ABSTRACT

ORIGIN, NATURE AND MODIFICATION OF THE FLOWERING STIMULUS IN THE TOMATO (LYCOPERSICUM ESCULENTUM)

by Sharad Chintaman Phatak

The origin and nature of the flowering stimulus, and the mechanism of flower formation in the tomato were studied by experimentally manipulating nutritional, environmental, chemical, and other factors. Effects of nutrient deficiency, nitrogen levels, root and top temperatures, carbon dioxide, light intensity, photoperiod, mutilation, exogenous application of chemicals, and reciprocal top-root grafting of diverse flowering types on the formation of the first inflorescence in the tomato were studied.

The tomato plants were exposed to various nutrient deficiencies and to different nitrogen levels. The optimum nitrogen level for the earliest flowering varied with the time of the year, higher quantities of nitrogen were more effectively utilized in the summer. Nutrient element deficiency studies revealed that the initiation of the first inflorescence occurred even in the absence of nutrient elements. Thus, it was concluded that the role of mineral nutrition in tomato flowering is probably an indirect one.

Reciprocal grafts of early and late cultivars, exposure of seedlings to different top and root temperatures, and mutilation of seedlings suggest that the flowering stimulus as well as the inhibitor of the flowering stimulus for the first inflorescence originates in the leaves, and under natural conditions plumule leaves play an important role in determining the initial flowering responses of tomato seedlings as influenced by external factors. Reciprocal top-root grafting of early and late cultivars revealed that flowering was not modified by hypocotyl grafts or grafting above the site of the plumule leaves in the absence of leaves on the rootstocks. Flowering was, however, influenced by the stocks when the scions were grafted above the intact plumule leaves. Exposure of seedlings of several tomato cultivars to different top and root temperatures for 2 to 3 weeks following cotyledon expansion resulted in significant differences in flowering, as indexed by nodes subtending the first inflorescence, by top but not by root temperature. The removal of cotyledons gave inconsistent results while root removal had no effects on flowering. Flowering was, however, modified by the removal of plumule leaves when interacted with the effects of temperature. There were no effects at 50-55°F., a delay at 60-65°F. and earlier flowering on plants at 70-75°F.

Studies with metabolic inhibitors suggest that the flowering stimulus in the tomato is possibly an isoprenoid or a steroid. Single foliage applications of 10^{-3} molar 2-thiouracil, 5-flurouracil and chloramphenicol did not affect flowering. Extended exposure, however, to these metabolic inhibitors at 10^{-5} and $5x10^{-5}$ molar maintained in the solution culture root media resulted in significant delays in flowering with marked suppressions of vegetative growth. One foliar application of 1.25 to 5.0 mg /ml of steroid synthesis inhibitors (SK&F 7732 and SK&F 7997), was sufficient to delay flowering without a reduction in vegetative growth.

Observations on tomato flowering following application of auxins, gibberellins and inhibitors of vegetative growth, and auxin contents of tomato plants at different temperatures, and the gibberellin content at different photoperiods suggested that the auxin-gibberellin balance or relationship modifies the expression of the flowering stimulus for the first inflorescence. Higher, but not super optimal, levels of auxin or auxin-like compounds promote earlier flowering while super optimal gibberellin

levels delay flowering. It is further suggested that modifications in the auxin-gibberellin levels may be the pathway by which certain environmental factors exert an influence on the flowering behavior.

Induction of anther development on flowers of a stamenless (sl_1sl_1), tomato mutant following GA_3 application through the solution culture root medium suggested that the gibberellins are functional in the development of the microgametophyte within the developing flower parts.

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By

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INTRODUCTION

Flower formation is a phenomenon of great significance in crop production. Any factor which regulates flowering in higher plants is concerned mainly with induced modifications of meristems. Flowering in many plants is dependent on environmental factors that in turn are easily controlled experimentally. Of these factors photoperiod, or the daily length of illumination is perhaps the best known and one of the easiest to manipulate. Thus, photoperiodic control of floral induction in long and short day plants has been extensively studied. Very little is known, however, about the mechanism and physiology of flowering of a large group of plants which are not dramatically responsive to photoperiod. These are commonly classified as day neutral. The tomato is in this category.

The environment during early growth of the tomato plant is important in the initiation of the first flower cluster. This inflorescence is formed within a few days following cotyledon expansion. The number of leaves subtending the first inflorescence is reduced when the plants, during this period of floral initiation, are grown at low temperatures, high light intensity, and short days (19, 20, 34, 42, 43, 76, 77, 93, 94, 100, 101, 132)

Up to the first 2 to 3 weeks following cotyledon expansion a tomato plant consists of roots, cotyledons, a stem, expanding plumule leaves, and an apical meristem. Elucidation of the possible role of each of these plant parts as the origin for the site of the flowering stimulus for the first inflorescence in the tomato was the first objective. This was followed by studies of factors affecting flowering, the flowering mechanism, and the chemical nature of the flowering stimulus. Limited observations are also included as to the effects of naturally occurring plant growth substances on the development of specific floral parts.

These studies are of significance not only with respect to fundamental aspects of the physiology of flowering in the tomato, a plant classically, perhaps erroneously (132) considered as day neutral in its flowering behavior, but should also be of practical value in tomato crop production.

LITERATURE REVIEW

Inheritance of the Flowering Responses

Flowering of the tomato is controlled by genetic and non-genetic factors. Information on the inheritance of earliness of flowering is fragmentary (32, 39, 91, 96). It has been suggested that flowering as indexed by the time interval from seeding to the first anthesis may be controlled by three or more major gene pairs (39, 91). Recently, however, Honma, Wittwer and Phatak (55) proposed that the two characters, days from seeding to first anthesis and nodes subtending first inflorescence are governed by a single major gene pair, with earliness as dominant. Their observations were different from those of others due to variations in the environmental conditions under which the investigations were conducted. These two characters, days to first anthesis, and number of nodes subtending the first flower cluster, are also subject to non-genetic variation, the details of which will be given in later sections.

The tomato inflorescence is described as a racemose cyme (24, 26, 104). Bouquet (14), however, has pointed out that the inflorescences occur in mixed populations of simple racemes, as well as racemose cymes having dichotomous and polychotomous branching. The simple raceme is determined by

a single dominant gene (30, 77). This dominant character occurs in all common varieties with the exception of Earliana (14). Many non-genetic (environmental) factors alter this dominance, among which are mineral nutrition, light, temperature, humidity, mutiliation, and plant growth substances. The latter include auxins, gibberellins, kinins, and growth inhibitors.

Factors Affecting Tomato Flower Formation

Mineral nutrition - Effects of nitrogen nutrition on flowering and fruiting in the tomato have received much attention since Kraus and Kraybill (65) proposed the concept of the carbohydrate-nitrogen relationship and its control on vegetative and reproductive responses. They concluded that fruitfulness in the tomato plant was dependent on the ratio of carbohydrate to nitrogen, or the C/N ratio. According to Kraus and Kraybill a moderate accumulation of carbohydrates favored flowering and fruiting, whereas if all carbohydrates were used up in new growth, a luxuriant vegetative condition resulted with little reproductive development. This conclusion, in general, was widely accepted and appears to have been valid for the particular conditions of their experiments. One should note, however, that Kraus

and Kraybill were chiefly interested in flower and fruit development, not in flower initiation. They did not record nodes subtending the first inflorescence which is, perhaps, the most objective measurement for flowering in the tomato. A study by Wittwer and Teubner (139) on the tomato does not support the hypothesis that high nitrogen favors vegetative growth at the expense of flowering. On the contrary the highest nitrogen levels in the solution cultures gave the earliest flowering even under optimal temperature conditions. Eguchi, Matsumara and Ashizawa (38) also observed in experiments where nutrient levels were a variable that the earliest flowering in the tomato also occurred at the highest levels of nitrogen and phosphate, with 8 or 9 nodes to the first inflorescence. Nitrogen or phosphate at the lowest levels delayed flowering to the 12th and 13th nodes.

Light - Photoperiodic control of floral induction in long and short day plants has been extensively studied. Little is known, however, of the large group of plants commonly classified as day neutral. The tomato has been considered as a day neutral and has been listed as the primary example of this class (82). Many others (5, 13, 49, 51, 89) have similarly classified it. It has been further concluded that since the tomato is completely day

length indifferent with respect to flowering, it offers an opportunity to study the purely vegetative effects of photoperiod and its relationship to temperatures (50).

The effect of the light regime upon flowering in tomatoes is now widely known. There has been some difficulty in interpretation relative to the failure of separating the influence of two factors; namely, light intensity and light duration which are partially independent in their effects and therefore should be studied separately. The former is significant through its control of the rate of photosynthesis, and the latter through the mechanism of photoperiodic control which, under certain circumstances, may be influenced by light intensities lower than those at which photosynthesis occurs.

There are many studies wherein high intensity lamps have been used as a supplement to the low light intensities and to extend the short winter photoperiod. This has promoted the development of greenhouse grown tomato plants. The greater length of the day was associated with more photosynthesis and resulted in earlier flowering (1, 72, 73, 119, 120, 128). Photoperiod differences and an enchanced photosynthesis were, however, not separated in these studies.

The effects of photoperiod on tomato flowering, however, has not been completely ignored by researchers. Reinders-Gouwentak (94) extended a basic photoperiod of 7-1/2 or 9 hours of high intensity artificial light with 1-1/2, 3, 4-1/2, or 7-1/2 hours of low intensity light. With each increase in photoperiod above 9 hours there was an increase in the number of leaves to the first inflorescence and in number days to flower initiation. Similar effects were observed by extending the duration of a 12-hour basic photoperiod. On the other hand, with the basic photoperiod of 7-1/2 hours the delay was shown only with the longest extension (7-1/2 hours) and enhancement of flower initiation with those of short extension (4-1/2 hours or less) of light period. Others (22, 95, 138) have similarly reported that exposure of tomato plants, during the critical stage of the formation of the first inflorescence to an extended photoperiod of low light intensity increased the number of leaves subtending the first inflorescence.

These observations led Wittwer (132) to an objective analysis of the photoperiodic behavior of flowering in the tomato. He reported that varieties differing widely in type and earliness responded to a short (9 hours) photoperiod by earlier flowering. This was indexed by the number

of nodes subtending the first inflorescence and the days to first anthesis. From the results obtained he suggested that tomato be classified as a facultative short day plant.

The number of leaves subtending the first inflorescence decreases with an increase in light intensity. This results in earlier flowering (22, 34, 69, 70, 121). Wittwer (132) confirmed the favorable effects of high light intensities. The extent of flower initiation and polychotomy within an inflorescence of the tomato was increased by high light intensities and extra illumination (76, 77, 81, 119, 120).

Photoperiod and light intensity not only influence the "position" of the first inflorescence as to node number and influence polychotomy, but may also modify the development of individual flowers. Under short days and low light intensities, the flower develops an extremely long pistil in relation to the stamens and this interferes with normal self pollination (17, 56, 104). This may be a contributing factor affecting flower abscission.

Effects of unfavorable photoperiodic cycles on the vegetative growth of tomato have been studied by many workers (21, 41, 10, 49, 50, 117, 129). These researchers, however, did not study the flowering response of the tomato to different cycles of light and dark.

Temperature - The response of the tomato plant to temperature is complicated. Roberts (97) suggested that night rather than the day temperature largely determines the response a particular plant makes to temperature. A diurnal fluctuation in temperature, designated as "thermoperiodicity", is of paramount importance in the development of the tomato plant (124). Went (126) also found a gradual shift of optimal night temperature for growth from 30°C. in the seedling stage to 18°C. in the early fruiting stage. He (126) further observed that the optimum night temperature increased with increased light intensity. Hoffman (53) suggested that for tomatoes, under greenhouse conditions in Ohio, the night temperature be kept around 15 to 16°C. and be raised to 21 to 24°C. during sunny days. Roodenberg (100) and Withrow and Withrow (129) observed that low temperatures prevented or moderated the injury to tomato plants caused by continuous illumination.

Seed vernalization, with the objective of earlier flowering, has given inconsistent results. Burr and Turner (18) found that plants grown from vernalized seeds lagged behind the controls in growth, fruiting, time of ripening, and yield of fruit. Subsequent experiment (118). indicated earlier flowering and fruit ripening from vernalized

seeds provided the seedlings were given 12 days of continuous light. Stier (106) obtained similar results and also observed that the accelerating effect of continuous light was negated by prolonged low temperature (32°F.) exposure. Stier concluded that vernalization was not important for early flowering in the tomato.

An increase in the number of flowers formed in the early inflorescences was observed by Goodall and Bolas (44) on tomato plants grown from vernalized seed, but there was no effect on the time of fruit maturity. Higher yields were obtained from vernalized seed and chilling at 3°C. for 10 to 15 days increased early yields (60, 108). Calvert (21) was unable to reproduce the results obtained by Junges (60). Likewise, Wittwer and Teubner (139) observed no effect from seed vernalization.

Diurnal temperature fluctuations during early seedling growth have striking effects on tomato flowering. Plants grown at 78°F. day and 55°F. night temperatures produced inflorescences that were more branched and had a greater number of flowers than plants at a lower day temperature accompanied by a higher night temperature (124, 125, 126, 127). Verkerk (119) suggested that temperature fluctuations favored earliness and increased yield. However, he observed that a difference of 10°F. or more between day and night retarded growth.

Lewis (77) reported that the temperature sensitive period for tomato seedlings with respect to flower number in the first inflorescence is shortly after cotyledon expansion. Cold exposure during this period will induce a larger number of flowers to form in the first inflorescence. This effect may carry over the fifth inflorescence. A series of papers from the John Innes Horticultural Institute (69, 70, 71) has reported a decrease in nodes to first inflorescence accompanied by an increase in flower number in tomato plants exposed to low temperatures (55°F.) at this seedling stage.

Wittwer and Teubner (136, 137, 138, 139) and Kurki and Wittwer (68) reported that the number of nodes subtending the first inflorescence was less and the number of flowers in the first cluster greater when tomato seedlings were exposed for 2 to 3 weeks following cotyledon expansion to a temperature of 40-55°F., as compared with the plants grown at 65-70°F. Further conclusions indicate no difference in the effectiveness of the cold treatment between day and night exposure. It was not the pattern of cold exposure but the accumulation of cold that determined the effectiveness of the treatment. This is in agreement with the conclusions of Calvert (19) and Went (126). Others (40, 58, 59, 67, 100, 101) have obtained similar results on tomato flowering by exposure of seedlings to lower temperatures. Howlett (57),

however, observed no significant differences in the number of flowers in the first four clusters following three weeks of cold exposure. This may have been related to the variety WR-7 (pink glove) used in the trials which apparently was less sensitive to the cold treatment.

