days after anthesis) of fruit development.

2. Fruit Dry Weight. After fresh weight recording, the same fruits were exposed to 70°C/72 h and reweighed for determining the fruit dry weight.

3. Fruit Diameter. Ten days after anthesis, 15 fruits were retagged in each plot using labels of colored paper. These fruits were then used, while attached, for measuring fruit diameter development following the same age order used in fruit fresh weight measurements. The first 8 days after anthesis were excluded in this analysis because of the minute differences between treatments.

4. Ethylene and ACC Sampling and Analysis. Following the same sampling procedures used for fruit fresh weight recording, fruits were harvested at the same ages and divided into two equal groups. Fruits of the first group were incubated for ethylene analysis; those of the second group were dipped immediately in liquid nitrogen and kept at –20°C for ACC analysis.

For ethylene analysis, fruits younger than 22 days were placed, immediately after harvesting, in 225-mL glass vessels; older fruits were incubated in 375-mL glass jars. The incubating containers were sealed and transferred carefully to the Horticultural Department of the above-mentioned institute. One-mL gas samples were withdrawn from the incubator headspace after an incubating period of 4 h and injected into a Varian 6000 gas chromatograph for ethylene analysis.

Two g of frozen fruit tissue was homogenized in 10 mL of TCA for ACC analysis using a mortar and pestle (a little washed silica sand was used to assist the grinding). The mixture was centrifuged at 7,000 rpm for 10 min. The supernatants were decanted, and the aliquots were assayed for ACC using the procedure of Atta-Aly et al. (1987) as a modified version of Lizada and Yang (1979).

5. Anatomic Observations. Tomato fruits were harvested 6, 9, 12, 15, and 18 days after anthesis. Triangular pieces from the middle portion of the fruit pericarp tissues were cut into transverse sections using a shaving stainless steel blade and then immersed immediately in FAA solution (5 mL of formalin, 5 mL of acetic acid, and 90 mL of 70% ethyl alcohol). The paraffin method technique (Johansen 1940) was followed. Sections of paraffin-embedded samples were obtained using a rotary microtome. Transverse sections of 10–12 μm were fixed on microscopic slides with albusol adhesive (Sass 1951). Staining was attained using a double combination of saffranin and light green. Sections were then mounted in Canada balsam. Photomicrographs were then obtained using a camera mounted on Carlzeiss (Jena) microscope. Using micrometer slides, the cell number as well as enlargement were examined and calculated.

The mesocarp cell number was estimated in 1 mm^2. Using five randomized replicates of the external mesocarp (distance between the exocarp and vascular bundles), the cell number was calculated. The same procedure was also used to estimate the internal mesocarp cell number (distance between the vascular bundles and internal epidermis).

For CE measurements, 20 randomized cells were measured at their maximum length using different loci of external and internal mesocarp.

6. Days to Red-Ripe Stage. Starting 2 days after tagging (fruit set), the days required for the fruits to reach the red-ripe stage were recorded using the same 10 plants left without fruit sampling and used for recording flower abscission.

Fruit Yield

With ripening initiation, fruits of the second trial were harvested at weekly intervals. At each harvest, fruits of each plot were counted and weighed. At the termination of the experiment, the average fruit weight (g), fruit number/plant, and total fruit yield (kg/plot) were calculated.

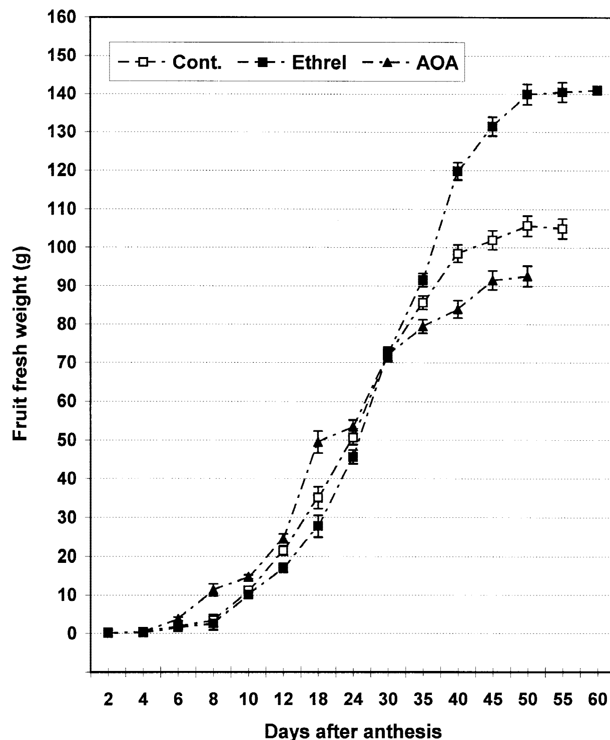

Fig. 1. Effect of flower treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 2 days after anthesis on tomato fruit fresh weight during growth and development. LSD values at each sampling date are shown as *vertical bars* at the 5% level.

