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EFFECT OF CYTOKININS AND GIBBERELLINS ON FLOWERING AND FRUIT SET OF TOMATO (LYCOPERSICON ESCULENTUM MILL.) UNDER HIGH TEMPERATURE

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EFFECT OF CYTOKININS AND GIBBERELLINS ON FLOWERING AND FRUIT SET OF TOMATO (*LYCOPERSICON ESCULENTUM* MILL.) UNDER HIGH TEMPERATURE

by

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Satti Mohamed ElZein Satti

entitled Effect of cytokinins and gibberellins on flowering and fruit set of tomato (Lycopersicon esculentum Mill.) under high temperature

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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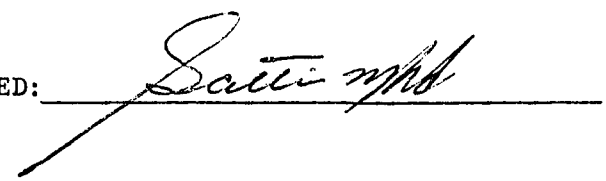
SIGNED: 

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ABSTRACT

Tomato plants (Lycopersicon esculentum Mill.) were grown in the greenhouse and in the field during 1979 and 1980. The inflorescences were treated with gibberellin (GA4/7) and/or benzyladenine (BA). Root exudate was collected at various stages of growth and development for the estimation of the levels of cytokinins in the plant. Soluble sugars and starch were determined in inflorescences at different stages of development. Partitioning of dry matter between the different plant portions was studied to evaluate growth of tomato plants in two different conditions.

The application of GA4/7 and BA to tomato inflorescences promoted the development and increased the number of flowers. These growth regulators substantially increased fruit set and yield of tomatoes in both greenhouse and field experiments.

Determinations of carbohydrates in inflorescences treated with growth regulators showed higher amounts of soluble sugars and starch over a considerable period of development.

The level of cytokinins in root exudate was higher during early phase of vegetative growth. At the time of bud formation and anthesis, the level of cytokinins declined. The quantity of translocated cytokinins in the greenhouse was 4 to 5 times higher than under high temperatures in the field. The low levels of cytokinins were associated with poor flower development.

Field grown tomato plants produced more vegetative growth and fewer inflorescences than plants in the greenhouse. Allocation of assimilates to newly developed leaves and low level of growth regulators in buds and inflorescences might contribute towards more vegetative growth but poor flowering under high temperatures.

CHAPTER 1

INTRODUCTION

Commercial production of tomatoes in many arid regions is limited because of unfavorably high temperatures during flowering and fruit set. Although differences are known to occur among varieties, flower formation in most tomatoes is drastically reduced, and flowers which are formed are often dormant and fail to reach anthesis and set fruits when temperatures exceed 30 C.

Currently, the physiological mechanisms responsible for high temperature inhibition of flowering and fruiting of tomatoes are not well understood, but it seems probable that reduced levels of growth regulators or unfavorable distribution of carbohydrates may be factors which contribute to prevention of proper flower development.

There is evidence that a measurable reduction in cytokinin activity in root exudate is observed when a plant is exposed to water stress, flooding and salinity or excess osmotica. In these cases stress is applied to the root and changes in shoot growth are the manifestation of the stress effect on the root. It has been suggested that roots are the site of cytokinin synthesis and that biosynthesis of cytokinin in the shoot has so far been reported only in fruitlets.

Studies of plant responses to an environmental factor such as water and salinity have indicated that root factors are involved in the regulation of growth and metabolism of the shoot. Poor shoot growth of

maize at low temperature was accompanied by lower gibberellin and cytokinin activity and a higher level of inhibitors in xylem exudate (Atkin, 1973). The use of the synthetic cytokinin, benzylaminopurine has been reported to overcome the reduction of stem growth in tomato caused by waterlogging the roots (Railton and Reid, 1973).

Kinet (1977b) found that localized applications of gibberellins 4 and 7 (GA4/7) and benzyladenine (BA) on inflorescences greatly enhanced flower development under low light intensities. Also, many farmers commonly remove developing vegetative regions to enhance flowering. Conceivably, this removal of "sinks" will make more carbohydrates available for the developing flowers and fruits.

The objectives of this study were: a) to determine if applications of BA and GA4/7 will influence flowering and fruit set of tomatoes grown in Arizona during the summer, when temperatures often exceed 35 C; b) to establish whether quantitative changes in cytokinins occur in root exudates of tomato grown under high temperature; and c) to gain a better understanding of how high temperature might influence carbohydrate distribution in the plant.

CHAPTER 2

LITERATURE REVIEW

The regulation of plant growth was first thought to be the exclusive function of auxin, and Went (1928) proclaimed "Without the growth substance there is no growth". But the concept of regulation had to be expanded as other hormones were found which affected growth processes. As our knowledge of the relationship of hormones to the cell and the entire plant improves, we are approaching a better understanding of the intra-and intercellular regulation of growth and the interaction with the environment.

Effect of Temperature on Flowering and Fruit Set

Growth, flowering and fruit set of tomato are profoundly modified by temperature. Lewis (1953) showed that tomato seedlings when exposed to a low temperature of 14 C in contrast to a high temperature of 25 to 30 C after cotyledon expansion, had more flowers on the first inflorescence. Subsequent reports by Wittwer and Teubner (1956) and Calvert (1957) have confirmed the positive effects of low temperature on tomato flowering. Exposure of tomato seedlings, after cotyledon expansion, to low temperatures of 10 to 13 C resulted in greater flower numbers on the first inflorescence (Calvert, 1959; Saito, 1962).

The differentiation and growth of floral structures of most plant species follows a regular pattern of development until anthesis.

At this point a stimulus for continued growth is required; otherwise the ovary will discontinue its development. In the tomato, pollen tube growth in the style stimulates fruit set and development.

Style exertion, as a cause of fruit set failure in tomato, occurs in greenhouse production in winter and in the field during periods of high temperature. The significance of style exertion in the cultivated types is related by many workers to the failure of fruit set and blossom drop especially at high temperature in some varieties (Johnson, 1955; Abdalla and Verkerk, 1968; Charles and Harris, 1972). Rick and Dempsey (1969) found that the lower stigma position affords better self pollination, hence improved fruit set. Hot dry winds might cause drying of the stigma and lead to reduction in fruit set (Coyne, 1968). During high temperature conditions, low fruiting was observed irrespective of the position of the stigma (Rick and Dempsey, 1969) so the stigma position did not account completely for high or low fruit set in tomatoes. High temperature might affect the substrate stored in the pollen grain, causing slow growth of the pollen tube (Abdalla and Verkerk, 1968) or possibly the dormant ovaries were not fertilized because of pollen sterility (Johnson and Hall, 1953).

A Role for Carbohydrates

Abdalla and Verkerk (1970) reported that high temperature conditions caused tomato vines to be generally small with thin elongated branches, resulting in reduced truss capacity to set and carry fruits of good size. Many workers have suggested that carbohydrate synthesis is low at high temperatures or that the rate of respiration exceeds

that of photosynthesis. Went (1957) showed that poor tomato flower development at high temperature was due to insufficient sugar supply to the growing point and whatever reaches the growing point is immediately used in growth. Hussey (1963) showed that there is competition for available assimilate between the developing leaves and the apex. Calvert (1969) also found that at earlier low temperature the apex enlarges rapidly, while the rate of leaf production is low; consequently flower initiation is achieved with a minimal leaf number before the first inflorescence. Some researchers believe that carbohydrate stress at high temperature is an important factor in blossom drop but there is little evidence to support this view. At high temperature there is a negative net photosynthesis since there is a progressive increase in respiration. Others have suggested that low carbohydrates are not critical in the failure of fruit set in heat sensitive cultivars (Johnson and Hall, 1952; Leopold and Scott, 1952). However, studies on flower development under low light conditions support the view that carbohydrate stress affect fruit set (Kinet, 1977b). Kinet (1977b) showed that there is a flowering inhibition in tomatoes caused by young leaves, and that removal of young leaves, particularly those produced just before inflorescence initiation, promoted flowering. It was assumed that defoliation was effective because it removed a primary sink for assimilates. Inadequate supply of assimilates, has been shown to cause microspore sterility and degeneration (Howlett, 1939).