There is a high correlation between the number of flowers in the first inflorescence and early yield (136) as well as the number of nodes to first cluster and days to the first anthesis (55, 78).

Low root temperatures also increase flower number in the first inflorescence (112). Tomato plants exposed to a cool soil temperature of 57 to 61°F. gave an increased yield (20) which may have been from a greater number of flowers in the earlier inflorescences.

The development of floral parts is also affected by the temperature. At night temperatures of 54 to 59°F., tomato flowers showed phyllody of the calyx and had a greater tendency to fasciate (124). High temperatures accompanied by low humidity caused styles to elongate abnormally (105). Self pollination under such conditions failed because the styles elongated several days earlier than the anthers dehisced.

Moisture supply - Availability of moisture has an effect on flowering, and low temperatures were effective only under optimum moisture supply. Fukushima and Masui (40) observed that leaf number to first inflorescence was increased by dry soil conditions during the first two weeks following cotyledon expansions and was not affected by night temperatures if moisture was limiting. Leaf number, however, was increased by high night temperatures under optimum soil moisture conditions.

Mutilation - Seedling mutilation alone and in combination with various temperatures invariably modified the flowering behavior of the tomato. DeZeeuw (34), 35) suggested that young expanding leaves exerted an inhibitory effect on flowering in tomato. The removal of these young expanding leaves advanced flower initiation and hastened the first anthesis. The number of flowers in the inflorescences was also increased. Shen (103) confirmed these findings but reported no difference in the number of nodes subtending the first inflorescence from defoliation. Heinze (48) and Leopold and Lam (75) observed an increase in the number of flowers in the first cluster and a decrease in time to the first anthesis following the removal of young expanding leaves for several varieties varying in earliness of fruiting.

This effect observed by Leopold and Lam (75) was much greater on late than on early varieties.

Hussey (58, 59) has suggested that at high temperatures leaves of the tomato grow vigorously and there is a competition for available assimilates between the apex and the expanding leaves. This delays flowering. He further observed that at high temperatures (25°C.) removal of the plumule leaves hastens flower initiation by 8 days and reduces the number of leaves to first inflorescence from 11.8±0.03 to 9.6±0.05. Cotyledon removal, however, delayed flowering. At low temperatures leaf removal had no significant effect on flowering. Calvert (22) has also reported a delay in flowering upon removal of portions of the cotyledons following germination.

Chemical - Auxins and auxin-like compounds - Auxins have been used in many experiments to alter flowering behavoir. Leopold (74) suggested that they may modify flowering in four ways: (1) earlier flowering from seed treatment; (2) promotion of flowering following plant treatment; (3) delay in flowering, and (4) altering flowering morphology.

Inconsistent results have been obtained with auxin treatment of tomato seeds. Cholodny (23) reported a

promotion of flowering. Thimann and Lane (1938) and Stier and DuBuy (107) observed a hastening of anthesis and an increase in the flower number following treatment of tomato seed with NAA and IAA at 100 ppm. Both earlier flowering and an accelerated vegetative development on IAA treated plants was reported by Tang and Loo (109). Barton (6) observed no beneficial effects from auxin treatment of tomato seeds.

The number of flower buds in the first inflorescence may be increased by treating the young seedlings with indoleacetic acid (111, 135). Flowering as indexed by nodes subtending the first inflorescence, however, was not affected by treatment with indoleacetic acid (135). Triiodobenzoic acid (TIBA) which was considered an auxin (82), and anti-auxin (4), and an auxin synergiest (115), increased the number of flower primordia in tomato (45, 46, 47, 142, 143). Alpha (2-naphthoxy) phenylacetic acid produced responses on the tomato plant similar to those of TIBA (86). TIBA, however, was quantitatively more active than alpha (2-naphthoxy) phenylacetic acid in respect to flower number in the first inflorescence. An increase in flower number

was observed subsequent to the application of N-m-tolylphthalamic acid and other N-aryl-phthalamic acids (103, 110, 111, 112, 113, 114). Under environmental conditions not favorable for flower formation the effects of N-m-tolylphthalamic acid on flower number was reflected in more fruit and noticeably higher yields of greenhouse tomatoes (110, 112).

Gibberellins - Flowering of gibberellin treated tomato plants is delayed as indexed by nodes to the first cluster. There are fewer flowers in the first cluster. Days from seeding to anthesis, however, did not differ because growth was accelerated in the treated plants (15, 16, 131).

Kinins - Flowering was delayed in tomato plants following exposure of the seedlings to various concentrations of kinetin maintained in the solution culture root media (135). This delay in flowering was reflected by an increase in the number of nodes to the first flower cluster and days to the first anthesis. Vegetative growth was also inhibited proportionately as the concentration of kinetin was increased from 10^{-8} to 10^{-5}M.

Growth Inhibitors - Many chemicals which inhibit vegetative growth also affect flowering in the tomato.

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2-Chloroethyltrimethylammonium chloride (CCC) and related compounds inhibit vegetative extension and at the same time promote earlier flowering. The time to first anthesis was reduced and the first inflorescence was formed one node earlier in a midwinter tomato crop (131, 140, 141). 2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylatemethyl chloride (Amo-1618) had no effect on tomato flowering, while maleic hydrazide and 2,4-dichlorobenzyl-trimethyl phosphonium chloride (phosphon D) delayed anthesis and increased the number of nodes subtending the first inflorescence (140).

GENERAL METHODS

Plant material - Several homozygous tomato cultivars, including F_1 hybrids, were used. The majority of the experiments were conducted with the variety Michigan-Ohio Hybrid (Wittwer, 130) supplied by Roy Burghart, Eureka Greenhouse, Greenville, Michigan. Tuckcross O was supplied by Joseph Harris Seed Company. Seeds of the remaining varieties were obtained from different sources and multiplied from individual plant selections during the course of these investigations.

Chemicals - The following chemicals were utilized and obtained from the sources indicated.

Indole-3-acetic acid (IAA), 6-furfurylaminopurine (kinetin) and 1,2-Benzopyrone (coumarin) were purhcased from Nutritional Biochemicals Corporation, Cleveland, Ohio.

2-isopropyl-4-dimethylamino-5-methylphenyl-1-piperidine-carboxylatemethyl chloride (Amo-1618) was supplied by the Rainbow Color and Chemical Co., Box 31, Northridge, California.

N-dimethylaminoscuccinamic acid (B995) and 1,2-dihydro-pyridazine-3,6-dione (Maleic hydrazide (MH-40)) were supplied by the Naugatuck Chemical Co., Naugatuck, Connecticut.

2-chloroethyltrimethylammonium chloride (CCC) was supplied by the American Cyanamide Co., New York 20, New York.

2,4-dichlorobenzyltrimethylphosphonium chloride (phosphon D) was obtained from Virginia-Carolina Chemical Corporation, Richmond, Virginia.

3,4,7-Trihydroxyflavonone (Naringenin) was supplied by California Corporation of Biochemical Research.

Gibberellins and gibberellic acid derivatives were supplied by Merck and Company, Rahway, New Jersey.

5-fluorouracil (5-FU) was supplied by Hoffman-LaRoche, Inc., Nutley 10, New Jersey.

2-Thiouracil (2-TU) was obtained from Nutritional Biochemicals Corporation.

D-threo-N-dichloroacetyl-1-p-nitrophenyl-2-amino-1, 3-propanediol (chloramphenicol) was supplied by Parke, Davis and Co., Detroit 32, Michigan.

SK&F 7732-A_3 (tris (2-dimethylaminoethyl)- phosphate trihydrochloride), SK&F 7997-A_3 (tris(2-diehtylaminoethyl)-phosphate trihydrochloride) was supplied by the Smith, Kline, and French Laboratories in Philadelphia.

Indole-3-acetyl-D,L-aspartic acid was provided as a gift by Dr. Norman E. Good of the Department of Botany and Plant Pathology, Michigan State University.

Anthogenes were obtained through the courtesy of Dr. R. H. Roberts, Professor Emeritus in Horticulture at the University of Wisconsin.

Chemicals were applied as sprays, dips, or plants were immersed for varying time intervals or added directly to the solution culture root media. For the solution culture treatments sufficient quantities of a stock solution were added to achieve the appropriate molar concentrations of the entire volume as indicated. Unless otherwise stated, replacement of evaporated and transpired solutions was by addition of half strength Hoagland solution only (see below).

Plant Growing - Plants were maintained in experimental greenhouses. Both soil and solution cultures were employed. One-half strength of the normal nutrient concentration

(Hoagland and Arnon, 52) was used for the solution cultures. That lost through transpiration and evaporation was replenished by the same solution unless stated otherwise.

A mixture of loam, sand, and muck in equal volume proportions was steam sterilized and used for the soil cultures. Fertility levels in the soil were maintained by watering once a week with a soluble fertilizer solution (1/2 ounce per gallon) containing equal amounts by weight of diammonium phosphate and monopotassium phosphate for the first three weeks following transplanting and 1 ounce per gallon thereafter until the experiment was terminated.

Seedlings were germinated in vermiculite at approximately 65°F. night and 75°F. day temperatures and were transplanted or grafted at cotyledon expansion. Generally seedling plants were exposed to the experimental variables during the first 24 hours after transplanting. Plants were trained to a single stem by periodic removal of the laterals.

The seedlings grown in soil cultures were first transplanted as single plants into three inch peat pots, and after two to three weeks into six inch clay pots of soil. For solution cultures, seedlings were transplanted directly

into one gallon containers of aerated one-half strength nutrient solution. The plants were suspended in the solution over the container by styrofoam corks placed around the hypocotyl. Light was excluded from the roots in the aerated solutions within the gallon glass jars.

Recording observations - Following observations were recorded: number of days from seeding to first anthesis, number of nodes subtending the first inflorescence, and number of flowers in the first inflorescence.

Nodes subtending the first inflorescence were determined by counting from cotyledonary leaves to the nodes formed prior to first inflorescence, excluding the plumule leaves. Node number is an objective measurement of flowering. Unless mentioned otherwise, flowering will be considered as node number subtending the first inflorescence.

Statistical methods - Unless otherwise stated replications consisted of single plants. Conventional experimental designs were followed. CDC 3600 and other facilities of the computor laboratory, Michigan State University were used for analyzing the data. The data were analyzed by appropriate statistical methods (Cochran

and Cox, 25; Panse and Sukhatme, 87) applicable to the experimental designs. Two treatment means were compared as to the least significant differences, and three or more by the Multiple Range Test (Duncan, 37). Significant differences (odds of 19:1) between means are designated by appropriate letter suffixes.

EXPERIMENTAL RESULTS

I. Mineral Nutrition

A. Effect of nutrient element deficiency during floral initiation and subsequent treatment.

Experiment 1

Seed of tomato (cv. Michigan-Ohio Hybrid) was sown on April 26, 1962 and the seedlings at cotyledon expansion were transferred to aerated solution cultures complete with all nutrients, lacking in all nutrients, or minus the single nutrients of nitrogen, phosphorus, potassium, calcium, or magnesium, or minus all the trace elements (Table 1). Half (six) the seedlings were transferred to one-half strength Hoagland solution after exposure to these nutrient treatments for three weeks (fig. 1). The remainder were planted in six inch pots of soil. The greenhouse temperatures were approximately 70°F., which varied with the prevailing outdoor temperatures. The experiment was terminated after all the plants flowered.

The results (Table 1) show that the plants grown in cultures that were minus in all nutrients and minus in magnesium flowered significantly later as indexed by

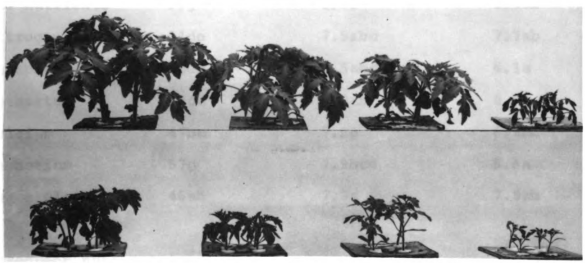

Figure 1. Vegetative growth of the tomato seedlings after a three week exposure to nutrient element deficiencies.

Top (L to R): Complete nutrient, -trace elements, -Ca, -Mg.

Bottom (L to R): -K, -P, -N, -all nutrients.

Table 1. Effects of nutrient element deficiencies during flower induction and subsequent exposure of roots to soil and solution cultures on the formation of the first inflorescence of the tomato, cv. Michigan-Ohio Hybrid

Initial nutrient treatment	Days to 1st anthesis	Nodes to 1st inflorescence	Flowers in 1st inflorescence
		(Means for 12 plants)	
Complete	44a	7.2a	8.0ab
-All nutrients	59g	8.8e	6.3ab
-Nitrogen	51de	7.5abc	7.7ab
-Phosphorus	53ef	7.5abc	6.1a
-Potassium	49cd	7.3ab	8.9b
-Calcium	47bc	7.2a	6.8ab
-Magnesium	57g	7.9bcd	5.5a
-Trace element	46ab	7.2a	7.8ab
Subsequent nutrient treatment		(Means for 48 plants)	
Solution cultures	49	7.7	8.6b
Soil cultures	52	7.4	5.7a

Means followed by the same letter not different at 5% level.

number of nodes subtending the first inflorescence and number of days to first anthesis. The first anthesis was also delayed significantly by growing plants in solution cultures lacking in nitrogen, phosphorus, potassium or calcium. Number of flowers in the first inflorescence was less on plants grown in the absence of magnesium or phosphorus and in the minus all nutrients cultures (distilled water) as compared with those maintained in minus potassium cultures. Flower number in the first inflorescence was significantly affected by the subsequent root treatment. Number of flowers formed on all the plants transferred from solution to soil cultures was less than those retained in solution cultures. All treatments which delayed flowering also suppressed the vegetative growth (fig. 1).

Experiment 2

Experiment 1 was essentially repeated and seed of the tomato (cv. Michigan-Ohio Hybrid) was sown on April 25, 1963. The seedlings were grown during the three weeks following cotyledon expansion in solution cultures complete with all nutrients, minus in all nutrient, or in solution cultures lacking in nitrogen, phosphorus, potassium, calcium, magnesium, or sulphur (Table 2). Half (six) of the

Figure 2. Development of tomato inflorescences following exposure of seedlings to nutrient element deficiencies during the sensitive period for the floral initiation.