Statistical Analysis

Data means were paired as the combined analysis of the results of each trial (two seasons). Because the results followed a similar trend, they were analyzed for significant statistical differences using the LSD test at the 5% level (Little and Hills 1978).

Results

First Trial

Tomato fruit fresh and dry weights showed an early period of slow growth during the first 6–8 days after anthesis (Figs. 1 and 2). During this period, fruits were less than 8 mm in diameter. These parameters increased rapidly as the fruit passed the age of 1 week, indicating that fruit transmission to a period of rapid growth lasted for 3 additional weeks. By that time, a second slow growth period was observed and existed until fruit reached its maturation (Table 1 and Figs. 1 and 2). During the early period of fruit growth (2–4 weeks after anthesis), fruits of AOA-treated flowers had the largest diameter (Table 1) and heaviest fresh and dry weights (Figs. 1 and 2), whereas those that had received 100 ppm of ethrel resulted in the smallest and the lightest fruits compared

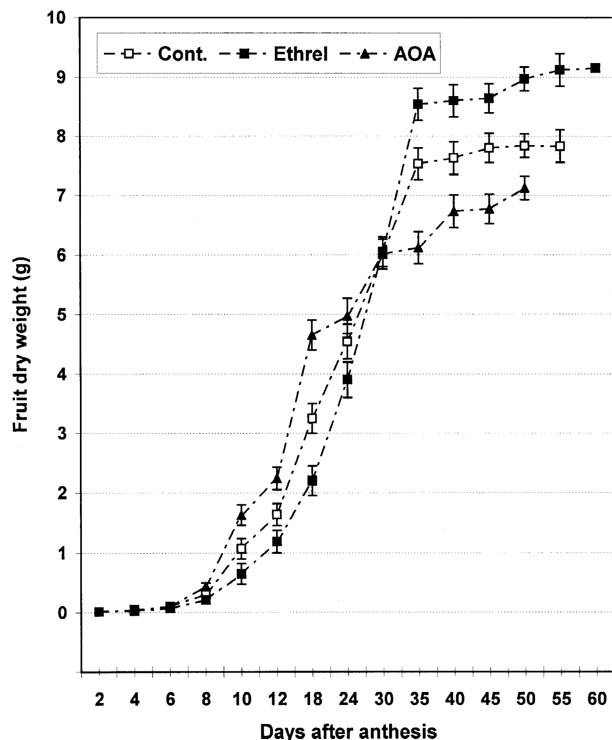

Fig. 2. Effect of flower treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 2 days after anthesis on tomato fruit dry weight during growth and development. LSD values at each sampling date are shown as *vertical bars* at the 5% level.

Table 1. Effect of flower treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 2 days after anthesis on tomato fruit diameter during development and on the time required for the fruits to reach breaker and red-ripe stages.

Days after anthesis	Fruit diameter (cm)		
	Control	Ethrel	AOA
10	1.25 b	1.14 b	1.56 a
12	2.51 b	2.18 c	2.79 a
18	3.11 b	2.73 c	3.67 a
24	4.64 b	4.57 b	4.83 a
30	5.15 b	5.71 a	5.06 b
35	5.33 b	5.87 a	5.23 b
40	5.51 b	6.14 a	5.39 b^X
45	5.86 b^X	6.45 a	5.75 b^Y
50	6.42 b^Y	6.93 a^X	5.86 c
55	6.49 b	6.98 a	
60		6.98^Y	

Means within each row followed by the same letter are not statistically different at the 5% level. X, time between anthesis and breaker stage. Y, time between anthesis and red-ripe stage.

with those of the control. No significant differences were found among all treatments in terms of flower or fruit abscission measured 3 weeks after anthesis (33 ± 2% abscission for all treatments). The significant descending order of fruit growth obtained with applications of AOA, H_2O, and ethrel was diminished 4 weeks after anthesis and reversed totally to an ascending order as the fruit age passed 30 days after antheses (Table 1 and Figs. 1 and 2). This new ascending significant order was strongly evident as fruit reached its red-ripe stage. Furthermore, fruits of AOA-treated flowers reached their red-ripe stage faster than control, and both were faster than those from ethrel-treated flowers (i.e. 45, 50, and 60 days after anthesis, respectively) following the same growth ascending order (Figs. 1 and 2). The time between red color initiation (breaker) and red-ripe stages (Table 1) was also extended but only in the fruits of ethrel-treated flowers compared with other treatments (10 vs 5 days, respectively).