Tanaka et al. (1974b) has shown that the translocation efficiency of tomato cultivars with an indeterminate growth habit is relatively poor. Less than 20% of fixed ^{14}C was exported from the leaf in 24 hours.

Root Cytokinins

Traditionally, the root has been viewed as an organ of water and salt absorption and most research in root physiology centered around these two functions. Recently it became evident that the root supplies cytokinins to the upper parts of the plant and that root cytokinins may exert hormonal control over certain metabolic functions of the shoot (Kende and Sitton, 1967). Went (1943) proposed that a growth hormone was supplied by the root and needed for stem growth. This hypothesis was based on the observation that growth of the shoot was inhibited when the root was removed and could be restored by treating leaves of rootless tomato plants with coconut milk or pea diffusate. Richmond and Lang (1957) discovered that kinetin delayed senescence of detached leaves; as reflected in higher protein and chlorophyll levels in the treated leaves. This idea was further strengthened by Mothes and Engelbrecht (1963) who exposed tobacco leaves to heat stress, and found that either root factors or kinetin applied to the leaf increased the heat tolerance of the tissue. According to these workers, kinetin or a root factor could stabilize normal metabolic processes occurring in the shoot, thus rendering it less susceptible to stress.

Plant responses to high or low temperature, drought or salinity have similar effects that vary with the plant age, growing conditions and the genetic makeup of the plant. Stress can result in profound metabolic changes in the plant; these could be rapid adaptive mechanisms such as in stomatal closure or slow developmental and morphological changes such as the tendency to flower or grow vegetatively. It has

been indicated that even a slight stress could affect such different phenomena such as cell growth, cell wall synthesis, nitrogen and chlorophyll metabolism and hormone balance of the plant (Hsiao, 1973). Root tips have been considered a major site of cytokinin synthesis in higher plants. This was documented by the finding that substantial amounts of cytokinins exist in root tips of peas (Short and Torrey, 1972) and that cytokinin could be detected in the xylem sap of various plants (Kende and Sitton, 1967; Skene, 1972; Van Staden and Davey, 1976). Studies of root temperature on growth are documented (Fujishige and Sugiyama, 1968; Watts, 1972a), but some reports have attempted to relate responses to possible changes in the growth substances exported from root to shoot. Skene and Kerridge (1967) showed that different root temperatures qualitatively changed the cytokinin activity in xylem exudate from grape vines, while Atkins, Barton and Robinson (1973) demonstrated that high root temperature for 17 days duration influenced growth substances exported from maize root to shoots. Thus the poor shoot growth was accompanied by lower cytokinin activity and a higher level of inhibition in xylem exudate. Menhenett and Wareing (1975) presented evidence showing that low root temperature leads to alterations in the hormone content of the sap from tomato, which may contribute to the reduction of shoot growth. Niimi and Torikata (1978) suggested that cytokinins synthesized in roots may play an important role in controlling the growth of flower clusters and floral organs. They also reported a high activity for cytokinins at the early stages of grape flower clusters, and there after decreased, but again the activity increased rapidly at

the time of full bloom. Phosphorus deficiency resulted in a decrease in the number of flowers in tomato; this was accompanied by a decrease in the cytokinin activity of the root exudate (Menary and Van Staden, 1976). Davey and Van Staden (1976) have indicated that zeatin and zeatin riboside are present in similar concentration in the root exudate over the period of growth encompassing flower bud formation, however, the zeatin riboside level is greater than zeatin before and after flowering in tomato plants.

Application of Benzylaminopurine and Gibberellin (GA4/7)

The literature contains reports of growth and fruit set induced by added hormones, but this is not necessarily an evidence for an endogenous regulatory role. The possibility remains that hormone might not be the primary regulator, but rather an interaction of numerous systems, is responsible for induction of flowering and fruit set. Crane (1969) proposed that fruit growth is controlled not directly by hormones, but by the fruit capacity to mobilize hormones and nutrients into the fruit. Growth regulators which exogenously stimulate fruit set could promote mobilization of endogenous hormones and nutrients to the fruit from other plant parts. Mobilization out of the leaves to the fruits is lacking at anthesis but becomes strong after fertilization (Linck and Swanson, 1960). Kinet (1977) applied benzyladenine and gibberellic acid to promote floral development. Tse et al. (1974) were able to enhance flower development in bougainvillea, following application of cytokinin. Mullins (1967) reported that cytokinins are necessary for

the normal development of grape flowers, while Skene and Antcliff (1972) showed that they affect flower number in grapes. Phosphorus deficiency resulted in a decrease in the number of flowers that develop on the first inflorescence of tomato. This was accompanied by a decrease in the cytokinin activity of root exudate while application of kinetin to the growing medium increased the number of flowers produced by the seedlings (Menary and Van Staden, 1976). After studying application of benzyladenine on seedlings of pharbitis, Ogawa and King (1979) suggested that cytokinins can have an indirect effect on photoperiod induction, by altering assimilate and, hence, floral stimulus translocation to the shoot apex. Weaver (1965) observed that application of cytokinins to flower clusters promoted fruit set in grapes. Aung and Byrne (1975) found that apical application of benzyladenine and gibberellin A4/7 alone or in combination, promoted hypocotyl and cotyledonary growth of tomato plants, foliar sprays of benzyladenine partially overcome the dwarfing effect of stress and restored the gibberellin content of tomato plants. Rylski (1979) has shown that deformation may occur in winter grown tomatoes both as a consequence of abnormal flower development under low temperature conditions and as a result of growth regulator treatment. Kinet (1978) has established the promotion of inflorescence development in tomato plants grown in adverse light conditions by application of benzyladenine and gibberellin localized on the inflorescence. Their study suggested that in combination with benzyladenine, GA4/7 was more effective than GA_3 in promoting the development of the inflorescence. The evidence of hormonal involvement in flowering and fruit set is

further substantiated by the work of Greene (1980) who reported that sprays of GA4/7 and BA at bloom time, increased fruit set in apple trees.

The mechanisms by which these growth substances favor the development of the inflorescence in tomato is not clear. A possible action could be by redirecting the flow of assimilates as has been previously shown for cytokinins and for gibberellins in other species. The question of the precise stimulating agent for fruit set becomes more clouded with the evidence of possible involvement of any one of the known plant hormones and a myriad of other growth regulating compounds. Crane (1965) has shown that parthenocarpic figs can be produced by application of either an auxin, gibberellin or cytokinin while other species show little responsiveness to any of these hormones. Van Overbeck (1966) considered that the differences in responses of various species to fruit setting agents could be explained by the succession of different physiological stages in the life of the fruit with each stage subject to different regulatory mechanisms involving the interaction and antagonism of different growth substances.