Top (L to R): Complete nutrient, -Trace elements, -Ca, -Mg.
Bottom (L to R): -K, -P, -N, -all nutrients

Table 2. Effects of nutrient element deficiencies during flower induction and subsequent exposure of roots to soil and solution cultures on the formation of the first inflorescence of the tomato, cv. Michigan-Ohio Hybrid

Initial nutrient treatment	Days to 1st anthesis	Nodes to 1st inflorescence	Flowers in 1st inflorescence
		(Means for 12 plants)	
Complete	42a	7.0a	6.5
-All nutrients	58d	8.8e	7.0
-Nitrogen	55d	7.8bc	6.2
-Phosphorus	55d	7.7bc	6.6
-Calcium	47bc	7.3ab	5.8
-Magnesium	56d	7.9bcd	6.6
-Sulphur	43ab	7.3abc	7.8
-Potassium	44ab	7.0a	7.8
Subsequent nutrient treatment			
		(Means for 48 plants)	
Solution cultures	49	7.7	8.1b
Soil cultures	51	7.5	5.5a

Means followed by the same letters not different at 5% level.

plants were then transferred to soil in pots and the remainder to solution cultures complete with all known essential mineral nutrients.

Flowering of plants in solution cultures lacking in all nutrients, nitrogen, phosphorus, or magnesium was significantly delayed as indexed by the number of days to first anthesis and number nodes subtending the first inflorescence (Table 2). The first anthesis was also delayed on plants grown in solution cultures lacking in calcium. Number of flowers in the first inflorescence was not affected by the initial nutrient deficiency treatments but was significantly altered by the subsequent treatments of solution versus soil cultures. Number of flowers formed on all plants transferred from solution cultures to soil cultures was once again less than those retained in solution cultures. Treatments which delayed flowering also suppressed vegetative growth.

Experiment 3

Experiment 2 was duplicated and paralleled with the cultivar, Farthest North (Table 3). Flowering as indexed by the number of days to first anthesis and number of nodes subtending the first inflorescence, was delayed on all plants grown in cultures of all mineral nutrients

Table 3. Effects of nutrient element deficiencies during flower induction and subsequent exposure of roots to soil and solution cultures on the formation of the first inflorescence of the tomato, cv. Farthest North

Initial nutrient treatment	Days to 1st anthesis	Nodes to 1st inflorescence	Flowers in 1st inflorescence
		(Means for 12 plants)	
Complete	39a	7.1e	11.1
-All nutrient	57f	7.1e	11.0
-Nitrogen	47cd	6.2bcd	11.5
-Phosphorus	48d	6.1bcd	10.0
-Potassium	43bc	5.9abc	11.5
-Calcium	44bc	5.9abc	10.1
-Magnesium	53e	6.8cde	9.5
-Sulphur	41ab	5.7ab	10.5
Subsequent nutrient treatment			
		(Means for 48 plants)	
Solution cultures	44	6.0	12.4b
Soil cultures	48	6.2	9.8a

Means followed by the same letter not different at 5% level.

and solution cultures lacking in nitrogen, phosphorus or magnesium. First anthesis was also delayed on plants in solution cultures lacking in potassium or calcium. Initial nutrient element deficiencies had no significant effect on the number of flowers in the first inflorescence. Plants subsequently maintained in solution cultures, however, had a greater number of flowers in the first inflorescence than those transferred to soil cultures.

Results of these three experiments suggest a consistent delay in flowering due to the lack of all nutrients and of magnesium during the period of the initiation of the first inflorescence. A significant delay in the flowering of plants from both cultivars grown in solution cultures lacking in nitrogen and phosphorus was observed in 1963. This delay in flowering was associated with a marked suppression of negetative growth. Flowers appear to have been initiated in the absence of added major nutrient elements in the root media.

B. Effect of nitrogen nutrition.

Experiment 1. Nitrogen levels and root temperature effects.

Seed of Michigan-Ohio Hybrid was sown on February 28, 1963. The seedlings were transplanted on March 7 into

solution cultures containing 0, 55, 110, or 220 ppm of nitrogen. The solution culture containers were then placed in the temperature controlled water tanks. Root (tank) temperatures of 70° and 60°F. were duplicated. There were two replicates of each nitrogen level containing two plants each within each temperature replication. In the analysis of the data each container with two plants was handled as a single replicate. After three weeks exposure to the various nitrogen levels and root temperatures the seedlings were transplanted into soil in 6 inch clay pots. The results are summarized in Table 4.

With increase (Table 4) in nitrogen level flowering was earlier as indexed by number of days to first anthesis and number of nodes to the first inflorescence. The highest nitrogen level (220 ppm) resulted in earliest flowering. The nitrogen effect was not modified by root temperature. Flower number in the first inflorescence was not significantly affected by the nitrogen level in the root medium. Root temperature had little effect on flowering. There were no significant interactions between root temperatures and nitrogen levels.

Table 4. Flowering of the tomato (cv. Michigan-Ohio Hybrid) following exposure of the seedlings to different nitrogen levels and root temperatures for three weeks following cotyledon expansion. (Spring 1963).

Nitrogen (ppm)	Days to 1st anthesis			Nodes to 1st cluster			Flowers in 1st cluster		
	70°F.	60°F	Means	70°F.	60°F.	Means	70°F.	60°F.	Means
	(Means for 8 plants)								
0	58b	60c	59.0c	8.9d	8.8c	8.85d	6.8	5.6	6.2
55	55ab	59bc	57.0bc	8.1bc	8.3bc	8.20bc	6.9	7.1	7.0
110	53a	55ab	54.0ab	7.5ab	7.8ab	7.65ab	6.1	6.3	6.2
220	53a	54a	53.a	7.4a	7.6a	7.50a	5.6	7.0	6.3
Means	55	57		8.0	8.1		6.4	6.5	

Means followed by the same letter not different at 5% level.

Experiment 2. Effect of nitrogen levels and full vs. partial (2/3) sunlight.

Seed of Michigan-Ohio Hybrid tomato was sown on June 20, 1963. Seedlings were transplanted on June 27 into solution cultures with 11, 55, 110, 220, 440, 660, or 880 ppm of nitrogen (Table 5). Sunlight was reduced to 2/3 by shading with muslin. Values in Table 5 constitute those from twelve plants, grown as three - four plant replicates. The treatments within each sunlight level were randomized and each container of four plants were considered as a single replicate. The plants were transferred to soil in 6 inch clay pots on July 8, 1963.

Increase in nitrogen promoted earlier flowering up to 440 ppm (Table 5). Plants at 440 ppm of nitrogen flowered earliest, as indexed by number of days to first anthesis and nodes to first flower cluster. Plants grown at nitrogen levels of 660 and 880 ppm cultures showed typical high nitrogen injury with suppressed vegetative growth. Flowering on these high nitrogen plants was delayed. Mean number of nodes subtending the first inflorescence was less at full sunlight than on plants at 2/3 sunlight. This difference was significant only at

Table 5. Flowering of the tomato (cv. Michigan-Ohio Hybrid) following exposure of seedlings to different nitrogen levels and to partial (2/3) or full sunlight for three weeks following cotyledon expansion. (Summer 1963)

Nitrogen (ppm)	Days to 1st anthesis			Nodes to 1st cluster			Flowers in 1st cluster		
	2/3	Full	Means	2/3	Full	Means	2/3	Full	Means
				(Means of 12 plants)					
11	59	59ab	59.0	7.9cd	8.0d	7.9	5.1	6.2	5.7
55	57	56ab	56.5	7.6abc	7.5abc	7.6	5.7	6.3	6.0
110	56	56.0	56.0	7.5ab	7.5abc	7.5	5.8	6.0	5.9
220	57	55a	56.0	7.5ab	7.2ab	7.4	6.0	6.5	6.3
440	56	55a	55.5	7.4a	7.1a	7.3	6.1	6.4	6.3
660	59	58ab	58.5	8.2d	7.9cd	8.1	6.1	6.0	6.1
880	60	60b	60.0	8.5e	8.1d	8.3	5.8	5.9	5.9
Means	57.7	57.0		7.8	7.5		5.8	6.2	

Means followed by the same letter not different at 5% level.

nitrogen levels of 220 ppm and above. Flower number was not influenced.

Experiment 3. Effect of nitrogen levels maintained throughout the growing period.

Seed of the Michigan-Ohio Hybrid was sown on January 27, 1964 and seedlings were transplanted at cotyledon expansion into solution cultures containing 11, 110, 220, 330, 440, 550, 660 and 880 ppm. The nitrogen levels were replicated three times with two plants in each container (replicate).

The data (Table 6) reveal that during winter when light limits growth any deviation in nitrogen level from 110 ppm significantly delayed flowering as indexed by days to first anthesis and nodes to the first inflorescence. At early growth stages all plants in 220, 330, or 440 ppm nitrogen showed injury and vegetative growth was inhibited. These plants recovered after 2 to 3 weeks perhaps as a result of an increased leaf area and greater photosynthesis. Flower number was not affected. Plants grown at 550 ppm or above did not survive.

Results of the three experiments on nitrogen levels in the root medium and flower formation in the tomato

Table 6

Nitroge (ppm)
11
110
220
330
440
550-880

Means f

Table 6. Effects of different nitrogen levels maintained throughout growing period on flowering of the tomato (cv. Michigan-Ohio Hybrid). (Winter 1964)

Nitrogen (ppm)	Days to 1st anthesis	Node to 1st inflorescence	Flowers in 1st inflorescence
		(Means of 6 plants)	
11	67b	9.2b	6.0
110	59a	8.3a	7.0
220	65b	9.2b	5.2
330	67b	9.4b	5.8
440	66b	9.5b	5.5
550-880	Plants did not survive		

Means followed by the same letter not different at 5% level.

suggest that the optimum level of nitrogen for flowering as well as growth varied with the time of the year. Tomato seedlings during the high light of summer can utilize more nitrogen than during winter when the light limits the normal growth. The observed effects of various nitrogen levels on tomato flowering appears indirect as they reflect an influence on seedling growth rather than direct effects on the flowering process.

II. LIGHT

A. Effects of light intensity and carbon dioxide concentration.

Seeds of tomato cultivars Farthest North, 146j, Michigan-Ohio Hybrid and Pennorange were sown July 25, 1963. The seedlings were transplanted into 3 inch peat pots August 2. Twelve seedlings from each cultivar were exposed to three light intensities (500, 1000, 2000 f.c.) maintained in two different growth chambers. The carbon dioxide concentration in the atmosphere of one of the growth chambers was maintained at 2000 ppm and in the other at the nearer normal level of 350 ppm (Table 7). The levels

Table 7. Flowering of the tomato following exposure of the seedlings to different light intensities and carbon dioxide during the sensitive period for the formation of the first inflorescnce.

Tomato cultivar	(CO_2 (ppm)	Light intensities in foot candles 500	1000	2000	Means
		(Means of 12 plants)			
Days to first anthesis					
Farthest	350	53	46	44	48b
North	2000	50	44	43	46a
146j	350	66	58	50	58b
	2000	63	54	47	55a
Michigan-Ohio	350	62	57	52	57b
Hybrid	2000	59	54	48	54a
Pennorange	350	68	64	61	64b
	2000	65	61	58	61a
Means		61c	55b	50a	
Nodes subtending the first inflorescence					
Farthest	350	7.6	6.1	5.1	6.1b
North	2000	6.6	5.3	5.0	5.6a
146j	350	9.0	7.9	6.8	7.9a
	2000	8.8	7.8	7.0	7.7a
Michigan-Ohio	350	9.4	7.6	6.9	8.0a
Hybrid	2000	9.1	7.4	7.0	7.8a
Pennorange	350	11.0	11.6	10.5	11.0a
	2000	11.5	11.5	10.8	11.3a
Means		9.1c	8.1b	7.4a	
Number of flowers in the first inflorescence					
Farthest	350	9.4	10.3	10.5	10.1
North	2000	9.4	10.1	10.8	10.1
146j	350	15.0	13.3	10.8	13.1
	2000	20.6	15.1	12.4	16.0
Michigan-Ohio	350	5.9	6.1	5.9	6.0
	2000	6.9	6.2	6.1	6.4
Pennorange	350	2.8	2.6	3.4	2.9
	2000	2.8	2.7	3.0	2.8
Means		9.1a	8.3a	7.9a	

Means by the same letter not different at 5% level.

of carbon dioxide were monitored at 12 minute intervals and automatically recorded. After three weeks exposure to the indicated light and carbon dioxide levels the seedlings were transferred to the greenhouse and planted in 6 inch clay pots of soil.

Increase in light intensity induced earlier flowering as indexed by days to first anthesis and number of nodes to first inflorescence in all the cultivars. The number of flowers in the first inflorescence was not affected by light intensity.

All cultivars reached anthesis earlier when exposed to high carbon dioxide level. Farthest North also flowered after fewer nodes when given 2000 ppm of carbon dioxide. The differences in carbon dioxide in the atmosphere did not influence number of flowers in the first inflorescence.

B. Effects of proportional duration of light and dark in 24 and 36 hour cycles.

Seeds of Michigan-Ohio Hybrid, 146j, Manapal, Spartan Pink 10, Ailsa Craig, Farthest North, Indian River, Moneymaker, Hot Set, Pennorange and 1-3 (selection from multiple flower cluster type) cultivars were sown

on November 15, 1963. Seedlings were transplanted in 3 inch peat pots November 29 and eight plants of each cultivar were transferred to each of the two controlled atmosphere chambers. One of the chambers was manually operated at a 36 hour cycle consisting of 15 hours of light and 21 hours of dark, and the other at a 24 hour cycle of 10 hours of light and 14 hours of dark. Thus plants maintained in both the chambers were exposed to approximately the same ratios and amounts of light and dark in total, but for varying durations in a given cycle. Seedlings were exposed to the same light intensities (1500 f.c.) and same temperatures (60-65°F.) in each growth chamber. After 18 days exposure (12 cycles of 36 hours or 18 cycles of 24 hours) the seedlings were transferred to a greenhouse and transplanted into 6 inch pots. Mean values in Table 8 represent eight plants.

The results (Table 8) show that all cultivars exposed to 36 hours cycle flowered later than those exposed to a 24 hour cycle. This was true of both days to anthesis and nodes to the first inflorescence. Number of flowers in the first inflorescence was not affected by the different cycles to which the plants were exposed.

Table 8. Effect of 18 days exposure of seedlings following cotyledon expansion to 24 hour (10 hr. light + 14 hr. dark) cycles and 36 hour (15 hr. light + 21 hr. dark) cycles on the formation of first inflorescence in several tomato cultivars.

Cultivar	Days to 1st anthesis		Nodes to 1st cluster		Flowers in 1st cluster	
	24 hr. cycle	36 hr. cycle	24 hr. cycle	36 hr. cycle	24 hr. cycle	36 hr. cycle
	(Means of 8 plants)					
Michigan-Ohio Hybrid	68	73	6.5	7.6	7.5	6.3
146j	74	80	6.5	7.6	7.1	8.6
Manapal	70	71	7.2	7.5	9.0	7.1
Spartan Pink 10	72	77	6.4	7.5	4.8	5.9
Ailsa Craig	69	73	7.2	8.4	8.3	9.5
Farthest North	65	69	5.5	7.0	12.5	8.9
Indian River	71	73	6.1	6.9	7.1	7.6
Money Maker	70	73	6.5	7.5	7.1	8.0
Hot Set	67	73	6.5	7.4	5.8	6.9
Pennorange	79	84	12.2	12.6	3.0	2.9
1-3 (multiple flower cluster type)	76	77	8.6	8.9	29.9	30.9
Means	71a	75b	7.2a	8.1b	9.2	9.3

Means followed by the same letter not different at 5% level.