According to the anatomic studies, the transsection of tomato fruit during its early stages of growth and development showed that fruit pericarp tissues consisted of exo- and mesocarp tissues. Exocarp tissue is formed from uniecuate parenchymatous cells coated with cuticle with two to three layers of cells located under the epidermis called hypodermis (Fig. 3). Mesocarp tissue, on the other hand, was formed from two different layers defined as outer and inner mesocarp layers. The outer mesocarp layer is located in the distance between the hypodermis and the vascular bundle of pericarp tissues; the inner mesocarp tissue is entirely parenchymatous cells located under the vascular bundle and extending to the loci of the ovules (Fig. 3). The transition phase from CD to CE occurred mostly in the outer and inner mesocarp layers (Fig. 3).

Fruits that developed from ethrel-treated flowers showed the highest level of CD in the outer mesocarp followed by control, whereas fruits of AOA-treated flowers were significantly the lowest (Figs. 3 and 4). The inner mesocarp, however, did not show CD activity 6 days after anthesis in either the control or AOA treatments (Fig. 3). Nine days after anthesis, however, CD was terminated in the fruit inner mesocarp of all treatments and only in the outer mesocarp of AOA-treated fruits. By that time the highest level of outer mesocarp CD was obtained with ethrel application (Fig. 4). Mesocarp cells were greater in number but smaller in size with the application of ethrel compared with other treatments 6 and 9 days after anthesis (Figs. 3 and 4). To define such results, the number of cells/mm^2 was counted as presented in Table 2. The number of cells was significantly higher in the ethrel treatment than in the control, whereas the AOA treatment resulted in the lowest cell number (Table 2). These results were observed in both the inner and the outer mesocarp up to 12 days after anthesis. The cell number in the outer mesocarp remained significantly higher with the application of ethrel than that of the control up to 18 days after anthesis, and both were significantly higher than in the AOA-treated fruits. In the

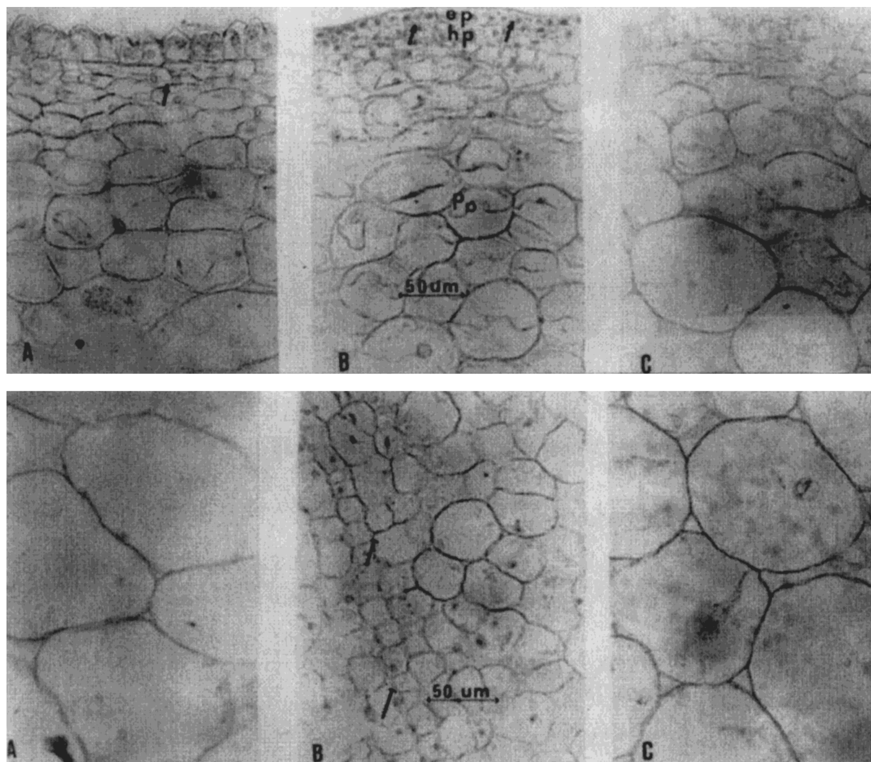

Fig. 3. Transection through tomato fruit (6 days after anthesis) showing the external (*upper*) and internal mesocarp (*lower*) layers as affected by previous flower application with H_2O as control (*A*), 100 ppm of ethrel (*B*), or 0.5 mM AOA (*C*) 2 days after anthesis. *ep, hp,* and *Pa* are the abbreviations for epidermis, hypodermis, and parenchymatous cells, respectively; *arrows* indicate cell division.

inner mesocarp tissues, however, the number of cells/mm^2 in ethrel-treated fruits remained significantly higher than in other treatments, but the differences between the control and AOA treatments diminished 15 days after anthesis (Table 2). The outer mesocarp exhibited a higher cell number/mm^2 than the inner mesocarp. On the other hand, CE could be observed easily as early as 6 days after anthesis with AOA treatment, and it was more pronounced in the internal than in the external mesocarp compared with that of the control and ethrel-treated ones (Fig. 3). In addition, the AOA-treated fruits showed the highest level of CE, in both the outer and inner mesocarp, followed by control, and the lowest level occurred with the application of ethrel as early as 6 days after anthesis (Table 2).