CHAPTER 3

MATERIALS AND METHODS

Growing Conditions

All the field experiments reported here were done at the University of Arizona, Campbell Avenue farm greenhouse and the University experiment farm in Marana from July through November 1979 and 1980. Tomatoes (Lycopersicon esculentum Mill.) cv. 'Walter' were grown in a soil mixture in Speedling trays for four weeks. Subsequently they were transplanted into 5 gallon containers and kept in the greenhouse at a day temperature of 30 C and night temperature of 18 C. Watering and management practices were done as required. The plants were arranged in a randomized complete block design with five replications. Each block was composed of three treatments, namely, benzylaminopurine (BA), BA + gibberellin (GA4/7), and a control with five plants in each treatment, five extra plants were maintained in each block for collecting root exudate thus 100 plants were kept in the greenhouse. The Marana experiment was designed and treated similarly to the greenhouse experiment, except that the prevailing temperatures at daytime were 32-37 C during August to October and a night temperature of 15 to 21 C during the same period (see appendix for temperature records).

Application of Growth Substances

Aqueous solutions of 25 ppm GA4/7 (obtained from Abbott Laboratories, North Chicago, IL.) and/or 10 ppm. BA (Calbiochem, San Diego,

CA.) were prepared with Tween-20 at a concentration of 0.1% as surfactant. The solution were applied to small cotton plugs using a syringe, these were then placed on the inflorescence at the time when they were just visible (macroscopic stage). The growth substances were applied at 3 day intervals for a period of 15 days to each of the first 3 inflorescences on the main stem of the plant. Control plants received only distilled water containing Tween-20.

Root Exudate

To obtain the root exudate, the plants were watered at 8:00 a.m. At 10:00 a.m. they were cut approximately 3 to 4 cm above soil level. Sterilized rubber tubes were attached to the cut stems and the exuded sap was collected for a period of 6 hours. Sap from each plant was measured, filtered and stored at -20 C until required for bioassay. Root exudates were collected from tomato plants in the greenhouse and Marana farm starting at the seedling stage and up to the time of fruit set to cover different physiological stages of growth and development. The cut shoot was used for growth data.

Growth and Development Data

a) For inflorescence development, the number of leaf nodes preceding the appearance of the first 3 inflorescences on the main stem was recorded as an index for earliness and onset of flowering. The number of open flowers in each inflorescence was also counted.

b) The number and weight of fruits obtained in each inflorescence was recorded.

c) Growth data were obtained from cut shoots including fresh and dry weights of leaf, stem and root separately. Stem heights were measured from the cotyledonary node to the apex.

The different fractions were oven dried at 80 C for 72 hours and their dry weights measured.

Cytokinin Bioassay

The frozen sap was thawed and partitioned with equal volumes of petroleum ether for purification. The aqueous phase with a pH of 5.2 to 5.4 was adjusted to 5.8 and used in the soybean callus (Glycine max cv. 'Acme') assay, described by Miller (1968) for detection of cytokinins. Exudate from five stages of growth was used, i.e., seedling, vegetative, flowering, post-anthesis and fruit set. For each stage, 3 ml exudate were used in Miller's media that was prepared without kinetin. The media containing exudate was set up into 10 ml bottles, which were inoculated with one piece of callus. A series of kinetin standards were cultured at the same time, each standard was replicated ten times, from this a dose response curve was plotted. After 3 weeks of incubation at 27 C, the callus was weighed fresh. For purpose of comparisons, assays of exudate from the greenhouse and Marana were conducted simultaneously.

Determination of Carbohydrate Content

Quantitative estimation of total soluble sugars and starch were done in freeze dried inflorescences using a modified Yemm and Willis (1954) and Ebell (1969) procedure. The tissue was extracted with 80% ethanol at 80 C for 5 minutes. After centrifugation (5,000 rpm at room

temperature for 10 min.) the residue was washed twice with hot 80% ethanol. The resulting supernatants were combined and ethanol soluble sugars were determined using freshly prepared anthrone reagent. The extract and anthrone were boiled for 12 minutes and readings were taken at room temperature in a spectrophotometer at 625 nm. A standard curve was prepared with glucose.

For starch determination, the ethanol insoluble fraction was treated with 0.1 N-NaOH and boiled for 30 minutes. The supernatant was neutralized with 0.3 N-acetic acid, then hydrolysed with equal volumes of the enzyme amyloglucosidase and incubated at 45 C for 12 hours. The hydrolysate was centrifuged at 1100 rpm at room temperature for 5 minutes. The starch glucose was determined with ortho-toluidine at room temperature in a spectronic 20 at 630 nm.

Glucose equivalent starch determinations with anthrone and ortho-toluidine have agreed, however, ortho-toluidine is glucose specific, rapid and stable for at least 3 hours (Ahmet Arslan, University of Arizona, personal communication).

Statistical Procedure

Computerized statistical analysis of variance was done with the assistance of the University Agricultural Experiment Station Quantitative Studies Center. Treatment means were compared using the least significant difference (LSD) and Student-Newman-Keul's multiple range test.

The data presented in this study was compiled from 1979 and 1980, since there were no significant differences between the two years results, the results shown represent means of observations from both years.

CHAPTER 4

RESULTS AND DISCUSSION

Effect of BA and BA/GA 4/7 on Flowering and Fruit Set

The number of flowers per inflorescence was increased significantly with BA alone or in combination with GA4/7 in both the Greenhouse and Marana Experiments (Table 1). There were no differences between the three inflorescence in the number of flowers obtained with the same treatment in the same location, however, the more favorable environment in the greenhouse had a promotive effect in increasing flowering in all the treatments. Under Marana conditions BA and BA + GA4/7 were not statistically different except when applied to the first inflorescence. Kinet (1977b) reported that BA alone had a promotive effect on inflorescence development. Promotion of flowering by GA in several rosette species is well known. The effect of GA is more pronounced when the inflorescence is initiated by cytokinin treatment as has been demonstrated in bougainvillea (Tse, 1974). The data presented in Table 1 clearly indicate a role for cytokinins in flowering of tomatoes. This supports the study on flowering of pharbitis (Ogawa and King, 1979) when BA was applied. Flowering was promoted in association with an enhanced export of assimilate from the leaf or possibly by enhancing floral stimulus translocation to the shoot apex. Although these data do not prove that cytokinins per se will increase

Table 1. Effect of BA and BA/GA4/7 on number of tomato flowers developed in the first 3 inflorescences.

	Control	BA	BA/GA4/7
	No.		
Greenhouse			
1st inflorescence	4.7 a[y]	7.0 b	8.7 b
2nd	4.3 a	7.0 b	8.5 b
3rd	4.4 a	7.2 b	8.3 b
Marana			
1st inflorescence	2.8 a	5.4 b	7.0 b
2nd	3.2 a	6.0 b	6.0 b
3rd	2.7 a	5.2 b	5.6 b

[y]Means followed by the same letter within the same row are not significantly different at the 5 percent level according to Student-Newman-Keuls procedure.

flower number, they clearly indicate that the endogenous level of these hormones is sub-optimal at least at the time of flowering.

Environmental conditions can greatly alter the capability of a plant to set fruit. Often fruit set failure is due to poor flower formation, including reduced inflorescence size and abnormalities in the flower structure that cause embryo abortion. The application of BA and BA with GA4/7 to the inflorescence of tomato significantly increased fruit set in both the greenhouse and at Marana (Tables 2 and 3). There was very little difference in fruit set within the three inflorescences receiving the same treatment at the same location, however, a very highly significant difference was obtained between greenhouse and Marana. The highest yield per inflorescence was 714 grams in the greenhouse, compared with 385 grams in Marana. Generally, the mean fruit weight decreased when the number of fruits increased in both locations.