Significant differences were observed between the cultivars in days to first anthesis, nodes subtending first inflorescence, and number of flowers in the first inflorescence.

III. TEMPERATURE

A. Effects of continuous and three week exposure of seedlings to different root temperatures.

Michigan-Ohio Hybrid seed was sown on June 29, 1962. Seedlings were transferred on July 8 to solution culture containers placed in temperature control water tanks within a greenhouse. The root (tank) temperatures were 54-58, 58-62, 62-66, and 66-70 degrees F. There were 20 plants in each temperature tank. Ten of these plants were transferred to soil in 6 inch pots after three weeks. The remainder were retained at the same root temperatures in the solution cultures. The effects on flowering are summarized in Table 9.

With decrease in root temperature there was a progressively delay in flowering (Table 9). The number of nodes subtending the first inflorescence was increased on plants maintained

Table 9. Effects of continuous and three weeks exposure of seedlings, following cotyledon expansion, to different root temperatures on the flowering of the tomato, cv. Michigan-Ohio Hybrid.

Root temperature (°F.)	Days to 1st anthesis			Nodes to 1st cluster			Flowers in 1st cluster		
	Cont.	3 weeks	Means	Cont.	3 weeks	Means	Cont.	3 weeks	Means
				(Means of 10 plants)					
66-70	45	50	48a	7.6	7.6	7.6a	6.3	6.1	6.2
62-66	49	53	52ab	8.6	8.4	8.5b	6.8	6.6	6.7
58-62	55	57	56bc	9.0	8.8	8.9b	8.0	7.7	7.9
54-58	58	60	59c	8.1	9.0	8.6b	8.2	8.0	8.1
Means	52	55		8.3	8.5		7.3	7.1	

Means followed by the same letter not different at 5% level.

at temperatures lower than 66°-70°F. Number of flowers in the first inflorescence was markedly (but not significantly) affected by root temperature. These effects were, however, not consistent when continuous and three week's exposure to these root temperatures were considered separately. This inconsistency may be following wider fluctuation in top temperatures because of lesser degree of control over greenhouse temperatures during the summer. To avoid this fluctuation in air temperature further experiments were conducted in controlled atmosphere chambers.

B. Effects of differential exposure of tops and roots of seedlings.

Michigan-Ohio Hybrid seed was sown on September 1, 1963. The seedlings were transferred on September 10 to solution culture containers maintained at two (60-65° and 50-55°F.) root (tank) temperatures within two plant growth chambers. One growth chamber was held at 60-65°F. and the other at 50-55°F. Light intensities at the plant levels in both chambers were 1200 f.c. A 24 hour cycle of 10 hours of light and 14 hours of dark was established. There were ten

plants for each temperature combination. Seedlings were transferred to pots of soil in a greenhouse after three weeks exposure to these temperatures.

The experiment was repeated with two other cultivars (Farthest North and 146j). Seedlings from seed sown October 1, 1963, were manipulated as described above for the Michigan-Ohio Hybrid.

The results for the three cultivars are summarized in Table 10. Flowering as indexed by nodes subtending the first inflorescence was significantly affected by top but not by the root temperatures. Low (50-55°F.) top temperatures induced the first flower cluster to form at a significantly lower node than high (60-65°F.) top temperatures.

The effect of top and root temperatures on numbers of flowers in the first inflorescence, were exactly opposite to those obtained for nodes subtending the first inflorescence. Here flower numbers in the first inflorescence were significantly increased by low (50-55°F.) root temperatures but not by low top temperatures.

These studies on flower formation in the tomato in relation to temperature suggest that top (air) temperatures determine the position of the first flower cluster as to

Table 10. Effects of differential exposure of roots and tops of seedlings during sensitive period on the formation of the first inflorescence of the tomato, cv. 146j, Farthest North (FN) and Michigan-Ohio Hybrid (MO).

Tomato Cultivar	Root temp. (°F.)	Top temperature (°F.) 60-65	50-55	Means
		(Means of 10 plants)		
Number of nodes				
146j	60-65	8.0	7.2	7.6
	50-55	7.9	7.2	7.6
	Means	8.0b	7.2a	
FN	60-65	5.7	4.5	5.6
	50-55	5.5	4.7	5.6
	Means	5.6b	4.6a	
MO	60-65	7.1	6.5	6.8
	50-55	6.9	6.3	6.6
	Means	7.0b	6.4a	
Number of flowers				
146j	60-65	5.8	5.5	5.6a
	50-55	7.8	9.3	8.6b
	Means	6.8	7.4	
FN	60-65	9.3	8.5	8.9a
	50-55	10.5	10.8	10.6b
	Means	9.9	9.5	
MO	60-65	5.2	5.4	5.3a
	50-55	7.8	8.2	8.0b
	Means	6.5	6.8	

Means followed by the same letter not different at 5% level.

node number while root temperatures control the number of flowers in the first inflorescence.

IV. MUTILATION

A. Effects of mutilation of seedlings.

Experiment 1.

Seed (cv. Michigan-Ohio Hybrid) was sown on March 3, 1962. Seedlings were mutilated on March 14 after cotyledon expansion. Treatments included (a) intact plants (controls) and plants with (b) roots or (c) cotyledons removed. The seedlings were transplanted into vermiculite in 3 inch peat pots. There were 8 plants for each treatment. The above experiment was duplicated on the cultivar Farthest North seed of which was sown on June 6, 1962. The seedlings were mutilated on June 15.

A summary of flowering responses for both cultivars is shown in Table 11. Removal of cotyledons significantly delayed flowering in terms of the first anthesis. There was also a delay but not a statistically significant one, in flowering following cotyledon removal as indexed by an increase in the number of nodes subtending the first flower

Table 11. Effects of mutilation of seedlings following cotyledon expansion on the flowering of the tomato, cv. Farthest North (FN) and Michigan-Ohio Hybrid (MO).

Mutilation treatment	Days to 1st anthesis			Nodes to 1st cluster			Flowers in 1st cluster		
	FN	MO	Means	FN	MO	Means	FN	MO	Means
				(Means for 8 plants)					
Control	45	64	55a	5.0	7.8	6.4	12.5	6.5	9.5
Roots removed	47	67	57ab	5.5	7.8	6.7	14.5	7.5	11.0
Cotyledons removed	54	70	62b	5.6	8.5	7.1	9.9	7.3	8.6
Means	48a	67b		5.4a	8.0b		12.3b	7.1a	

Means followed by the same letter not different at 5% level.

cluster for both cultivars. The number of flowers in the first cluster was not influenced by cotyledon removal nor by root removal.

Experiment 2.

Michigan-Ohio Hybrid seed was sown on June 6, 1962. Seedlings were mutilated on June 15. Treatments were: (a) intact plants (controls), (b) roots removed (rerooted), (c) cotyledons removed, (d) one cotyledon removed, (e) 1/4 cotyledons removed, and (f) 1/2 cotyledons removed. There were eight plants for each treatment. Plant growing procedures were same as in Experiment 1 above.

The anthesis was delayed by 8 days when both cotyledons were removed. Number of nodes subtending the first inflorescence and number of flowers in the first cluster were not affected by mutilation of seedlings at cotyledon expansion (Table 12).

In these two mutilation experiments, seedlings grown in the greenhouse were exposed to wide range of fluctuating day to day temperatures. To avoid a differential exposure of seedlings to fluctuating temperatures, seedlings in further experiments were transferred to growth chambers following mutilation and were maintained in a controlled

Table 12. Effects of mutilation following cotyledon expansion on flowering of the tomato, cv. Michigan-Ohio Hybrid

Mutilation treatment	Days to 1st anthesis	Nodes to 1st inflorescence	Flowers in 1st inflorescence
	(Means for 8 plants)		
Control	56a	7.1	6.4
Roots removed	59a	7.0	6.4
Both cotyledons removed	64b	7.2	6.4
One cotyledon removed	56a	7.0	5.8
1/4 cotyledons removed	56a	7.0	5.3
1/2 cotyledons removed	57a	6.9	5.5

environment during the period when flowers for the first inflorescence were initiated.

Experiment 3.

Cultivars Farthest North and 146j were seeded June 29, 1962 and the seedlings mutilated on July 12. There were two treatments, the intact plants (controls), and those with cotyledons removed, with eight plants per treatment for each cultivar. Experimental seedlings were planted in peat pots and moved to a controlled environment chamber. The seedlings were maintained at 65-70°F. and 8 hours of 1500 f.c. light with 16 hours of darkness in a 24 hour cycle. After three weeks, plants were transferred to a greenhouse and moved to 6 inch pots.

Flowering (Table 13) was delayed by cotyledon removal in the 146j cultivar, but not in Farthest North. Days to first anthesis and numbers of flowers in first cluster were not significantly altered by cotyledon removal.

Experiment 4.

The cultivar Michigan-Ohio Hybrid was seeded June 29, 1962. Seedlings were mutilated and transferred to a controlled environment chamber on July 12. Treatments were the same as those for Experiment 2 above (Table 12).

Table 13. Effects of mutilation on flower formation in the tomato cultivars Farthest North (FN) and 146j.

Mutilation Treatment	Days to 1st anthesis			Nodes to 1st inflorescence			Flowers in 1st infloresnce		
	FN	146j	Means	FN	146j	Means	FN	146j	Means
				(Means for 12 plants)					
Control	62	73	67.5a	5.7	7.0	6.4a	11.2	18.5	14.8a
Cotyledons removed	64	78	71.0a	5.8	9.0	7.4b	9.5	16.2	12.8a
Means	63.0a	75.5b		5.7a	8.0b		10.3a	17.3b	

Means followed by the same letter not different at 5% level.

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The results (Table 14) indicate that there was a significant delay in days to first anthesis following removal of both the cotyledons, one cotyledon or 1/2 of both botyledons. No differences were observed in number of nodes subtending first inflorescence and number of flowers in the first cluster when mutilated.

Experiment 5.

Michigan-Ohio Hybrid tomato seed was sown on June 8, 1962 and the newly emerged seedlings were transplated into peat pots on June 14. Treatments included (a) intact plants (controls), (b) plants with cotyledons removed, (c) those with plumule leaves removed, and (d) plants with cotyledons as well as plumule leaves removed. The cotyledons were removed June 15 and plumule leaves were removed June 19 as they became visible. Plants were grown in a greenhouse at night temperatures 65-70°F.

All mutilation treatments (Table 15) delayed flowering. The delay in flowering following removal of only the plumule leaves was greater than the delay caused by the removal of only the cotyledons. Removal of both (cotyledons and plumule leaves) caused delay greater in flowering than removal of either but was not any greater

Table 14. Effect of mutilation of flowering of the Michigan-Ohio Hybrid tomato grown under controlled conditions for three weeks following cotyledon expansion

Mutilation treatment	Days to 1st anthesis	Nodes to 1st inflorescence	Flowers in 1st inflorescence
	(Means for 8 plants)		
a-Control	71a	8.7	6.4
b-Roots removed	74ab	9.0	6.6
c-Both cotyledons removed	78b	9.1	5.9
d-One cotyledon removed	77b	9.0	7.1
e-1/4 cotyledons removed	75ab	9.1	6.1
f-1/2 cotyledons removed	77b	9.0	6.5

Means followed by the same letter not different at 5% level.

Table 15. Effects of mutilation of seedlings on flowering of the tomato, cv. Michigan-Ohio Hybrid

Mutilation treatment	Days to 1st anthesis	Nodes to 1st inflorescence	Flowers in 1st inflorescence
		(Means for 12 plants)	
Control	58a	7.4a	6.4
Cotyledons removed	63b	8.5b	6.0
Plumule leaves removed	67c	9.1e	6.2
Cotyledons and plumule leaves removed	69c	8.9bc	5.4

Means followed by the same letter not different at 5% level.

than the removal of only the plumule leaves. These results suggest that the plumule leaves play an important role in the initiation of the first inflorescence. The numbers of flowers in the first inflorescence were not affected by removal of either cotyledons, plumule leaves, or both.

B. Effect of mutilation, temperature and light intensity.

Seeds of cultivars Farthest North (FN), Michigan-Ohio Hybrid (MO), and Pennorange (PO) were sown on February 13, 1964. Seedlings at cotyledon expansion were transplanted into 3 inch peat pots and moved to controlled environment chambers maintained at 50-55°F. and 70-75°F. on March 3. Light intensities were 400, 1000 and 2000 f.c. and 24 hour cycle (10 hour light and 14 hour darkness). Treatments included the (a) controls (intact plants), (b) plants with cotyledons removed, (c) those with plumule leaves removed, and plants with (d) plumule leaves plus cotyledons removed. There were six plants for each treatment. The seedlings were mutilated on March 9, six days after cotyledon expansion and were moved to the greenhouse on April 7, 1964.

Except for significant delay in anthesis (Table 16) following removal of plumule leaves and cotyledons there

Table 16. Effects of mutilation, temperature, and light intensity on flowering of the tomato cv. Farthest North (FN), Michigan-Ohio Hybrid (MO) and Pennorange (PO).

Mutilation treatment	Temp. °F.	Light intensity in foot candles									Means	Means
		400			1000			2000				
		FN	MO	PO	FN	MO	PO	FN	MO	PO		
		(Means for 6 plants)										
Days to first anthesis												
Control	50-55	78	86	97	69	77	88	63	72	74	78	76.5
	70-75	74	88	96	62	78	89	51	68	72	75	
Cotyledons removed	50-55	89	99	101	74	78	84	65	73	76	82	79.5
	70-75	80	93	99	63	76	81	53	69	75	77	
Plumules removed	50-55	82	89	98	72	76	87	65	71	76	80	77.5
	70-75	75	87	93	65	75	82	54	67	74	75	
Plumules and cotyledons removed	50-55	91	95	112	77	87	96	68	78	81	87	84.5
	70-75	82	93	107	67	88	93	55	76	79	82	
	Means	81a	91b	100c	69a	79b	88c	59a	72b	76c		
	Means		91c			79b			69a			
Nodes to the first inflorescence												
Control	50-55	7.6	8.5	13.5	6.4	7.1	13.3	4.9	6.5	12.4	8.9a	9.4
	70-75	8.3	10.5	14.2	7.4	8.5	14.2	6.3	7.2	13.1	10.0b	
Cotyledons removed	50-55	7.7	8.8	12.9	6.6	7.7	11.5	5.2	6.8	11.1	8.7a	9.0
	70-75	8.7	10.0	13.5	6.4	8.5	11.5	6.2	7.4	11.4	9.3b	
Plumules removed	50-55	8.0	9.0	12.1	7.2	6.8	11.3	5.0	6.5	10.9	8.5a	8.7
	70-75	7.7	10.0	12.9	6.2	7.7	11.5	5.7	7.1	11.2	8.9a	
Plumules and cotyledons	50-55	8.4	10.3	13.8	8.0	8.8	13.5	5.8	8.4	12.8	10.0a	10.3
	70-75	8.9	10.2	14.5	7.4	10.5	14.2	6.0	9.5	14.0	10.6b	
	Means	8.2a	9.5b	13.4c	7.0a	8.2b	12.5c	5.6a	7.4b	12.1c		
	Means		10.4c			9.2b			8.4a			

Means followed by the same letter not different at 5% level.