In terms of ethylene production, tomato fruits produced their highest ethylene level at the stage of fruit set (2 days after anthesis). At this stage, fruits of ethrel-treated flowers produced a higher ethylene level compared with that of the control (Fig. 5). Fruits of AOA-treated flowers, however, produced the lowest ethylene level (Fig. 5). Shortly after anthesis, ethylene production decreased to its basal level (the lowest constant level). Fruits of AOA-treated flowers were the first to reach their basal level of ethylene production because the time required was only 6 days after anthesis. Fruits of control and ethrel-treated flowers, however, reached their basal level, which was comparable to that of the AOA-treated flowers, 10 and 18 days after anthesis, respectively. In contrast to ethylene, the tomato fruit ACC content showed a gradual but significant increase during the early period of growth. This increase was noticed for a period of 10 days after anthesis in the fruits of ethrel treatment but only for 8 days in the fruits of other treatments (Fig. 6). This was followed by a sharp decrease in ACC to the lowest level when fruits of AOA, H_2O, and ethrel treatments reached their ACC basal level 10, 12, and 18 days after anthesis, respectively (Fig. 6). At the age of 40 days after anthesis, however, both ethylene and ACC levels restored their significant increases following the same significant increase order found with AOA, H_2O, and ethrel treatments during the early stages of fruit development (Figs. 5 and 6).

Second Trial

When tomato plants received AOA, H_2O, or ethrel treatment only 1 week after flower anthesis of the first cluster, fruits of AOA-treated plants reached their harvest peak 4 and 12 days earlier than that of control or ethrel-treated plants, respectively (Fig. 7). Furthermore, the fruit yield of AOA-treated plants was significantly higher than that of other treatments only during the early harvests. An opposite trend, however, was obtained in the late harvests when ethrel-treated plants became superior in their yield to those of control, and both were significantly higher than AOA-treated plants (Fig. 7). In

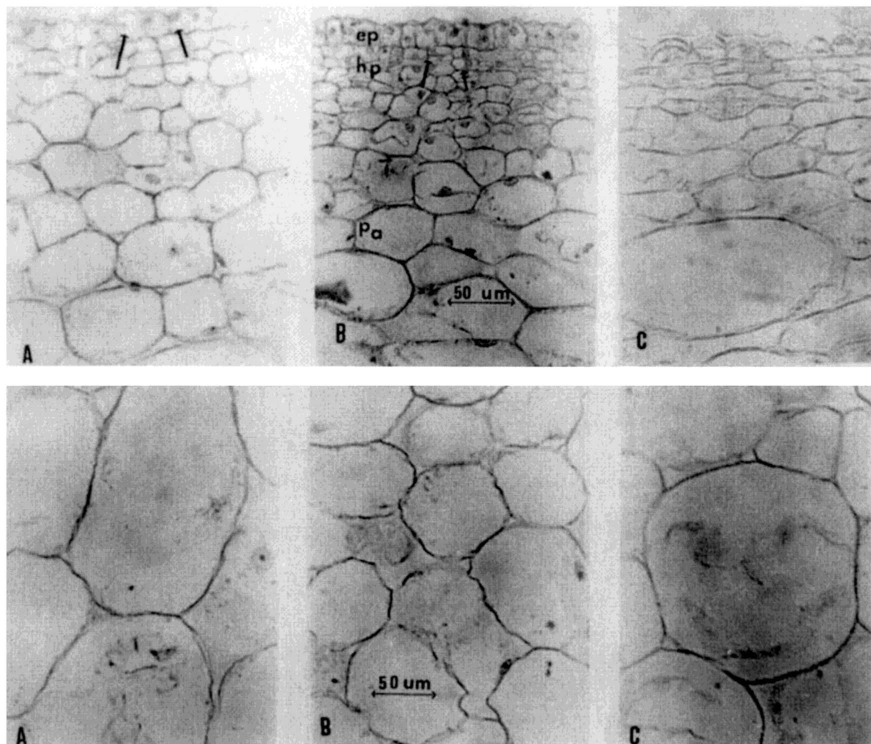

Fig. 4. Transection through tomato fruit (9 days after anthesis) showing the external (*upper*) and internal mesocarp (*lower*) layers as affected by previous flower application with H_2O as control (*A*), 100 ppm of ethrel (*B*), or 0.5 mM AOA (*C*) 2 days after anthesis. *ep, hp,* and *Pa* are the abbreviations of epidermis, hypodermis, and parenchymatous cells, respectively; *arrows* indicate cell division.