There are numerous cases of fruit set or growth induced by added hormones, but this is not necessarily evidence for a direct or endogenous regulatory role. The possibility remains that hormones may not be the primary regulators of fruit set. The interaction of numerous systems in fruit development led Crane (1969) to propose that fruit growth is controlled not by hormones directly but by the fruit capacity to mobilize hormones and nutrients into the fruit. Cytokinins are transported to the shoots via the transpiration stream (Van Staden and Davey, 1979) and most the ^{14}C was incorporated into the leaves and side shoots, with only a small proportion detected in the fruits. Recent studies have cast doubt on the capability of developing fruits to synthesize their entire cytokinin complement (Van Staden and Button,

Table 2. Percent fruit set in tomato as affected by growth regulator treatment.

	Control	BA	BA/GA4/7
	%		
Greenhouse			
1st inflorescence	33 a	71 b	69 b[y]
2nd	57 a	85 b	78 b
3rd	45 a	81 b	71 b
Marana			
1st inflorescence	17 c	47 a	57 a
2nd	20 c	42 a	42 a
3rd	26 c	56 a	46 a

[y]Means followed by the same letter are not significantly different at the 5% level, according to Student-Newman-Keuls procedure.

Table 3. Tomato fruit number and weight as affected by BA and BA/GA4/7 in the first 3 inflorescences.

Treatment	Inflorescence	No. of Fruits	Total Fruit Wt per Inflorescence	Mean Fruit Wt
		no.	g	g
Greenhouse				
Control	1st	1.55 a[y]	250 a	161 a
	2nd	2.48 a	311 b	125 b
	3rd	2.00 a	278 ab	139 b
BA	1st	5.02 b	706 c	140 b
	2nd	6.00 b	646 c	107 c
	3rd	5.80 b	634 d	109 c
BA/GA4/7	1st	5.95 b	714 e	119 c
	2nd	6.60 b	656 c	99 c
	3rd	5.90 b	663 c	112 c
Marana				
Control	1st	0.47 a	68 a	145 a
	2nd	0.63 a	86 b	136 a
	3rd	0.69 a	89 b	129 a
BA	1st	2.56 b	322 c	126 a
	2nd	2.63 b	286 d	108 b
	3rd	2.90 b	266 d	92 b
BA/GA4/7	1st	3.96 c	385 e	97 b
	2nd	2.63 b	288 d	109 b
	3rd	2.60 b	281 d	108 b

[y]Means followed by the same letter within the same column are not significantly different at the 5 percent level, according to Student-Newman-Keuls procedure.

1978; Summons et al., 1979). There is thus considerable evidence for the demand of hormones during fruit set particularly when the endogenous levels are low under high temperature.

Soluble Sugars and Starch Content in Inflorescences

The levels of soluble sugars in the tomato inflorescence is shown in Table 4. The levels of soluble sugars were almost similar at Stage 1 (7 days from initiation) in the three treatments in the greenhouse experiment (Figure 1). There was steady increase of soluble sugars in inflorescence treated with BA and in the control, whereas in BA + GA4/7 a sharp increase was noticeable until at anthesis the levels in the three treatments were almost the same. The post-anthesis levels showed a sharp increase in BA treated inflorescences. In contrast the control showed a sharp drop in the levels of soluble sugars, whereas BA + GA4/7 treatments remained at almost the same level.

Under Marana conditions, there was a remarkable response to hormonal applications (Table 4 and Figure 2) as compared with the control. The levels of soluble sugars were higher at all stages than in the control. The levels of soluble sugars in BA treatment were noticeably high reaching 10.2 mg/g dry wt. This response was similar to BA treatment in the greenhouse experiment.

Since the inflorescence size differed remarkably in the treatments in both the greenhouse and Marana experiments, it was revealed that the total soluble sugars in the whole tomato inflorescence would illustrate the point (Table 5). At both locations, a higher level was

Table 4. Effect of growth regulator on soluble sugars content in tomato inflorescence (mg/g dry wt).

Treatment	Stage of Development[y] 1	2	3	4	5
	Soluble Sugars (mg/g) Dry Wt				
Greenhouse					
Control	6.64 a[z]	6.94 a	7.48 a	5.07 a	4.75 a
BA	6.87 a	6.55 a	7.18 a	7.55 a	10.64 b
BA/GA4/7	6.99 a	8.31 a	7.87 a	7.83 a	8.14 a
Marana					
Control	5.43 a	4.91 a	4.63 a	5.36 a	5.41 a
BA	5.51 a	8.3 b	6.47 a	6.83 a	10.2 b
BA/GA4/7	5.87 a	7.10 a	6.32 a	6.10 a	5.96 a

[y]Stage 1: 7 days from initiation; visible buds
2: 12 " " " mature buds
3: 21 " " " anthesis
4: 25 " " " post-anthesis
5: 30 " " " fruit-set

[z]Means followed by the same letter within the same treatment are not significantly different at the 5 percent level (Student-Newman-Keuls)