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were no overall effects of mutilation on anthesis. However, with every increase in light intensity there was significant decrease in number of days to first anthesis. The number of nodes subtending first inflorescence was significantly affected by temperature, light intensity and mutilation. The first flower clusters appeared on the lowest nodes following removal of plumule leaves when all plants maintained at 70-75°F. are compared. With a few exceptions all other mutilation treatments including controls when grown in high (70-75°F.) temperatures significantly increased number of nodes subtending first inflorescence as compared with low (50-55°F.) temperatures. With an increase in light intensity resulted in fewer numbers of nodes to first flower cluster. This effect of light intensity followed a definite trend being most effective on the earliest cultivar (Farthest North) and least effective on the late cultivar (Pennorange).

C. Effects of mutilation, photoperiod and light intensity.

Seeds of tomato cultivars Farthest North, Michigan-Ohio Hybrid and Pennorange were sown on March 27, 1964. Seedlings were transplanted and moved to controlled environment chambers

on April 8. Plants in one of the growth chambers were exposed to short days (9 hours of light and 15 hours of darkness) and in the other to long days (18 hours of light and 6 hours of darkness). Light intensities (1000 and 2000 f.c.) and temperatures (60-65°F.) were the same in both chambers. Cotyledons and plumule leaves were removed on April 14. There were eight plants for each cultivar in each treatment. Plants were removed to a greenhouse on May 4 and transplanted to 6 inch clay pots of soil.

The number of days from seedling to first anthesis (Table 17) was not affected by mutilation or by the length of day, but was reduced by the high (2000 f.c.) light intensity. There were significant differences among cultivars as to the effects of light intensity on days to the first anthesis. The number of nodes subtending the first inflorescence was significantly affected by all the experimental variables. Long days significantly increased the number of nodes to the first inflorescence as compared with the short days. Plants maintained at low (1000 f.c.) light intensity flowered later than those that received high (2000 f.c.) light. Except for Pennorange removal of plumule leaves significantly delayed flowering as indexed by nodes subtending the first inflorescence under both

Table 17. *Effects of mutilation, photoperiod, and light intensity on flowering of the tomato, cvs. Farthest North (FN), Michigan-Ohio Hybrid (MO) and*

Table 17. Effects of mutilation, photoperiod, and light intensity on flowering of the tomato, cvs. Farthest North (FN), Michigan-Ohio Hybrid (MO) and Pennorange (PO).

Mutilation treatment	Photoperiod	Light intensity in foot candles						Means	Means
		1000			2000				
		FN	MO	PO	FN	MO	PO		
		(Means for 8 plants)							
Days to first anthesis									
Control	Short day	55	61	70	49	55	64	59	
	Long day	53	59	71	44	55	63	57	58
Cotyledons removed	Short day	56	62	69	51	56	66	60	
	Long day	52	60	70	47	57	63	58	59
Plumules removed	Short day	53	64	72	53	58	65	61	
	Long day	57	61	72	48	58	63	61	61
	Means	54a	61b	71c	49a	56b	64c		
	Means		62b			56a			
Number of nodes to first inflorescence									
Control	Short day	6.6	6.9	10.9	5.6	6.4	10.5	7.8a	
	Long day	7.0	7.5	12.0	5.8	6.9	11.8	8.5b	8.1a
Cotyledons removed	Short day	6.5	7.1	10.6	5.8	6.3	10.6	7.8a	
	Long day	7.2	7.5	11.6	6.3	7.4	11.9	8.6b	8.2a
Plumules removed	Short day	7.5	8.1	10.5	6.0	7.0	10.4	8.3a	
	Long day	7.8	8.9	11.8	6.5	8.1	11.7	9.1b	8.7b
	Means	7.1a	7.7b	11.2c	6.0a	7.0b	11.1c		
	Means		8.7b			8.0a			

Means followed by the same letter not different at 5% level.

photoperiodic treatments. Varietal differences for node number were significant.

Removal of roots did not influence flowering in the tomato except in Experiment 4. Effects of cotyledon removal on tomato flower formation were inconsistent. Following cotyledon removal, tomato plants reached anthesis significantly later but the flower clusters did not appear at different nodes than in the controls. The effects on the removal of plumule leaves on the number of nodes subtending the first inflorescence, varied with temperature. Plumule leaf removal had no effect on the flowering of plants grown at 50-55°F. while at 70-75°F., plumule leaf removal induced the first inflorescence at a lower node. At 60-65°F., however, the first inflorescence occurred at a higher node if the plumule leaves were removed. Plumule leaves apparently play an important role in the initiation of flowers of the first inflorescence of the tomato.

V. CHEMICALS

A. Effects of application of indole compounds.

Michigan-Ohio Hybrid seed was sown on July 5, 1963. Seedlings were transplanted on July 17 and sprayed with

indole chemicals on July 18, 20 and 22. Indole-3-acetic acid (IAA) and Indole-3-acetyl-D,L-aspartic acid (IASP) were applied at 10^{-4}, 10^{-3}, and 10^{-2} molar concentrations. There were ten plants per treatment.

Number of days (Table 18) from seeding to first anthesis were not affected. Flowering as indexed by the number of nodes subtending first inflorescence was initiated earlier only by IAA at 10^{-3} and 10^{-4} molar concentrations. The number of flowers in the first inflorescence was not affected.

B. Effects of various concentrations of gibberellic acid (GA_3) applied through root media, and the duration of the GA_3 treatment.

Seed of the tomato (cv. Michigan-Ohio Hybrid) was sown on July 25, 1962. Seedlings were transferred to nutrient solution cultures on August 2. Aliquotes of a 10^{-3} molar stock solution of GA_3 were added to the containers to achieve the desired concentrations of GA_3 in the nutrient cultures. Concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} molar were established. Each treatment container was replicated

Table 18. Flowering response of the tomato (cv. Michigan-Ohio Hybrid) to Indole-3-acetic acid (IAA) and Indole-3-acetyl-D,L-aspartic acid (IASP), sprayed on the foliage during sensitive period.

Chemical	Molar concentration	Days to 1st anthesis	Nodes to 1st inflorescence	Flowers to 1st inflorescence
		(Means of 10 plants)		
Control	0	58	8.1cd	6.2
IAA	10^{-4}	56	7.4ab	6.8
	10^{-3}	55	7.3a	6.6
	10^{-2}	58	8.4d	6.5
IASP	10^{-4}	57	7.7abc	6.6
	10^{-3}	59	8.4d	5.7
	10^{-2}	60	8.1bcd	8.2

Means followed by the same letter not different at 5% level.

five times and there were five plants in each container. One plant from each container was removed and planted in soil in 6 inch pots 4, 8, 12 and 16 days after the GA_3 treatments were initiated. The remaining one plant in each container was grown continuously in the GA_3 solution. During the first two weeks of chemical treatment, the solutions lost through transpiration and evaporation was replenished with nutrient 1/2 concentration of standard Hoagland solution containing GA_3 at the same concentration originally applied. The containers were then emptied, rinsed and refilled with fresh nutrient solution containing the same GA_3 concentration. Growth responses of plants exposed to gibberellic acid at 10^{-7} molar were the same as the controls.

The number of days from seeding to first anthesis and the number of flowers in first inflorescence were not affected by GA_3 concentration nor by the duration of the treatments (Table 19). The number of nodes, subtending the first inflorescence, was significantly increased by GA_3 concentration and also by the duration of the treatments. The first flower cluster appeared after a higher node with increase in GA_3 concentration. Exposure of seedling to GA_3 for 8 days was most effective. An extension was no more effective. Plants with roots grown

Table 19. Effects of various concentrations of gibberellin A_3 and duration of treatment following cotyledon expansion on flowering of the tomato (cv. Michigan-Ohio Hybrid)

Molar Concentration GA_3	Duration of the treatment					Means
	4 days	8 days	12 days	16 days	Continuous	
	(Means for 5 plants)					
Days to first anthesis						
0	51	51	51	49	49	50
10^{-6}	51	51	52	50	49	51
10^{-5}	51	53	50	50	49	51
10^{-4}	52	51	51	50	48	50
Means	51	52	51	50	49	
Number of nodes to first inflorescence						
0	7.6	8.2	7.8	7.6	7.6	7.8a
10^{-6}	8.8	9.2	9.0	8.8	8.6	8.9b
10^{-5}	9.2	9.6	9.2	9.2	9.2	9.3bc
10^{-4}	9.2	10.2	10.2	9.4	9.6	9.7c
Means	8.7a	9.3b	9.0ab	8.7a	8.7a	
Number of flowers in first inflorescence						
0	6.4	5.6	6.0	5.4	8.4	6.4
10^{-6}	4.8	6.6	8.0	8.0	5.8	6.6
10^{-5}	6.6	4.4	7.0	5.4	6.8	6.6
10^{-4}	5.2	4.4	4.4	3.8	7.2	5.0
Means	5.7	5.2	6.3	5.6	7.0	

Means followed by the same letter not different at 5% level.

in the higher concentrations of GA_3 flowered after later nodes than the controls but because of more rapid growth (fig. 3) rate reached anthesis at about the same time.

C. Effects of various gibberellins applied on meristems.

Seed (cv. Michigan-Ohio Hybrid) was sown June 10, 1963. The seedlings were transplanted June 21, and treated June 22. The chemicals in solution were applied to the meristem with a micro-pipette. One and 10 micrograms of gibberellin A_3, the butylcellosolve ester of gibberellin A_3, the anhydride of gibberellin A_3 and gibberellin A_4 were applied. There were twenty plants in each treatment including the non-treated controls.

Number of days from seeding to first anthesis, and number flowers in the first inflorescence were not influenced by gibberellins (Table 20). All gibberellin compounds at the higher dosage (10 microgram) significantly increased the number of nodes subtending the first inflorescence. At the one microgram level only gibberellin A_3 delayed flowering by increasing node number. Gibberellin A_3 and its derivatives were more effective than gibberellin A_4.

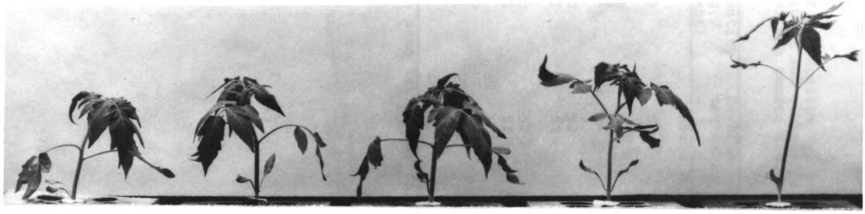

Figure 3. Comparative vegetative growth of the tomato, cultivar Michigan-Ohio Hybrid following three weeks of exposure to various molar concentrations of gibberellin A_3 in the solution culture root media.

Left to right: 0 (control), 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} molar.

Table 2

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Table 20. Effects of gibberellin A_3, some derivatives of gibberellin A_3 and gibberellin A_4 on the flowering response of the tomato (cv. Michigan-Ohio Hybrid)

Gibberellin	Quantity (µg./ plant)	Days to 1st anthesis	Nodes to 1st cluster	Flowers to 1st cluster
		(Means of 20 plants)		
Control	0	52	6.9a	5.1
Gibberellin A_3	10	52	8.5d	6.8
	1	52	7.8c	6.6
Butylcellosolve ester of GA_3	10	52	8.5d	7.3
	1	51	7.1ab	6.0
Anhydride of GA_3	10	52	8.7d	7.1
	1	51	7.5abc	7.0
Gibberellin A_4	10	52	8.2d	6.8
	1	51	7.5abc	6.7

Means followed by the same letter not different at 5% level.

D. Effect of chemical growth inhibitors.

Seed of the tomato cultivars Michigan-Ohio Hybrid (MO) and Farthest North (FN) were sown November 21, 1962. Seedlings were transferred to the solution cultures December 3. The treatments included 10^{-4}, 10^{-5}, and 10^{-6} molar concentration of MH, Amo-1618, B995, CCC, and Phosphon D, plus a control of 1/2 nutrient strength of Hoagland solution. Treatments were replicated twice for each of the cultivars with five plants of each cultivar in one container (one replication). Appropriate amounts of the stock solutions of each growth inhibitor were added to the solution cultures on December 4. Plants were removed to soil in 6 inch pots on December 20. Mean values for the response of each cultivar to the various growth inhibitors are given in Table 21.

The five chemical inhibitors of vegetative growth had contrasting effects on flowering of the tomato. The flowering was delayed 10-13 days by MH and Phosphon D at 10^{-5} molar. Plants treated with CCC at all concentrations reached anthesis 7 to 10 days earlier in the Farthest North cultivar but not in the Michigan-Ohio Hybrid. Other chemical treatments had no effect on anthesis.

Table 21. Effects of chemical growth inhibitors on formation of the first inflorescence of the tomato cv. Farthest North (FN) and Michigan-Ohio Hybrid (MO).

Chemical inhibitor	Molar concentration	Days to anthesis			Node number			Number of flowers		
		FN	MO	Means	FN	MO	Means	FN	MO	Means
		(Means for 10 plants)								
MH	10^{-4}	--	--	--	--	--	--	--	--	--
	10^{-5}	84	96	90e	8.6	9.4	9.0i	7.8	6.5	7.1
	10^{-6}	74	84	79bc	8.0	8.0	8.0f	9.4	7.5	8.4
Amo-1618	10^{-4}	71	86	78bc	7.9	8.9	8.4gh	8.8	6.7	7.7
	10^{-5}	73	84	78bc	7.4	8.5	7.9defg	8.6	6.2	7.4
	10^{-6}	71	81	76abc	6.6	7.6	7.1b	8.6	5.9	7.2
B995	10^{-4}	71	81	76abc	6.7	7.9	7.3cd	9.4	6.8	8.1
	10^{-5}	70	83	76abc	7.0	8.0	7.5d	8.2	6.2	7.2
	10^{-6}	71	82	76abc	7.1	8.2	7.6de	9.2	6.3	7.7
CCC	10^{-4}	61	81	71a	5.2	7.2	6.2a	8.8	8.1	8.4
	10^{-5}	64	84	74ab	5.4	7.8	6.6ab	9.2	6.2	7.7
	10^{-6}	64	83	78bc	6.2	8.0	6.9bc	8.4	6.4	7.4
Phosphon D	10^{-4}	--	--	--	--	--	--	--	--	--
	10^{-5}	84	92	88e	8.4	9.4	8.9hi	8.0	6.0	7.0
	10^{-6}	72	83	77bc	7.0	8.2	7.6de	8.8	7.0	7.9
Control		71	83	77bcd	7.2	8.2	7.7def	8.4	7.3	7.8

Means followed by the same letter not different at 5% level.