Table 2. Effect of flower treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 2 days after anthesis on tomato fruit mesocarp cell number and elongation during early stages of fruit growth and development.

Days after anthesis	Cell number/mm^2			Cell length (μm)		
	Control	Ethrel	AOA	Control	Ethrel	AOA
External mesocarp						
6	1,333 b	1,867 a	1,035 c	53 b	49 b	64 a
9	1,261 b	1,459 a	815 c	64 b	53 c	87 a
12	898 b	1,219 a	658 c	90 b	74 c	104 a
15	702 b	873 a	533 c	105 b	94 c	127 a
18	549 b	733 a	455 b	135 b	108 c	148 a
Internal mesocarp						
6	428 b	973 a	78 c	70 b	34 c	114 a
9	141 b	235 a	47 c	128 b	79 c	192 a
12	92 b	141 a	42 c	213 b	143 c	241 a
15	45 b	110 a	39 b	249 b	206 c	285 a
18	24 b	78 a	25 b	288 b	264 c	324 a

Means within each row in each parameter followed by the same letter are not statistically different at the 5% level.

addition, no significant differences were found among all treatments in terms of flower or fruit abscission, which supports the results obtained during the first trial. Similarly, no significant differences among treatments were found in terms of the fruit number/plant. The fruit average weight, however, was reduced significantly with the application of AOA but increased markedly with the application of ethrel compared with that of the control (Fig. 8). Fruit yield followed the same pattern of fruit average weight because the application of AOA reduced the tomato fruit yield by about 7%, but a 15% increase was obtained with the application of ethrel (Fig. 8).

Discussion

In a preliminary experiment conducted earlier in this work, it was found that applying ethrel to tomato flowers 2 days after anthesis at concentrations higher than 100 ppm (i.e. 300 ppm or more) induced flower abscission, whereas concentrations of 50 ppm or less had no impact on fruit growth as measured by fruit diameter and fresh weight. Applying 100 ppm, however, proved to be the best ethrel concentration for eliminating flower abscission and shifting the fruit growth pattern. It has been reported that ethylene induced CD in rice (Metzer 1984), pine (Barker 1979), and potato tubers (Ilker et al. 1977), but an opposite result was obtained by Apelbaum and Burg (1972) in pea apex and its root meristem. On the other hand, Maxie & Crane (1968) reported that ethylene inhibited CD and promoted CE in fig fruit. Therefore to test the impact of ethylene on CD and CE as well as on the transition phase between both developmental processes, tomato flowers were sprayed at fruit set (2 days after anthesis as reported previously by El-Beltagy et al. 1976) with 100 ppm of ethrel, 0.5 mM AOA, and H_2O (as

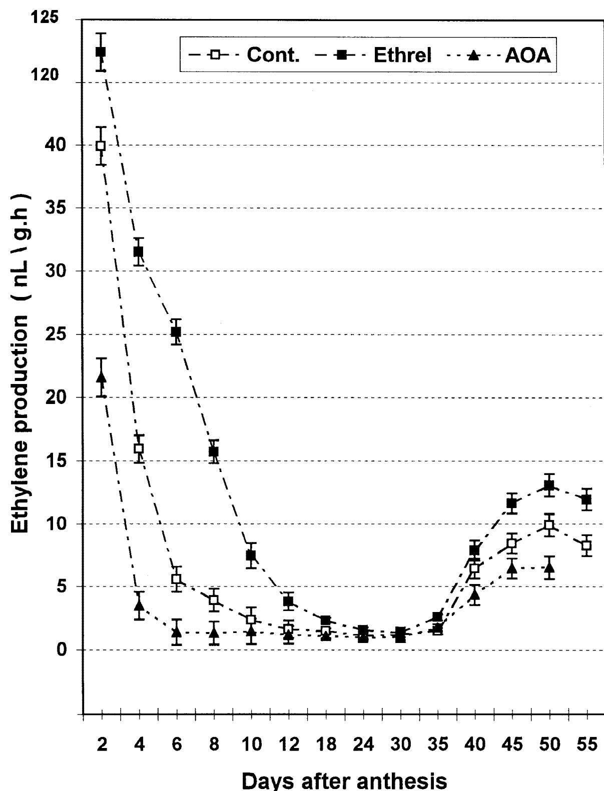

Fig. 5. Effect of flower treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 2 days after anthesis on ethylene production by tomato fruit during growth and development. LSD values at each sampling date are shown as *vertical bars* at the 5% level.