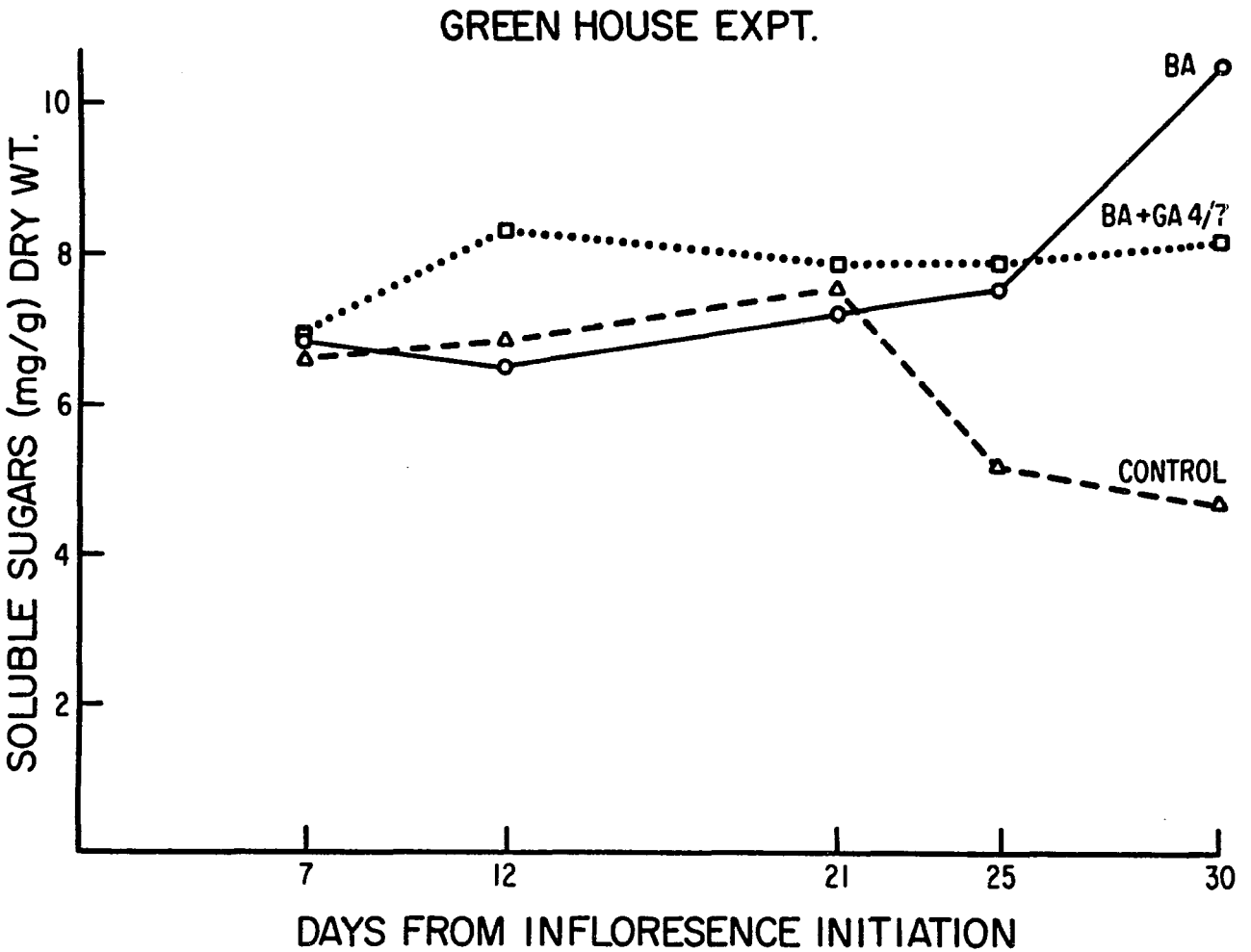

Figure 1. Effect of growth regulators on soluble sugars contents in tomato inflorescence (Greenhouse Experiment).

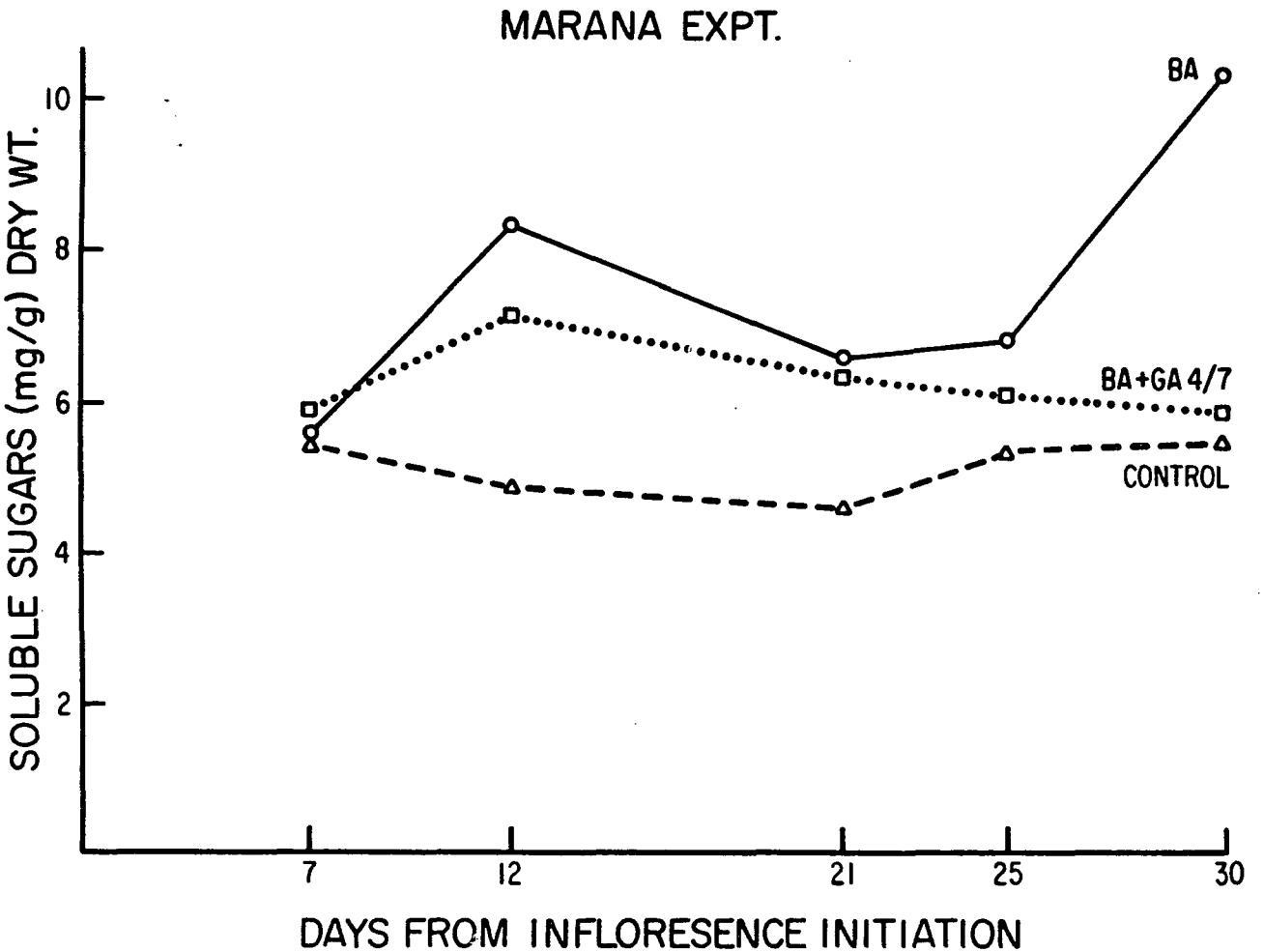

Figure 2. Effect of growth regulators on soluble sugars contents in tomato inflorescence (Marana Experiment).

Table 5. Soluble sugar content of whole tomato inflorescence (mg/ inflorescence).

Treatment	Stage of Development[y] 1	2	3	4	5
Greenhouse					
Control	1.73 a[z]	2.15 a	3.07 a	3.00 a	3.00 a
BA	1.99 a	2.30 a	4.24 b	5.00 b	8.72 c
BA + GA4/7	2.10 a	3.10 a	4.96 b	5.56 b	7.10 c
Marana					
Control	1.18 a	1.42 a	1.73 a	2.21 a	2.35 a
BA	1.41 a	2.50 a	3.13 a	3.97 b	7.90 c
BA/GA4/7	1.90 a	3.10 a	3.24 a	3.77 b	4.93 b

[y]Stage 1: 7 days from initiation; visible buds
2: 12 " " " mature buds
3: 21 " " " anthesis
4: 25 " " " post-anthesis
5: 30 " " " fruit set

[z]Means followed by the same letter within the same treatment are not significantly different at the 5 percent level (Student-Newman-Keuls).

achieved in inflorescences receiving BA + GA4/7 until shortly after anthesis, inflorescence treated with BA only, reached the highest level at the time of fruit set.

The starch content of the greenhouse controls remained at a lower level (Table 6 and Figure 3) than the treated plants throughout all stages of the inflorescence development. In BA treated inflorescence, a significantly higher level was attained at the time of anthesis. The highest starch content was obtained when BA + GA were used, a significant increase was noticeable from the time of anthesis and thereafter.

Under high temperature, the levels of starch were significantly lower than in the greenhouse at any stage (Table 6 and Figure 4). However, the control plants gave the lowest starch content, whereas there was no significant differences between BA and BA + GA plants.

The starch content of the whole tomato inflorescence is shown in Table 7. Plants treated with BA or BA + GA have accumulated higher starch in their inflorescence than the untreated controls both in the greenhouse and under high temperature in Marana. It is noteworthy to mention that the lower carbohydrate content (soluble sugars and starch) of the high temperature plants was associated with a much reduced inflorescence size, when compared with the greenhouse.