Flowering as indexed by number of nodes subtending first inflorescence, was delayed by both MH and Phosphon D at 10^{-5} and by Amo-1618 at 10^{-4} molar. All plants exposed to 10^{-4} molar of MH and Phosphon D did not survive. On the other hand CCC at all concentrations, and Amo-1618 at 10^{-6} reduced node numbers preceding the first flower cluster. With CCC the higher the concentration lower was the node number to the first flower cluster. This effect was more pronounced on Farthest North than in MO. The chemical treatments had no effect on the number of flowers in the first inflorescence.

Experiment 2.

Seeds (cv. Michigan-Ohio Hybrid) were sown on March 20, 22, 24, 26, and 28, 1964. On April 6, after the last planting of seed germinated, ten seedlings from each seeding date were immersed in beakers containing solutions of naringenin and coumarin at 0, 0.5 and 1.0 mg/ml. After two hours immersion the seedlings were transplanted in soil in 6 inch clay pots. Data for number of nodes subtending the first inflorescence are summarized in Table 22.

Both naringenin and coumarin at the higher (1.0 mg/ml) concentration significantly delayed flowering as indexed by

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Table 22. Flowering of the tomato (cv. Michigan-Ohio Hybrid) as indexed by the number of nodes subtending the first inflorescence as influenced by immersing seedlings of different ages in Naringenin and Coumarin.

Chemical inhibitors	Concentration (mg./ml.)	Age of seedling in days following emergence					
		9	7	5	3	1	Means
		(Means of 10 plants)					
Control	0.0	7.2	7.3	7.5	7.5	7.5	7.4a
Naringenin	0.5	7.4	7.6	7.6	6.9	7.4	7.4a
	1.0	8.3	8.0	7.7	8.0	7.3	7.9b
Coumarin	0.5	7.3	7.8	7.5	7.4	7.1	7.4a
	1.0	8.1	8.3	8.1	8.0	7.1	7.9b
Means		7.7	7.8	7.7	7.6	7.1	

Means followed by the same letter not different at 5% level.

an increase in nodes subtending the first inflorescence except with one day old seedlings. Both chemicals inhibited vegetative growth at the 1.0 mg /ml concentration.

E. Effects of 2-thiouracil, 5-flurouracil, and chloramphenicol.

In an earlier study roots and tops of the tomato (cv. Michigan-Ohio Hybrid) seedlings were dipped momentarily once in 10^{-4}, and 10^{-3} molar solutions of 2-thiouracil, 5-flurouracil, and chloramphenicol. This chemical treatment had no effect on tomato flower formation. In another experiment seedlings were grown for an extended period in nutrient solution containing the above chemicals.

Seeds of the tomato (cv. Michigan-Ohio Hybrid) were sown July 1, 1963. Seedlings were transferred to solution cultures on July 8. Treatments consisted of 10^{-5} and $5x10^{-5}$ molar concentrations of 2-thiouracil, 5-flurouracil and chloramphenicol in the solution culture root media plus non-treated controls grown in 1/2 nutrient strength (Hoagland solution). There were five replications with two plants per replicate for each treatment. After a 15 day exposure to the above chemical treatments, the plants were transferred on July 23 to soil in 6 inch clay pots.

Results are summarized in Table 23. All plants grown in $5x10^{-5}$ molar, 2-thouracil were killed. Days to first anthesis was significantly delayed by 2-thiouracil at 10^{-5} molar, and by chloramphenicol at both concentrations. 5-flurouracil did not modify days from seeding to first anthesis. The first flower cluster appeared after a higher node when plants were exposed to 10^{-5} molar 2-thiouracil and $5x10^{-5}$ molar, 5-flurouracil or chloramphenicol. Other treatments did not change the nodal position of the first flower cluster. Number of flowers in the first cluster were not affected.

F. Effect of inhibitors of steroid biosynthesis.

Experiment 1. Effect of various concentrations.

Michigan-Ohio Hybrid seed was sown on October 7, 1963. Seedlings were chemically treated on October 19 by applying a drop of solution to the growing point. Chemicals used were SK&F 7732 and SK&F 7997 at 1.25, 2.50 and 5.00 mg per ml. They were dissolved in distilled water and the pH immediately adjusted to 6.5. There were eight plants in each treatment including the controls.

These inhibitors (Table 24) significantly delayed the first anthesis and markedly increased the number of nodes subtending the first inflorescence, but they had

Table 23. Effects of 2-thiouracil, 5-flurouracil and chloramphenicol applied through the solution culture root media on the formation of first flower cluster in the tomato, cv. Michigan-Ohio Hybrid

Inhibitor	Concentration ($x10^{-5}M$)	Days to Anthesis	Node Number	Number of flowers
		(Means of 8 plants)		
2-thiouracil	5	--	--	--
	1	55bc	8.7d	6.2
5-flurouracil	5	54abc	8.3bcd	6.7
	1	52ab	7.9abc	6.4
Chloramphenicol	5	57c	8.3bcd	5.3
	1	55bc	7.8abc	5.9
Control		51a	7.2a	6.0

Means followed by the same letter not different at 5% level.

Table 24. Effects of different concentrations of inhibitors of steroid biosynthesis on the formation of the first flower cluster in the tomato cv. Michigan-Ohio Hybrid

Steroid Inhibitor	Concentration mg./ml.	Days to anthesis	Node Number	Number of flowers
		(Means of 8 plants)		
SK&F 7732	1.25	49ab	8.0bc	6.2
	2.50	49ab	8.1bc	5.8
	5.00	50b	8.4c	5.5
SK&F 7997	1.25	48ab	7.5ab	6.2
	2.50	49ab	8.2b	6.2
	5.00	51b	8.6c	5.8
Control		47a	7.0a	5.8

Means followed by the same letter not different at 5% level.

no effect on flower number. Both SK&F 7732 and SK&F 7997 at 5.00 mg /ml delayed anthesis. Flowering occurred at a later node following treatment with either compound and all concentrations except SK&F 7997 at 1.25 mg /ml The node number preceding flowering increased as the concentrations increased. The numbers of flowers in the first inflorescence were not influenced by these treatments nor was the rate or amount of vegetative growth modified.

Experiment 2. Effect of duration of treatment.

Seed of Michigan-Ohio Hybrid was sown on March 20, 1964 and seedlings were treated on March 31. Seedlings were immersed or dipped into solutions of SK&F 7997 (2 mg /ml) at pH 6.5 Treatments consisted of momentary dipping whole seedling, and immersion of seedlings for 30 minutes, 1 hour, 2 hours, 4 hours and 8 hours. Control seedlings were immersed in distilled water for 8 hours. There were eight single plant replicates for each treatment.

All plants immersed in SK&F 7997 for 4 or 8 hours were killed. Treatments did not modify days from seeding to first anthesis or the number of flowers in first inflorescence but markedly influenced node number to the first inflorescence (Table 25). All treatments

Table 25. Effects of duration of treatment with steroid synthesis inhibitor (SK&F 7997) on the formation of the first inflorescence of the tomato cv. Michigan-Ohio Hybrid

Duration of treatment	Days to anthesis	Node number	Number of flowers
	(Means for 8 plants)		
Control	60	7.4a	6.0
Dip	60	8.3b	6.4
1/2 hour	60	8.4bc	6.0
1 hour	61	8.8bcd	5.6
2 hour	61	9.0d	5.6

Means followed by the same letter not different at 5% level.

significantly delayed flowering as indexed by the number of nodes subtending the first inflorescence. With each increase in duration of treatments there was a progressively greater delay in flowering as indexed by nodes subtending the first inflorescence.

Experiment 3. Effectiveness when applied at different stages of seedling growth.

Lots of tomato (cv. Michigan-Ohio Hybrid) seed were sown March 20, 22, 24, 26, and 28, 1964. On April 6 after the last planting of seed germinated, 10 seedlings from each seeding date were immersed in the solutions of SK&F 7732 and SK&F 7997 (1.0 and 2.0 mg/ml each) at pH 6.5 for two hours. (Controls were immersed in distilled water). After the two hours immersion the seedlings were transplanted into soil.

The number of nodes subtending the first inflorescence are given in Table 26. Flowering was significantly delayed by SK&F 7732 at 2.0 mg/ml and SK&F 7997 at 1.0 and 2.0 mg/ml. Both of these chemicals were most effective in delaying flowering when 7 and 9 day old seedlings were treated. SK&F 7732 had very little delaying effect on flowering of 1, 3, and 5 day old seedlings. SK&F 7997 was effective in delaying flowering on seedlings of all ages.

Table 26. Flowering of the tomato (cv. Michigan-Ohio Hybrid) as indexed by number of nodes to first inflorescence following immersion of seedlings at different stages of growth in solutions of steroid synthesis inhibitors

Steroid inhibitor	Concentration mg./ml.	Age of seedlings in days following emergence					
		9	7	5	3	1	Means
		(Means of 10 plants)					
Control	0	7.2	7.3	7.5	7.5	7.5	7.4a
SK&F 7732	1	7.8	7.8	7.6	7.3	7.3	7.6ab
	2	8.0	8.0	7.9	7.8	7.9	7.6bc
SK&F 7997	1	8.1	8.3	8.0	8.2	8.1	8.1c
	2	8.5	8.5	8.3	8.2	8.3	8.3c
Means		7.8	7.9	7.8	7.7	7.7	

Means followed by the same letter not different at 5% level.

G. Effect of anthogenes applied at different stages during seedling growth.

Seeds of the tomato (cv. Michigan-Ohio Hybrid) were sown on January 17, 20, 23, 26, 29 and February 1, 1964. On February 16, after the last planting of seed had germinated, 10 seedlings from each seeding date or age lot were immersed in solutions of anthogenes 1, 2, 3, or 4 for four hours. Anthogene stock solutions were diluted 1:200 with distilled water. After four hours immersion the seedlings were transplanted into pots of soil.

Node numbers were altered by treatment and the results are summarized in Table 27. Flowering was consistently delayed by anthogene 1. Anthogene 2 induced early flowering only when 4, 10, and 13 day old seedlings were treated. Anthogene 3 accelerated flowering when supplied to 4, 7, and 13 day old seedlings. Flower cluster formed after fewer nodes when anthogene 4 was applied to 4 and 7 day old seedlings. These inconsistent results shed little light on the possible roles of anthogenes in floral initiation in the tomato.

The effects of various plant growth substances, including inhibitors of vegetative growth, metabolic

Table 27. Effect of anthogenes supplied to tomato (cv. Michigan-Ohio Hybrid) seedlings at different stages of growth on flower formation as indexed by nodes subtending the first inflorescence

Treatment	Age of seedlings in days following emergence						
	16	13	10	7	4	1	Means
	(Means of 10 plants)						
Controls	8.6	8.2	8.5	8.6	8.7	8.2	8.5bc
Anthogene 1	9.1	8.6	8.1	9.0	9.0	8.8	8.8c
Anthogene 2	8.5	7.6	7.3	8.8	8.1	8.4	8.1ab
Anthogene 3	8.1	7.5	8.1	7.6	7.9	8.1	7.9a
Anthogene 4	9.0	9.0	8.5	7.8	7.8	8.1	8.4abc
Means	8.7	8.2	8.1	8.4	8.3	8.3	

Means followed by the same letter not different at 5% level.

inhibitors, and anthogenes have been evaluated as they modify flower formation in the tomato. Many diverse effects on vegetative growth and flowering have been recorded. Indole-3-acetic acid induced earlier flowering but the effect was not as marked as for other auxins already reported by other workers. Indole-3-acetyl-D,L-aspartic acid the endogenous tomato auxin (102) had no effect. All gibberellins were effective in delaying flowering. Even single applications on growing points were effective. Gibberellin A_3 (GA_3) delayed flowering when applied through the solution culture root media. Duration of treatment extending beyond 8 days was no more effective than a 8 days exposure. Inhibitors of vegetative growth had contrasting effects on flowering MH, Phosphon D, naringenin, and coumarin delayed flowering while CCC at all concentrations and Amo-1618 at 10^{-6} molar induced early flowering, and B995 was ineffective. Inhibitors of DNA-RNA synthesis (2-thiouracil and 5-flurouracil) and protein synthesis (chloramphenicol) had no influence on flowering when applied as a single treatment, but significantly delayed flowering after two weeks of contact through the solution culture root media.

This delay in flowering was accompanied by a marked suppression of vegetative growth. Single foliar application of steroid biosynthesis inhibitors (SK&F 7732 and SK&F 7997) significantly delayed flowering without restricting vegetative extension. These steroid biosynthesis inhibitors were increasingly effective in delaying flowering as the duration of the exposure was extended. Results with anthogenes were inconclusive.

VI. GRAFTING

A. Effects of reciprocal grafting.

Experiment 1.

Seeds of the cultivars (Farthest North, Michigan-Ohio Hybrid, 146j, Tuckcross O, and Pennorange) were sown December 28, 1961. Reciprocal root/top epicotyl grafts were prepared in which the rootstocks were grafted above the plumule leaves (first two true leaves). All five above mentioned cultivars were used in all possible rootstock-scion combinations. The plumule leaves were left intact on the rootstocks. The method of grafting was wedge and cleft. Sealtex Latex Bandage was used to hold the grafts together until a union was formed and, if needed, toothpicks were

used to support the grafted plant parts (fig. 4). Previously grown plants at 3 to 4 leaves were used as rootstocks. Grafting was done January 11-13, 1962. There were 12 grafts in each combination and 12 non-grafted controls of each cultivar. Subsequent to grafting all plants were kept in plastic chambers at high humidity for 8-10 days. Six grafted plants with acceptable unions were selected for final observations. Following similar procedures, hypocotyl grafts (fig. 4) were made from March 12 through 14, 1962. The results on flowering of the most effective combinations are presented in Table 28.