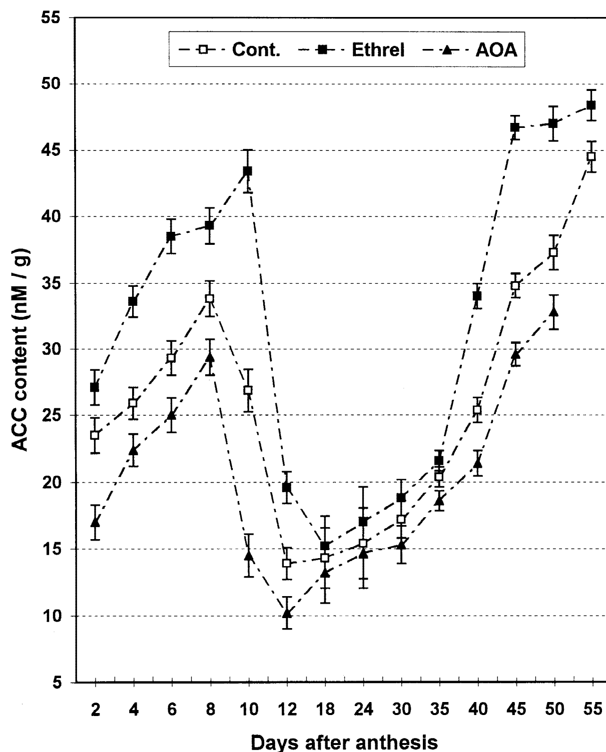

Fig. 6. Effect of flower treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 2 days after anthesis on ACC content in tomato fruit during growth and development. LSD values at each sampling date are shown as *vertical bars* at the 5% level.

control). AOA is known for its inhibition of ethylene biosynthesis by inhibiting ACC synthesis (Yang 1980). It has also been found that 0.5 mM is the most suitable AOA concentration to be used for inhibiting ethylene biosynthesis in tomato fruits (Atta-Aly 1992). Silver ion is used widely for antiethylene action. To increase its efficiency, silver ion has to be fed through the plant or organ vascular tissues because most of the flowers were abscised when silver was applied foliarly (Atta-Aly 1988, Atta-Aly et al. 1987). In this work, therefore, inhibiting ethylene biosynthesis with AOA is preferred to avoid flower abscission.

According to the growth parameters measured during the early stages of tomato fruit growth and development, it was found that the fruit of AOA-, H_2O-, and ethrel-treated flowers followed a significant descending order in their growth parameters measured as fruit diameter (Table 1) and fruit fresh and dry weights (Figs. 1 and 2). This significant descending order of growth existed early and lasted up to 24 days after anthesis. This was strongly established through the significant increase in fruit cell size obtained with the application of AOA and measured as cell elongation in the fruit outer and inner mesocarp tissues (Table 2). It has been reported that the early period of tomato fruit slow growth is dominated by CD (Davies and Cooking 1965, Gillaspy et al. 1993), whereas the fruit rapid growth period is caused entirely by CE (Iwahori 1967, Nitsch and Nitsch 1961). In contrast to the parameters of fruit and cell growth described above, fruits of ethrel-treated flowers showed the most significant CD activity followed by control and then fruits of AOA-treated flowers (Figs. 3 and 4). This was true for the outer mesocarp tissue. In the inner mesocarp tissue, however, CD was terminated 6 days after anthesis in treatments other than ethrel (Fig. 3), which prolonged the period of CD by about 3 additional days (Fig. 4). In contrast to the results obtained by Maxie and Crane (1968) on fig fruits, which reported that ethylene inhibited CD and promoted CE, AOA treatment shortened the period of CD and accelerated fruit transition to CE (Figs. 3 and 4). This may be the result of the differences between tomato and fig fruits in their response to their basal level of ethylene. The significant increase in mesocarp cells number obtained with an early application of ethrel (Table 2) strongly indicated that ethylene induced CD and delayed the transition phase to CE by prolonging the period of CD. This was also emphasized by the opposite trend obtained with the application of AOA (Table