Flowering and fruit set are phenomena that are influenced by a hormonal and nutritional factors. The involvement of cytokinins in the onset of flowering and inflorescence development has been previously shown in many species (Ginzburg, 1974; Goh, 1977; Mullins, 1968; Tse et al., 1974). An evidence for a promotive effect in tomato flowering

Table 6. Starch content in tomato inflorescence (mg/g dry wt).

Treatment	Stage of Development[y] 1	2	3	4	5
	mg/g dry wt				
Greenhouse					
Control	1.95 a[z]	2.55 a	1.85 a	1.39 a	0.94 a
BA	2.83 a	2.22 a	2.70 a	2.87 a	4.35 b
BA/GA4/7	2.01 a	2.63 a	3.47 a	4.48 b	4.72 b
Marana					
Control	1.28 a	1.63 a	1.31 a	1.28 a	0.89 a
BA	1.40 a	1.86 a	1.39 a	1.53 a	2.08 a
BA/GA4/7	1.38 a	1.92 a	1.68 a	1.40 a	2.00 a

[y]Stage 1: 7 days from initiation: visible buds
2: 12 " " " mature buds
3: 21 " " " anthesis
4: 25 " " " post-anthesis
5: 30 " " " fruit set

[z]Means followed by the same letter within the same treatment are not significantly different at the 5 percent level (Student-Newman-Keuls).

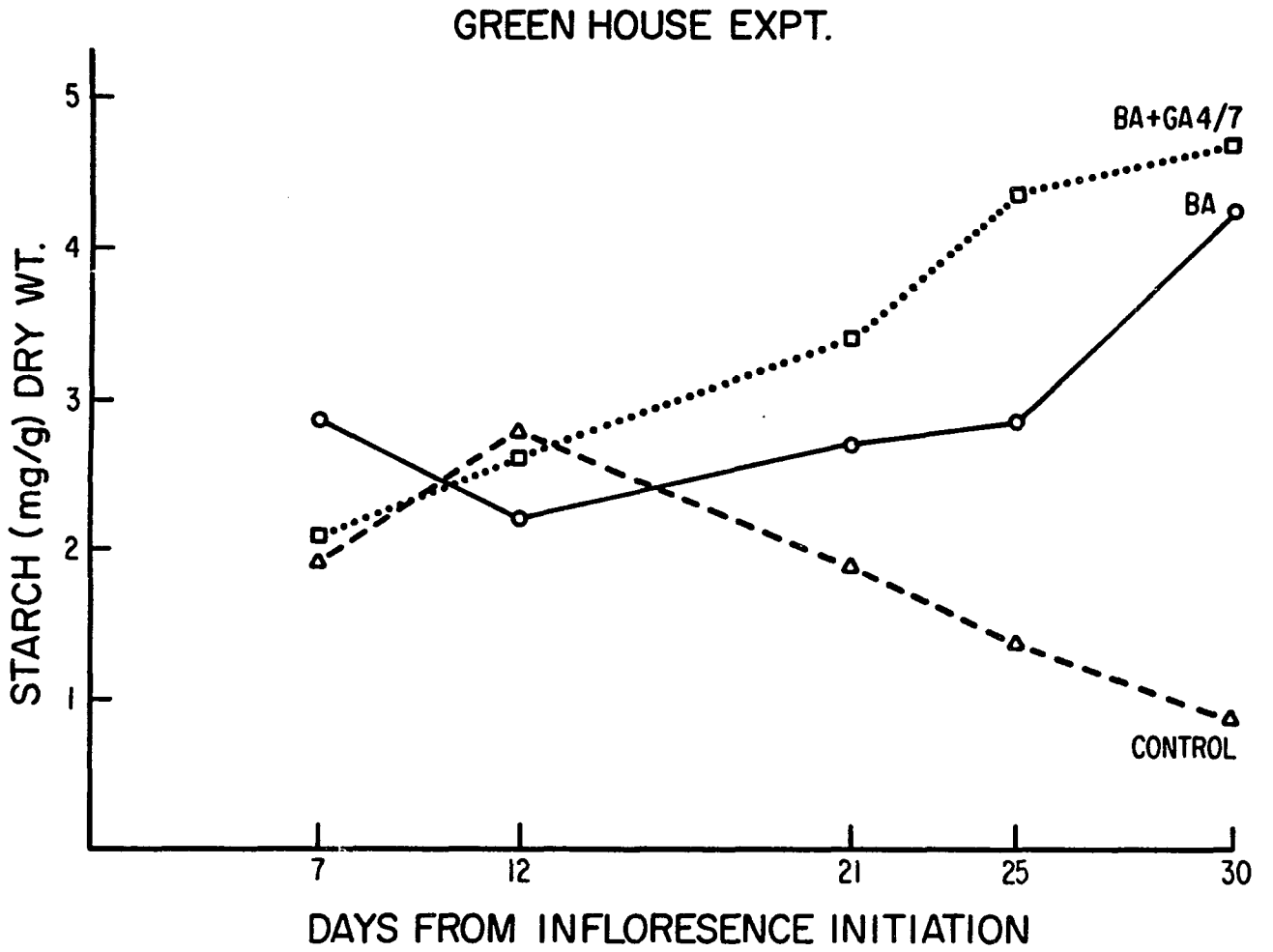

Figure 3. Effect of growth regulators on starch contents of tomato inflorescence (Greenhouse Experiment).