The node to first inflorescence and number of flowers in the first inflorescence were significantly affected when scions were grafted on rootstocks with intact plumule leaves. This was not true for hypocotyl grafts. As compared with non-grafted controls, rootstocks with plumule leaves of Farthest North (fig. 5) in which 6-7 leaves ordinarily formed prior to the first inflorescence, induced the first flower cluster after lesser number of nodes on scions of both Farthest North and of Pennorange. Farthest North rootstocks also formed more flowers in the first clusters on FN scions, but had no effects on flowering when used as a rootstock

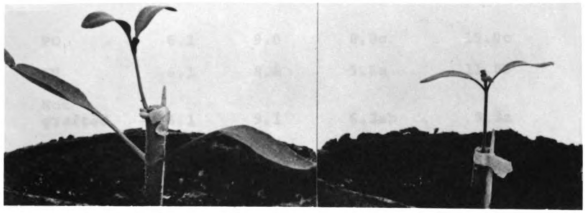

Figure 4. Grafting technique. (L to R) Epicotyl graft (above the cotyledons), Hypocotyl graft (below the cotyledons)

Ta

Sc

FN

FN

FN

PO

PO

PO

Me

no

Table 28. Influence of grafting and reciprocal top-root grafting of an early (Farthest North (FN)) and a late (Pennorange (PO)) cultivar on formation of the first inflorescence in the tomato.

Treatment		Hypocotyl grafts		Grafts above plumules	
Scion	Rootstock	Node number	Number of flowers	Node number	Number of flowers
		(Means of 6 plants)			
FN	PO	6.2	9.0	8.8c	15.0c
FN	FN	6.1	8.8	5.5a	11.8b
FN	Not grafted	6.1	9.1	6.3ab	9.3a
PO	PO	10.7	2.8	17.3c	3.8c
PO	FN	10.0	3.0	12.0a	2.5a
PO	Not grafted	10.2	2.7	15.1b	2.6ab

Means followed by the same letter within the same cultivar not different at 5% level.

Figure 5. (Top) Early and late tomato cultivars used in reciprocal top/root grafting studies. (l to R) Pennorange (late cultivar), Farthest North (early cultivar). (Bottom) Effects of rootstocks with intact plumule leaves on flowering in the Farthest North tomato. (L to R) Farthest North on Farthest North rootstock, Farthest North non-grafted, Farthest North on Pennorange rootstock.

in hypocotyl grafting. On the other hand, Pennorange (fig. 5) in which 12-15 nodes ordinarily subtend the first inflorescence, rootstocks with intact plumule leaves delayed flowering. This was indexed by the number of nodes subtending the first inflorescence on scions of both Farthest North (fig. 5) and Pennorange. Pennorange rootstocks also caused the initiation of larger numbers of flowers in the first inflorescence and had no effects on flowering when used as rootstock in hypocotyl grafts. Non-grafted plants were used for comparison.

It is possible that the above effects on scion flowering may have been positional and not related to the presence of plumule leaves on the rootstocks since graft unions were made at different levels on the rootstocks.

Experiment 2.

Seeds of Farthest North and Pennorange were sown April 15, 1964 and the seedling scions grafted April 24 on rootstocks derived from plants which were at the 3 to 4 leaf stage. Grafting procedures were the same as described in Experiment 1, above, except that all unions were made at the same time and above the plumule leaves.

Plumule leaves from half (twelve) of the rootstocks were removed. Six plants with good graft unions were selected for observations.

The data in Table 29 confirm the findings reported in Experiment 1, above. The presence of plumule leaves on the rootstocks of Farthest North induced significantly earlier flowering on both FN and PO scions, and the presence of plumule leaves on Pennorange significantly delayed flowering as indexed by nodes subtending the first inflorescence, on both the scions. Pennorange scions were not affected as to number of flowers by grafting irrespective of the rootstocks used but grafting increased the number of flowers on the Farthest North scions. This increase in flowers on Farthest North scions was highest when plumule leaves were present on the rootstocks. Non-grafted plants were used for comparisons.

These grafting experiments suggest that the flowering stimulus originates in above ground plant parts; specifically in the plumule leaves and perhaps those of subsequent development. Delayed flowering in late cultivars is the function of an inhibitor which is also produced in the above ground plant parts, particularly in the leaves.

Table

Tr
Scion

FN

FN

FN

PO

PO

PO

Years
same

Table 29. Influence of grafting and reciprocal top-root grafting of an early (Farthest North (FN)) and a late (Pennorange (PO)) cultivar on the formation of the first inflorescence in the tomato.

Treatment		Grafting above plumule leaves			
		Without plumule leaves		With plumule leaves	
Scion	Rootstock	Node number	Number of flowers	Node number	Number of flowers
		(Means of 6 plants)			
FN	PO	6.5	10.0b	7.2c	14.0b
FN	FN	6.4	11.0b	4.2a	16.0b
FN	Not grafted	6.6	7.0a	6.6b	7.0a
PO	PO	12.0	2.2	13.5c	2.1
PO	FN	12.0	2.5	11.0a	2.2
PO	Not grafted	12.0	2.3	12.0b	2.3

Means followed by the same letter within the scion of the same cultivar not different at 5% level.

VII. EXTRACTION OF GIBBERELLIN-LIKE SUBSTANCES FROM THE TOMATO

A. The gibberellin contents of Farthest North (FN), an early cultivar, and Pennorange (PO), a late cultivar.

Seedlings were started in a greenhouse held at 65°F. night temperature and under natural light and photoperiod during early fall. All plants at 3 to 4 leaf stage were harvested the same day.

All plant parts above soil level were utilized. Tissues were macerated with 90 percent methanol in a blender and held overnight in a refrigerator. Each sample was filtered, pressed, and then re-extracted with 90 percent methanol. Samples were again filtered, pressed, and the combined extracts concentrated in vacuo at 36°C. The residue was treated with a slurry of basic lead acetate to precipitate impurities. An excess of lead acetate was shown by adding a drop of dilute hydrochloric acid to the solution which produced a white precipitate of lead chloride (92). The purified solutions were then centrifuged. The supernatant was acidfied to a pH. of 2.5 and extracted three times with equal volumes of ethyl acetate. The combined ethyl acetate fractions were

concentrated to about 150 ml and extracted with three 50 ml volumes of 1 percent sodium bicarbonate solution. These sodium bicarbonate extracts were combined and re-extracted with three equal volumes of ethyl acetate. The combined ethyl acetate solutions were evaporated to dryness in vacuo at 36°C. The residue was then dissolved in 8 ml. of 50 percent methanol and the methanol evaporated.

Extracts of these residues were bioassayed on Blue Lake bean, Burpee Hybrid cucumber, and Little Marvel dwarf pea seedlings. The solutions were applied on growing tips. After 3 to 5 days, differences in vegetative extension were noted, and comparisons made with known amounts of gibberellic acid (GA_3).

Bioassays on beans, cucumber and pea seedlings (134) revealed that Pennorange tomato seedlings contained more gibberellin-like substances than Farthest North. The comparative amounts of the gibberellin-like substances (based on GA_3 activity) were estimated in the dwarf pea bioassay (Table 30). These extracts containing gibberellin-like compounds from two tomato cultivars when applied to seedlings of the Michigan-Ohio Hybrid tomato delayed flowering significantly by increasing the number of nodes subtending the first inflorescence. The

Table

Extrac

FN ext

PO ext

Contro

Means

Table 30. Amounts of gibberellin-like substances in an early tomato cultivar (Farthest North (FN)) and late tomato cultivar (Pennorange (PO)) as estimated by the dwarf pea (cv. Little Marval) seedling bioassay, and the effects of those extracts on the flowering of the Michigan-Ohio Hybrid tomato.

Extracts	Micrograms equivalent GA_3 per kilogram fresh weight	Node number to 1st inflorescence
FN extract	20-22	8.2b
PO extract	56-58	9.0c
Controls	--	7.5a

Means followed by same letter not different at 5% level.

delay induced by Pennorange extracts was significantly greater than that from Farthest North (Table 30).

Significant differences in cucumber hypocotyl growth were induced by the tomato plant extracts which suggested the presence of gibberellins A_4, A_7 or A_9. These are three known gibberellins that are most effective on cucumber hypocotyl growth (134). Thin layer chromotography (61, 80) with the known gibberellins eluted along with the extracts revealed that the gibberellins in the tomato plants may possibly include GA_4 and GA_7 and possibly small quantities of GA_6 and GA_9. The gibberellins separated by the thin layer chromotography were not bioassayed.

B. Effects of photoperiod on gibberellin content.

The seedlings of Michigan-Ohio Hybrid with cotyledons expanded were grown for 15 days in controlled environment chambers under long (18 hr. light + 6 hr. dark) and short days (9 hr. light + 15 hr. dark). Light intensities (1000 f.c.) and temperatures (60-65°F.) were the same at both photoperiods.

Extraction and purification procedures for native gibberellins were the same as already outlined, except

that dry ice was added while initially macerating the seedlings at 2 to 3 leaf stage. The extracts were again bioassayed on bean, cucumber, and dwarf pea seedlings. Growth responses from the various extracts were compared with those derived from known amounts of gibberellin A_3.

The results of the bioassays revealed that plants grown in long days had higher content of gibberellin-like substances than those grown in a short photoperiod. Growth responses with bean seedlings were inconsistent, but very reproducible on cucumber hypocotyl elongation. This again suggested the presence of gibberellins A_4, A_7 or A_9. Quantitative extractions derived from the growth of dwarf pea seedlings after the application of tomato plant extracts and from known amounts of gibberellin A_3 revealed that plants grown in long days had 68-70μg/K equivalents compared with 26-28 micrograms equivalent GA_3 for those maintained in short day (Table 31).

These results suggest that the higher gibberellin content in seedlings of Pennorange may be one of the factors contributing to the delay in flowering of the late cultivar compared to that of the early cultivar, Farthest North. The delaying effects of long days in relation to node to the first flower cluster may be explained on the

Table 31. Amounts of gibberellin-like substances in the Michigan-Ohio Hybrid tomato following exposure to long and short days, as bioassayed on the Little Marvel dwarf pea seedlings.

Photoperiod treatment	Micrograms equivalent GA_3 per kilogram fresh weight
Short day	26-28
Long day	68-70

basis of higher quantity of gibberellin-like substances, observed in long day grown plants as compared with those grown in the short days.

VIII. GIBBERELLIN INDUCED MICROGAMETOPHYTE DEVELOPMENT IN A STAMENLESS (sl_1sl_1) TOMATO (8)

One approach in the study of flowering is a consideration of factors influencing the formation of floral parts (stamens, pistils, petals, sepals). The tomato offers an excellent opportunity for such studies in that mutants are available that are lacking in important floral structures. Thus, the effects of gibberellin A_3 were observed on the development of stamen and pollen formation on otherwise male sterile and stamenless mutant.

In the preliminary study uniformly rooted cuttings of the stamenless mutant were exposed to IAA, kinetin, GA_3, and casein hydrolysate at 25 ppm in the solution culture root media, during the Spring of 1963. Plants exposed to GA_3 showed good anther development in the flowers which appeared 3 to 4 weeks after the initiation of the treatment. Microscopic observations showed normal

pollen to be present in these GA_3 induced anthers. Progeny raised from the seed obtained by sibbing gave 100 percent stamenless plants, which would have had to otherwise be propogated from a segregating population of 1 stamenless: 3 normal.

A more detailed experiment was planned in the Spring, 1964. Uniformly rooted cuttings were exposed to GA_3 treatments on April 4, 1964. These consisted of 0, 10^{-5}, 10^{-4} and $3x10^{-4}$ molar concentrations in the solution culture root media; and foliage spray applications of 0, 10^{-5}, 10^{-4} and 10^{-3} molar. A split-plot design was utilized with the foliage sprays as the main and root application as the sub-plots. There were 3 double plant replicates for each treatment. All solutions were renewed at six day intervals. After 18 days of exposure to the various gibberellin levels in the root media the containers were washed with water and replenished with 1/2 strength Hoagland solution only.

Spray treatments of gibberellin A_3 produced no observable effects on the development of microgametophytes, and are thus omitted in the presentation of the results in Table 32. All root treatments, however,

Table 32. Formation of microgametophytes in the flowers of a stamenless (sl_1sl_1)* tomato line through the application of gibberellin A3 in the solution culture root media.

GA_3 concentration in root media	Number of inflorescence under observation					Fruit set as
	1	2	3	4	5	
	percent flowers with one or more matured anthers (Means of 6 plants)					
0	0	0	0	0	0	0.0
10^{-5}	0	0	5	7	0	0.3
10^{-4}	0	0	98	100	0	2.0
$5x10^{-4}$	0	0	100	100	0	3.0

*Originally obtained from Dr. T. O. Graham of the Department of Horticulture, Ontario Agricultural College, Duelph, Ontario, Canada. Author wishes to express his gratitude to Dr. Graham for the permission to use the line for these studies.

produced some effect. Anther development was not influenced on the first two inflorescences that developed after treatment. Buds of these clusters were visible when the treatments were initiated. The subseuquent two inflorescences, which appeared after 3 to 4 weeks showed a marked response to gibberellin applied through the root medium. There was little response from 10^{-5} molar gibberellin A_3. Molar concentrations of 10^{-4} and $3x10^{-4}$, however, resulted in most all flowers having one or more (usually all) fully developed anthers (fig. 6). Microscopic examinations revealed that these gibberellin induced anthers contained normal pollen. The flowers with developed anthers were vibrated every day. This resulted in a small number of parthenocarpic fruits. Honma and Bukovac (54) similarly observed parthenocarpic fruits on stamenless tomato mutant following whole plant sprays with GA_3. They, however, observed no anther or pollen formation.

Gibberellin developed anthers did not form a regular tube or stamen cone around the stule and stigma, but were curled and twisted (fig. 6). This curling of anthers was most prevalent at the highest gibberellin A_3 concentration ($3x10^{-4}$M).

Figure 6. Effect of various concentrations of gibberellin A_3 applied through the solution culture root medium on the development of stamens in the flowers of a stamenless (sl_1sl_1) tomato mutant.
(L to R) Top to bottom: 0 (control), 10^{-5}, 10^{-4}, and $3x10^{-4}$M.

Flower clusters which appeared 3 to 4 weeks after the discontinuation of the gibberellin treatments showed no anther development, thus confirming that anther development was induced by the presence of an exogenous supply of gibberellin. In another parallel study it was observed that application of a drop of 10^{-2} molar GA_3 on the maristem, induced anther development on the flowers that appeared 3 to 4 weeks later.