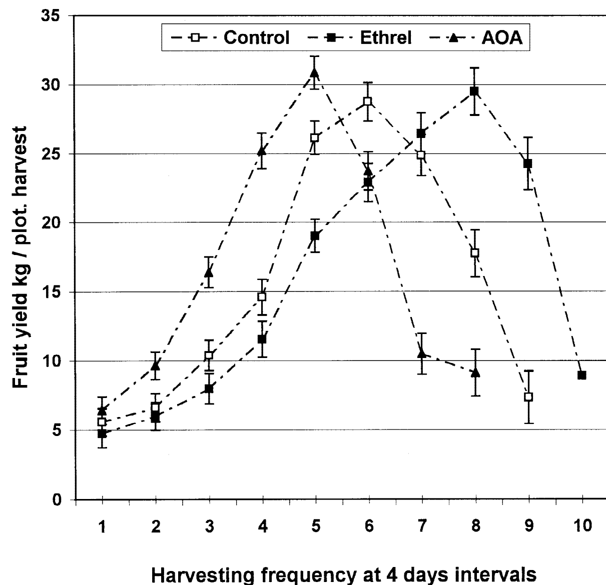

Fig. 7. Effect of plant treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 1 week after flower anthesis of the first cluster on tomato fruit ripening and yield/plot (42 m^2) at the subsequent harvests. LSD values at each harvest are shown as *vertical bars* at the 5% level.

2 and Figs. 3 and 4). This result was also supported by measuring the ethylene emanating from these fruits. In all treatments, the onset of the fruit rapid growth period (Tables 1 and Figs. 1 and 2) as well as the significant increase in CE (Table 2) and the near termination of CD (Figs. 3 and 4) proved to coincide with the time of the ethylene drop to its basal level (Fig. 5). It was suggested by El-Beltagy et al. (1976) that ethylene induced the transition from CD to CE during the early stages of tomato fruit development by inhibiting the first and promoting the second process. In contrast, the data here suggest that such a transition is a negative response to the ethylene level produced by the fruit. This was based on the fact that inhibiting ethylene biosynthesis with the application of AOA accelerated the fruit transition from CD to CE (Figs. 3 and 4). This early transition phase coincided with the earlier drop in ethylene production to its basal level (Fig. 5) accompanied with the control or the opposite fruit response to the application of ethrel. It is of interest to note that the early drop in ethylene production was accompanied by a significant increase in the fruit ACC content (Figs. 5 and 6). Compared with the control, the ethylene drop accelerated with the application of AOA but delayed markedly when ethrel was applied (Fig. 5). This order in ethylene drop was also found in terms of the fruit ACC content when the fruit passed 8 and 10 days after anthesis and after an initial period of substantial increase (Fig. 6). The higher ethylene and ACC levels noticed during the early stage of fruit development with the application of ethrel compared with those of control or AOA-treated ones may be caused by the higher levels of the ethylene autocatalysis mechanism (Atta-Aly et al. 1994). On the other hand, the significant increase in the ACC level in all treatments during the early fruit development may be caused by the presence of a natural biological activity regulating ACC conversion to ethylene (i.e. ACC malonization or ACC oxidase-reduced activity) to allow the natural fruit transition from CD to CE, which may be altered by the early application of ethrel.

Differences in the parameters of fruit growth obtained with AOA, H_2O, and ethrel at fruit set (i.e. fruit fresh and dry weights as well as diameter) disappeared 30 days after anthesis (Table 1 and Figs. 1 and 2). Furthermore, the descending order in fruit growth noticed with AOA, H_2O, and ethrel treatments, respectively, as mentioned, during the first 4 weeks after anthesis was totally reversed to an ascending order 35 days after anthesis until fruit reached the red-ripe stage. Therefore, delaying or enhancing the transition phase from CD to CE in tomato fruit with the application of AOA or ethrel, respectively, strongly affected fruit growth pattern toward maturation.

Based on fruit response to a C_2H_4 releaser or inhibitor applied at fruit set, fruit growth, therefore, can be divided into two periods of response. First is the period when fruit growth responds negatively to C_2H_4, which occurs after fruit set up to 30 days after anthesis. Second is the period of positive growth response to C_2H_4 which occurs 30 days after anthesis until fruit maturation. During the first period of growth, inhibiting the fruit C_2H_4 production with AOA application at fruit set shortens the period of CD and accelerates the CE process. This treatment results in higher rates of fruit growth only during the first period of growth as a result of early cell expansion; an opposite trend is obtained with the application of ethrel. During the second period of growth, however, fruits of AOA-treated flowers become limited in their growth (Table 1 and Figs. 1 and 2) and cell number (Table 2) because of the CD short period (Figs. 3 and 4). In contrast, fruits of ethrel-treated flowers pass through a longer period of CD and produce larger fruits as they reach maturation or the red-ripe stage. It was also evident, based on the data presented in Table 1 and Figs. 1 and 2, that the application of ethrel at fruit set produces fruits larger in diameter and heavier in fresh weight than those of AOA-treated flowers by about 19% and 52%, respectively. These values are reduced to 8% and 34%, respectively but are still highly significant compared with those of control.