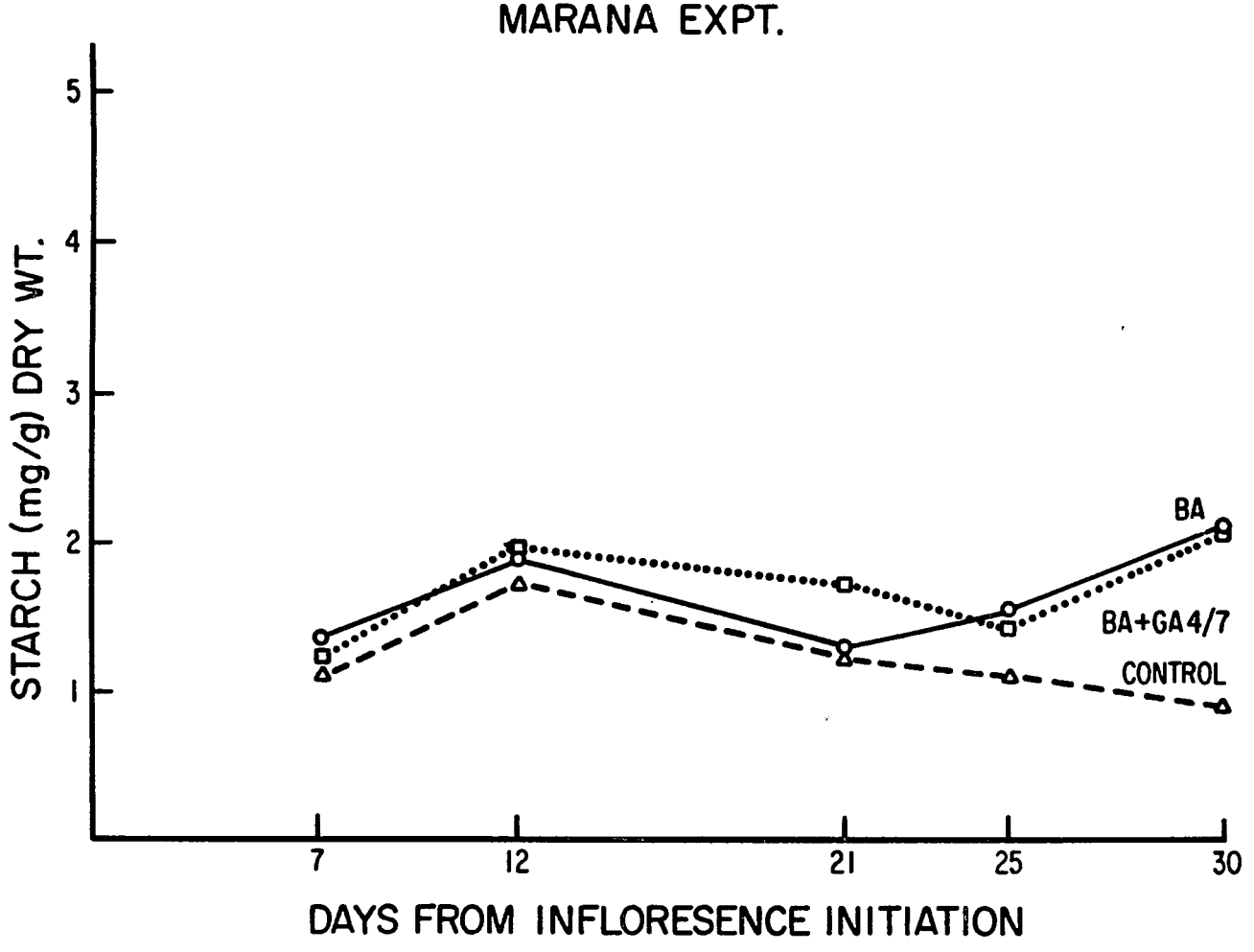

Figure 4. Effect of growth regulators on starch contents of tomato inflorescence (Marana Experiment).

Table 7. Starch content of whole tomato inflorescence (mg/inflorescence).

Treatment	Stage of Development[y] 1	2	3	4	5
	mg				
Greenhouse					
Control	.66 a	.78 a	0.85 a	.90 a	1.00 a
BA	.78 a	.82 a	1.60 a	1.90 a	3.57 b
BA/GA4/7	.69 a	.97 a	2.19 ab	3.18 b	4.11 b
Marana					
Control	.35 a	.47 a	.48 a	.53 a	.57 a
BA	.35 a	.71 a	.76 a	.88 a	1.64 a
BA/GA4/7	.44 a	.84 a	.86 a	.90 a	1.66 a

[y]Stage 1: 7 days from initiation: visible buds
2: 12 " " " mature buds
3: 21 " " " anthesis
4: 25 " " " post-anthesis
5: 30 " " " fruit set

[z]Means followed by the same letter within the same treatment are not significantly different at the 5 percent level (Student-Newman-Keuls)

was presented (Menary and Van Staden, 1976). The increase in the levels of carbohydrates and the size of the inflorescence is in agreement with Kinet (1978) who found that plants grown under insufficient light but treated with BA and GA, exhibited increased levels of carbohydrates in their inflorescences. This suggested that, the treatment of inflorescences under adverse conditions, modified the distribution of assimilates within the plant. Johnson and Hall (1953), after analyzing fruiting and non-fruiting plants, confirmed that low carbohydrates were not critical in the failure of fruit set while Osborne and Went (1953) showed that at high temperatures, fruit set can be improved by removal of vegetative growing points. This reveals a competition between vegetative and reproductive growth. Hussey (1963) showed that, the apex of tomato plants under high temperature, enlarges more slowly than at low temperature. This points towards insufficient supply of assimilates possibly because of competition. This was confirmed by Calvert (1969) who found that at earlier low temperature, the apex enlarges rapidly, and the rate of leaf production is low. The question of inflorescence initiation and development by the use of growth regulators is apparently due to changes in the direction of assimilates transport. However, when the initiation of inflorescence is poor, it is possible that an indirect effect over floral stimulus transport is likely.

Cytokinins in Root Exudates of Tomato Plants

The dry weight of the tomato plants increased from 18.6 grams at the seedling stage to 72.8 grams at the time of fruit set in the greenhouse study (Table 8 and Figure 5). The translocation of sap was

Table 8. Levels of cytokinins in the root exudate at different physiological age (Greenhouse Experiment).

Age of Plants (Days)	Shoot Dry Wt.	Volume of Exudate Per Plant	Bioassay[x] Callus Yield	Kinetin Equivalent
	— g —	——— ml ———	—— g ——	— µg —
40[y]	18.6 a[z]	17.5 a	0.37 a	2.1
47	29.8 b	22.8 b	0.34 a	1.9
60	45.3 c	30.7 c	0.29 a	1.6
68	54.0 c	36.6 c	0.36 a	2.0
82	72.8 d	13.2 a	0.49 a	2.8

[x]Bioassay media was prepared using 20 ml of Miller's media without kinetin and 3 ml exudate. In the control, callus yield was .06 g.

[z]Means followed by the same letter within columns are not significantly different at the 5 percent level (Student-Newman-Keuls).

[y]Stages in growth and development of tomato:

40 days coincides with vegetative growth
47 " " " flower bud formation
60 " " " anthesis
68 " " " post anthesis
82 " " " fruit set