DISCUSSION

Experimental modification of the flowering:

Kraus and Kraybill in 1918 proposed the concept of the carbohydrate-nitrogen relationship. Many studies and numerous observations have subsequently considered the effect of nitrogen nutrition on flowering and fruiting in the tomato. Most of these, however, have been directed toward effects on flower and fruit development and not on floral initiation. Wittwer and Teubner (139) exposed tomato plants to several levels of nitrogen in the root medium and obtained the earliest flowering with highest nitrogen levels. Similarly the highest nitrogen-phosphorus levels gave the earliest flowering in experiments conducted by Eguchi, Matsumura and Ashizawa (38). In the present studies the optimum nitrogen level in relation to flowering was dependent on the rate of growth and photosynthesis. Thus, during summer earliest flowering was obtained with 440 ppm, but in winter when light was limiting 110 ppm was best. High nitrogen levels were best utilized as photosynthetic rates were increased. Data on plant growth rates

as altered by various nutrient element deficiencies suggest that flowers are initiated even in the absence of added major nutrient elements. The effects of nitrogen nutrition on the flowering of the tomato are probably indirect.

The number of nodes subtending the first inflorescence decreases with increase in the light intensity. This results in earlier flowering (22, 34, 121, 132). The present studies further confirm these results wherein light intensities enhance earliness of flowering. However, this induction of early flowering in relation to node number does not seem to be a direct result of increased photosynthesis as carbon dioxide enrichment of the atmosphere which invariably increases photosynthesis failed to induce early flowering in all except one cultivar.

A large number of workers (22, 93, 94, 95, 138) have reported that exposure of tomato plants, during the sensitive stage of flower initiation of the first inflorescence, to an extended photoperiod of low light intensity increased the number of leaves subtending the first inflorescence. Wittwer (132) after an objective analysis of the photoperiodic behavior of flowering in the tomato suggested that the tomato be classified as a

facultative short day plant. The present findings support the proposal of Wittwer (132) and further suggest that the delay in flowering following exposure to long days may be from higher gibberellin production in the long day grown plants as compared to those grown in the short days.

The delay in the flowering observed herein following exposure of seedlings to a 36 hour cycle as compared to those grown in a 24 hour cycle may have been from higher gibberellin levels following exposure to the longer light period (15 hr.) to which the plants were exposed during a given interval in 36 hour cycles.

Many reports (20, 21, 68, 70, 71, 76, 103, 119, 120, 123, 124, 126, 136, 137) indicate that low temperatures during the early growth of tomato seedlings reduces the number of nodes to the first inflorescence, and increases the number of flowers. The effects of root and top temperatures have not, however, heretofore been separated. Teubner and Wittwer (112) distinguished between the effects of root and top temperatures, and observed that low top temperatures initiated the first flower clusters earlier while low root temperatures were responsible for a greater number of flowers in the first inflorescence. Observations in the present studies confirm

the findings of Teubner and Wittwer (112) that the node number is influenced by the top (air) temperatures and flower number by root temperatures. These differential effects of root and top temperatures suggest that the stimulus for the initiation of the first inflorescence originates in the above ground plant parts.

Number of workers (34, 35, 48, 75, 103) reported that defoliation increased the number of flowers on the first cluster and decreased the time of the first anthesis. Only Shen (103), however, recorded the number of nodes subtending the first inflorescence. She, like others, started defoliation after the first inflorescence was already initiated. Thus, it is obvious why she observed no difference in the node number to the first inflorescence following defoliation. Hussey (58, 59) in microscopic examinations of meristems observed earlier initiation of flowering following removal of plumule leaves of plants grown at high temperatures, but found that cotyledon removal delayed flowering. The results of the present study suggest that removal of plumule leaves induces early flowering when plants are grown at a high temperature (70-75°F.) but there was no delay in flowering when

cotyledons were removed. The present studies confirm the reports of Hussey (58, 59) that removal of cotyledons or plumule leaves had no effects on plants grown at low (50-55°F.) temperatures. A delay in flowering was noted, however, when plumule leaves were removed from plants grown at 60-65°F.

It is suggested that at high temperatures plumule leaves of the tomato grow vigorously and there is a competition for available assimilates between the apex and the expanding leaves (58, 59). The data support this assumption and suggest that there is also a competition for the auxins which may be present in smaller amounts in plants grown at higher temperatures (64).

These observations further suggest that plumule leaves play an important role in determining the flowering responses of tomato seedlings to external factors, particularly temperatures.

Flower number in the first cluster has been reported to be increased when young tomato seedlings were treated with IAA (112, 135). In this study, IAA did not influence flower number but reduced the number of nodes to first inflorescence thereby inducing early flowering. Indole-3-acetyl-D, L-aspartic acid the endogenous tomato auxin (102)

did not modify flowering in the tomato. This confirms other reports that IASP is inactive in some plant systems (3). Results of numerous studies (35, 45, 46, 47, 86, 103, 110, 111, 114) suggest that auxins and auxin-like substances play a crucial role in tomato flower initiation and development. Indole and non-indole auxins have been isolated from different parts of the tomato plant (64, 85). Kramer and Went (64) reported that the auxin content of tomato stem tips of seedlings exposed to low temperatures (46°F.) was double that of auxin in seedlings maintained at 72°F. Temperatures comparable to those that increase the auxin content also initiate earlier flowering and increase the number of flowers in the first inflorescence (20, 21, 68, 69, 70, 71, 76, 103, 119, 120, 123, 124). This is further evidence of the important role of auxins and auxin-like substances in the initiation and formation of the first flower cluster in the tomato.

Applied as a spray, gibberellin A_3 delayed flowering, and reduced the number of flowers in the first inflorescence. There was no effect on the net time to first anthesis since growth rates were accelerated at the same time that node number was increased (16, 131). Except for flower number similar results were obtained when GA_3 was added to the

solution culture root media. An 8 day exposure to GA_3 was most effective in delaying flowering. Extending the exposure time did not increase the effectiveness of GA_3. This suggests that the first flower cluster was initiated within 8 days after cotyledon expansion. Gibberellin A_3, some derivatives of GA_3, and GA_4 also delayed flowering when applied as a single drop on the apical meristems at cotyledon expansion. These results suggest that gibberellins play a role in modifying flowering of the tomato. Furthermore, gibberellin-like substances derived from different tomato cultivars and applied exogenously delayed flowering differentially. Quantitative differences in gibberellin-like substances in long and short day grown tomato plants further suggest that the effects of photoperiod may be mediated through a varied synthesis of gibberellin-like substances.

Chemical inhibitors of vegetative growth had contrasting effects on tomato flowering. Time to first anthesis was reduced and the first inflorescence was formed one node earlier in a mid-winter tomato crop following solution culture root media treatments with CCC (131, 140, 141). Similar results were obtained in these studies. CCC may inhibit the biosynthesis of

gibberellin in tomato plants as has already been reported for Fusarium sp. (62, 84). Amo-1618 though reported to be ineffective as foliage apray on tomato flowering (140) delayed flowering at 10^{-4} molar induced earlier flowering at 10^{-6} molar but had no effect at 10^{-5} molar when applied through the solution culture root media. MH delayed flowering both in terms of time to the first anthesis and node number to first inflorescence. The observations of Wittwer and Tolbert (141) were thus confirmed. Phosphon D delayed anthesis but did not alter node number in soil application (140). When applied through solution culture root media Phosphon D delayed anthesis and also increased nodes to the first inflorescence. B995 had no effect on tomato flowering.

The inhibitors of DNA-RNA biosynthesis; 5-flurouracil, and 2-thiouracil, which inhibit flower induction in Xanthium (12), and chloramphenicol, a specific inhibitor of protein synthesis, delayed flowering in the tomato only after extended periods of exposure. There was also an inhibition of vegetative growth. The delay in flowering following extended exposure may be from the inhibition of cell division which one would expect to occur following the inhibition of DNA-RNA and protein synthesis. Inhibitors

of steroid biosynthesis, which inhibit floral induction in Xanthium (11), also inhibited flowering in the tomato. A single application delayed flowering without affecting vegetative growth. This suggests that the flowering stimulus in the tomato may be a steroid or an isoprenoid.

Inconsistent results with anthogenes shed little light on the possible role of these lipid hormones (98, 99) in floral initiation in the tomato.

Reciprocal top-root grafting of late and early cultivars, involving hypocotyl grafts, grafts above plumule leaves, and epicotyl grafts with or without leaves on the rootstocks showed that flowering was not modified by hypocotyl grafts or grafts without plumule leaves on the rootstocks. Flowering was, however, significantly modified by rootstocks with plumule leaves. The rootstocks of an early flowering type with plumule leaves induced early flowering on the scion of a late cultivar while rootstocks of a late one with plumule leaves delayed flowering on scions of an early cultivar. This suggested that the origin of the flowering stimulus as well as that for an inhibitor for flowering may be in the plumule leaves.

Origin of flowering stimulus:

Differential exposure of tops and roots of tomato seedlings, mutiliation of seedlings, and reciprocal top-root grafting of early and late cultivars suggest that the flowering stimulus for the first inflorescence in the tomato originates in the above ground plant parts and that the plumule leaves play an important role in determining the response of seedlings to the external environment.

Nature of flowering stimulus:

Results of studies with DNA-RNA synthesis inhibitors and inhibitors of protein synthesis showed a delay in flowering on plants exposed to these chemicals for extended periods. DNA-RNA and protein synthesis is essential for cell multiplication, and therefore, for growth and development of every living organism. This is also true of floral initiation since cell multiplication is necessary.

Single applications of inhibitors of steroid biosynthesis, delayed flowering in the tomato without affecting the normal vegetative growth. This strongly

suggests that the flowering stimulus for the first inflorescence in the tomato may be a steroid or an isoprenoid.

Mechanisms of flowering:

Reciprocal grafts of early and late cultivars suggested the presence of both a flowering stimulus and an inhibitor for flowering in the plumule leaves. Honma, Wittwer and Phatak (55) have reported that the node number to the first inflorescence is controlled by one major gene pair with earliness being dominant. It is thus possible that the major gene, which controls node number, in its homozygous recessive form produces an inhibitor which in turn delays flowering in the late tomato cultivars.

There is some evidence that the auxin-gibberellin balance significantly modifies flowering (15, 16, 34, 35, 45, 46, 47, 64, 86, 103, 110, 111, 113, 114, 131, 133, 135). Higher auxin levels promote earliness. This is reflected by direct induction by auxin of earlier flowering and the results from exposure to higher light intensity, and to cold temperatures both of which increase the amounts of endogenous auxin. Conversely high gibberellin levels inhibit flowering reflected by the results from exogenous

gibberellin application, long day exposure, and CCC induced early flowering. The data presented by Honma, Wittwer and Phatak (55) also suggest the presence of minor genes or modifiers affecting the expression of the major gene controlling the node number to first inflorescence. These minor genes or the modifiers may be controlling auxin-gibberellin synthesis in the tomato thereby modifying the expression of the major gene. Thus formation of the flowering stimulus in the tomato is a continuing process and earliness or lateness of cultivars is determined by an inhibitor for the flowering stimulus. The auxin-gibberellin balance may also be the mechanism through which tomato cultivars respond to the external environment.

Gibberellin induced microgametophyte development:

The significant role of gibberellins in the development of microgametophyte in monoecious and gynoecious cucumbers is well known (41, 88). The present studies suggest that the gibberellins probably play a significant role in the development of micro-gametophyte (androecium), not only in the plants having unisexual flowers, but also on plants such as the tomato with bisexual or hermaphroditic flowers.

SUMMARY

The objectives of this study were to identify the origin and nature of the flowering stimulus in the tomato and to regulate the appearance of the first inflorescence. The procedure was to expose young seedlings to the various treatments during the critical period for the initiation of the first inflorescence, which is 1 to 2 weeks interval immediately following cotyledon expansion. Environmental factors (nitrogen nutrition, mineral nutrient deficiencies, photoperiod, light intensities, temperature, atmospheric carbon dioxide), reciprocal top-root grafting of early and late cultivars, removal of plant parts, treatment with various plant growth substances and metabolic inhibitors, analysis of naturally occurring plant growth substances in the seedlings and development of specific floral parts following application of plant growth substances constituted the experimental approach.

Flowering was delayed either from the lack of all nutrients, or from the absence of magnesium, phosphorus, and nitrogen. The optimum nitrogen levels for earliest

flowering related to season of the year. During the summer higher nitrogen was utilized than in the winter. The first inflorescence was initiated in the absence of added nutrients in the solution cultures. Plants flowered earlier under short days and a high light intensity. Flowering on plants was delayed when they were exposed to 36 hour as compared to 24 hour cycles of the same light-dark ratio and total exposures of light and dark. The numbers of flowers in the first inflorescence were increased by low root temperatures but not by low top (air) temperatures. Conversely low top (air) temperatures induced earlier flowering as to the node number. Anthesis occurred earlier in plants exposed to a high atmospheric carbon dioxide level.

Flowering was not modified by hypocotyl grafting or grafting without plumule leaves on the rootstocks. It was, however, greatly modified by grafting scions onto the rootstocks with plumule leaves. Root removal was without effect, and the results for cotyledon removal were inconsistent. The effects of plumule leaf removal on flowering were dependent on temperature.

Indole-3-acetic acid induced early flowering. Gibberellin delayed flowering. Inhibitors of vegetative growth had contrasting effects on tomato flowering

dependent upon the chemical used. DNA-RNA synthesis inhibitors and an inhibitor of protein synthesis delayed flowering only after the exposure of the tomato seedlings for extended period. This was accompanied by a marked suppression of vegetative growth. Single applications of steroid synthesis inhibitors markedly delayed flowering with no effect on vegetative growth. Effects of anthogenes were inconsistent.

The endogenous gibberellin contents of long day grown seedlings were higher than those grown in short days. Seedlings of late cultivar had higher gibberellin content than those of early cultivar.

Gibberellin applied through solution culture root media promoted the development of anthers containing normal pollen in the flowers of a stamenless mutant.

CONCLUSIONS

The present studies and those reported elsewhere suggest that the flowering in the tomato is a continuing process. A flowering stimulus and possibly an inhibitor originates in the leaves. Differences in number of nodes subtending the first inflorescence between the two cultivars may be related to a differential biosynthesis of an inhibitor. Synthesis of this inhibitor is probably controlled by one major gene. This gene in its homozygous recessive form produces an inhibitor which delays flowering.

The auxin-gibberellin balance seems to further modify the expression of the flowering stimulus for the first inflorescence. High auxin levels promote flower initiation. Conversely, high gibberellin levels delay floral initiation. Responses of tomato seedlings to certain environmental factors appears to be mediated through differential biosynthesis of auxins and gibberellins.

High auxin, low gibberellin, some growth inhibitors, low temperature, short days, high light intensity, high atmospheric carbon dioxide, and the presence of plumule leaves on rootstocks of early cultivars promote flowering.

Conversely, low auxin, high gibberellin, other growth inhibitors, high temperature, long days, low light intensity, low atmospheric carbon dioxide, plumule leaves on rootstocks in late cultivar, and metabolic inhibitors delayed flowering.

Gibberellin plays a significant role in the development of the microgametophyte.

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