It seems that there is a positive correlation between the period that fruit spends in CD and the days to reach the red-ripe stage because extending the period of CD, by the application of ethrel, delayed fruit ripening by about 15 days more than those of AOA-treated flowers and by about 10 days compared with control fruits (Table 1). This may also indicate that the application of ethrel at

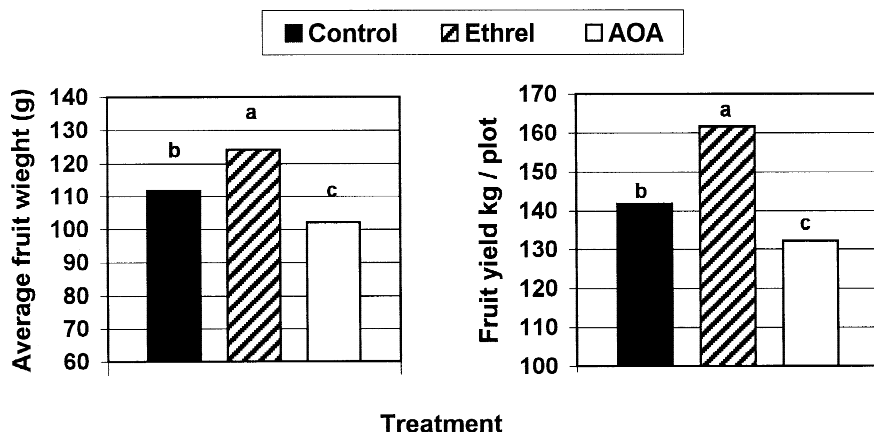

Fig. 8. Effect of tomato plant treatment with H_2O (control), ethrel (100 ppm), or AOA (0.5 mM) 1 week after flower anthesis of the first cluster on average fruit weight and fruit yield/plot (42 m^2). Means separation are presented according to the LSD test at the 5% level.

fruit set extends not only the period of CD but also the period of CE and fruit maturation.

Fruits of ethrel-treated flowers had higher levels of C_2H_4 (Fig. 5) and ACC (Fig. 6) than those of control or AOA-treated flowers during the fruit's early and last periods of growth. It has also been suggested by Atta-Aly (1988) that tomato fruit ripens when its sensitivity to C_2H_4 rises to meet its basal C_2H_4 level. Increasing the fruit basal level of C_2H_4 with an early application of ethrel, therefore, may increase the gap between ripening sensitivity and the new basal C_2H_4 level, and this may be the reason behind the ripening delay in the fruits of ethrel-treated flowers and the ripening enhancement of those produced from AOA-treated ones. This was also emphasized by the time required for breaker fruits to reach the red-ripe stage in the ethrel-treated fruits compared with those obtained with the application of AOA (Table 2).

It could be concluded from these results that the application of ethrel at fruit set extends the period of tomato fruit CD and delays the transition phase to CE as well as increasing the fruit size and fresh weight with a pronounced delay in ripening, whereas an opposite trend is obtained with the early inhibition of C_2H_4 production by the application of AOA.

The application of ethrel to tomato flowers proved to be a promising treatment in increasing tomato fruit size and may be the yield with a ripening delay as was found in the first trial. It is also of interest to note that ethrel has been registered internationally for pre- and postharvest use in several fruits including tomatoes (Abeles et al. 1992). It was also found that treating tomato flowers 2 days after anthesis is not practical for commercial treatment. Tomato plants therefore were exposed to the same treatments of the first trial 1 week after flower anthesis of the first cluster. By that time, flowers of the third cluster were close to anthesis stage, and the corolla was opened in the fourth cluster, meaning that the majority of the flowers may benefit from such application.

Ripening acceleration and delay obtained in the first trial were also apparent during the second trial when AOA and ethrel were, respectively, applied to tomato plants but not flowers. This was based on the fact that tomato fruit yield during the early harvests was greater with the application of AOA, but greater superiority was obtained at late harvests with the application of ethrel (Fig. 7). Furthermore, AOA, H_2O, and ethrel treatments showed the same ascending order of the time to harvesting peak found in the first trial (Fig. 7). The increase in the fruit average weight obtained with ethrel (Fig. 8) without affecting flower or fruit abscission as well as fruit number/plant strongly increased the tomato fruit yield of the second trial (Fig. 8). This increase was about 15% over control, whereas a significant reduction of about 7% was obtained with the application of AOA. The yield increase or decrease obtained with ethrel or AOA treatment, respectively, is due entirely to the size variability of the obtained fruits which relies on modulating fruit CD and consequently its growth pattern by modifying the fruit basal ethylene level during the early stages of fruit development.

Based on the results obtained from both trials, it can also be indicated that the application of ethrel during flowering or at the stage of tomato fruit set increases not only fruit yield but also fruit size as one of the fruit major quality factors.

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