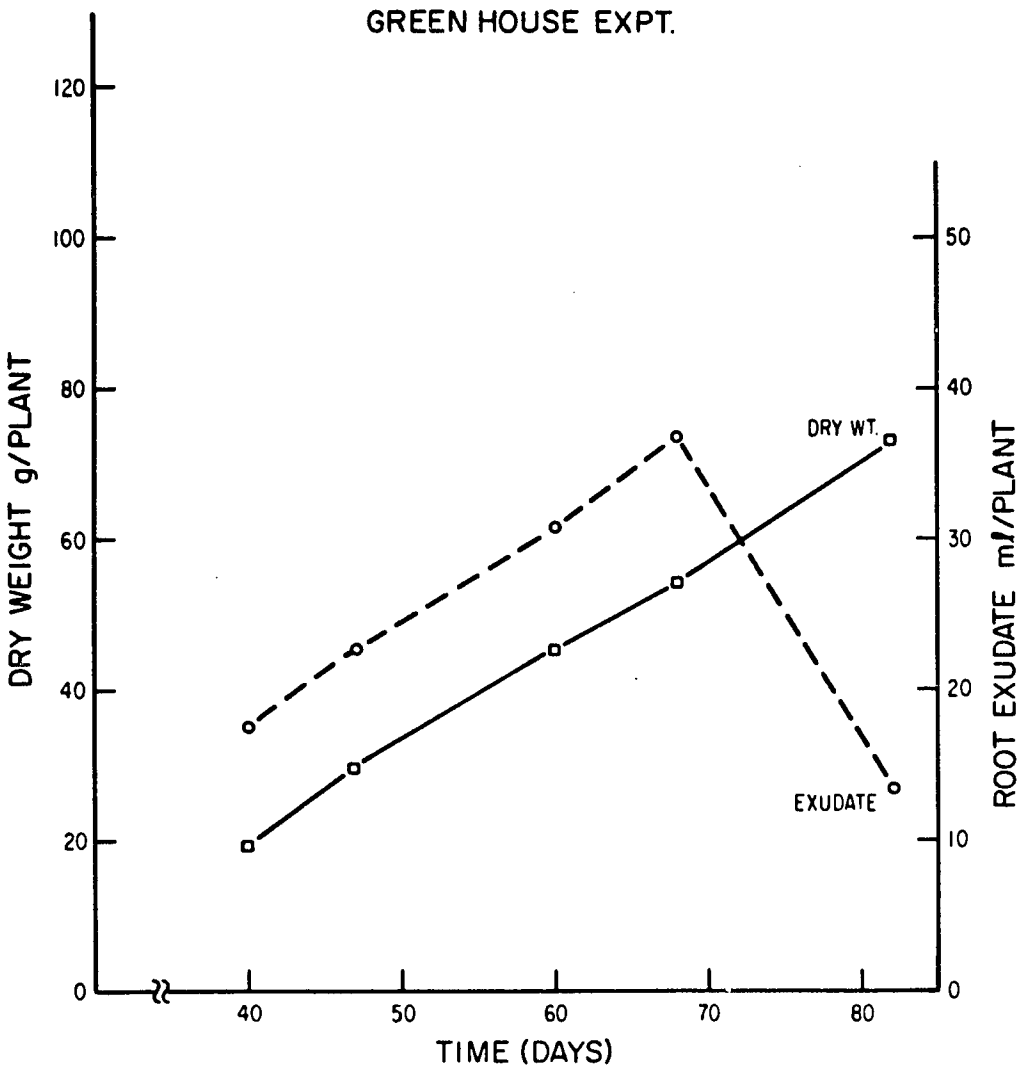

Figure 5. Dry weight increase and production of exudate of tomato with age (Greenhouse Experiment).

high until shortly after anthesis and then it declined rapidly as fruits were developing. Under high temperature (Table 9 and Figure 6), the dry weight of the tomato plants continued to increase at a slow rate until at the age of 60 days, when a very rapid phase of growth was noticeable. In contrast to the greenhouse, root exudate continued to flow at a linear increasing rate until termination of the experiment.

Figure 7 shows a quantitative bioassay for cytokinins in the root exudate of tomato plants grown in the greenhouse and in Marana at different stages of growth and development. It would appear that important quantitative changes in the cytokinin complement occur during growth and development. A relatively higher level was detected during vegetative growth in both locations, then decreased as flower buds developed and reached the lowest level at the time of anthesis. This was followed by a continuous gradual increase as fruits developed in the greenhouse. Whereas under high temperature a slight increase in the level of cytokinins was detected, and then the level decreased.

Throughout this investigation, it was assumed that the quantity of cytokinins collected from root exudate indicates the quantity which might reach the shoot if the plant was left intact. In the present work, using xylem exudate or sap as a basis for measuring the amount of cytokinins produced by the root and translocated to the shoot, new information has been gained that might help our understanding of the growth and development of tomatoes under high temperature. These results indicate that the amount of cytokinins in tomato root exudate reach their lowest level at the times of flower bud formation and

Table 9. Levels of cytokinins in the root exudate at different stages of growth (Marana Experiment).

Age of Plants (Days)	Shoot Dry Wt.	Volume of Exudate Per Plant	Bioassay[y] Callus Yield	Kinetin Equivalent
	g	ml	g	µg
40	19.8 a[z]	10.0 a	0.15 a	0.85
47	23.88 a	12.5 a	0.07 a	0.39
60	33.68 b	26.6 b	0.07 a	0.39
68	59.60 c	33.0 b	0.16 a	0.91
82	120.0 d	46.4 c	0.12 a	0.68

[y]Bioassay media was prepared using 20 ml of Miller's media without kinetin and 3 ml exudate. In the control, callus yield was .06 g.

[z]Means followed by the same letter within columns are not significantly different at the 5 percent level (Student-Newman-Keuls).

[y]Stages in growth and development of tomato:

40 days coincides with vegetative growth
47 " " " flower bud formation
60 " " " anthesis
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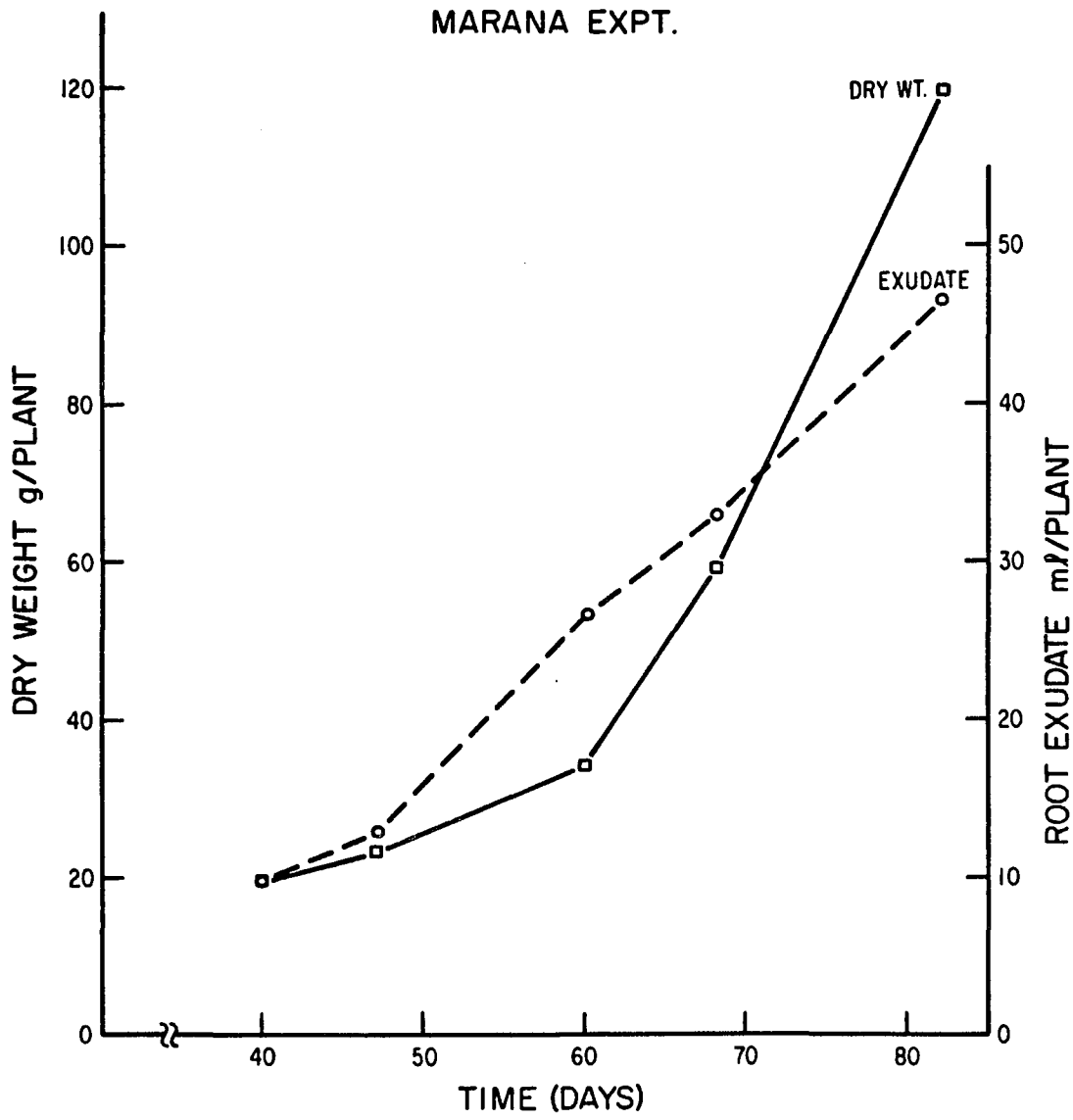

Figure 6. Dry weight increase and production of exudate of tomato plant with age (Marana).

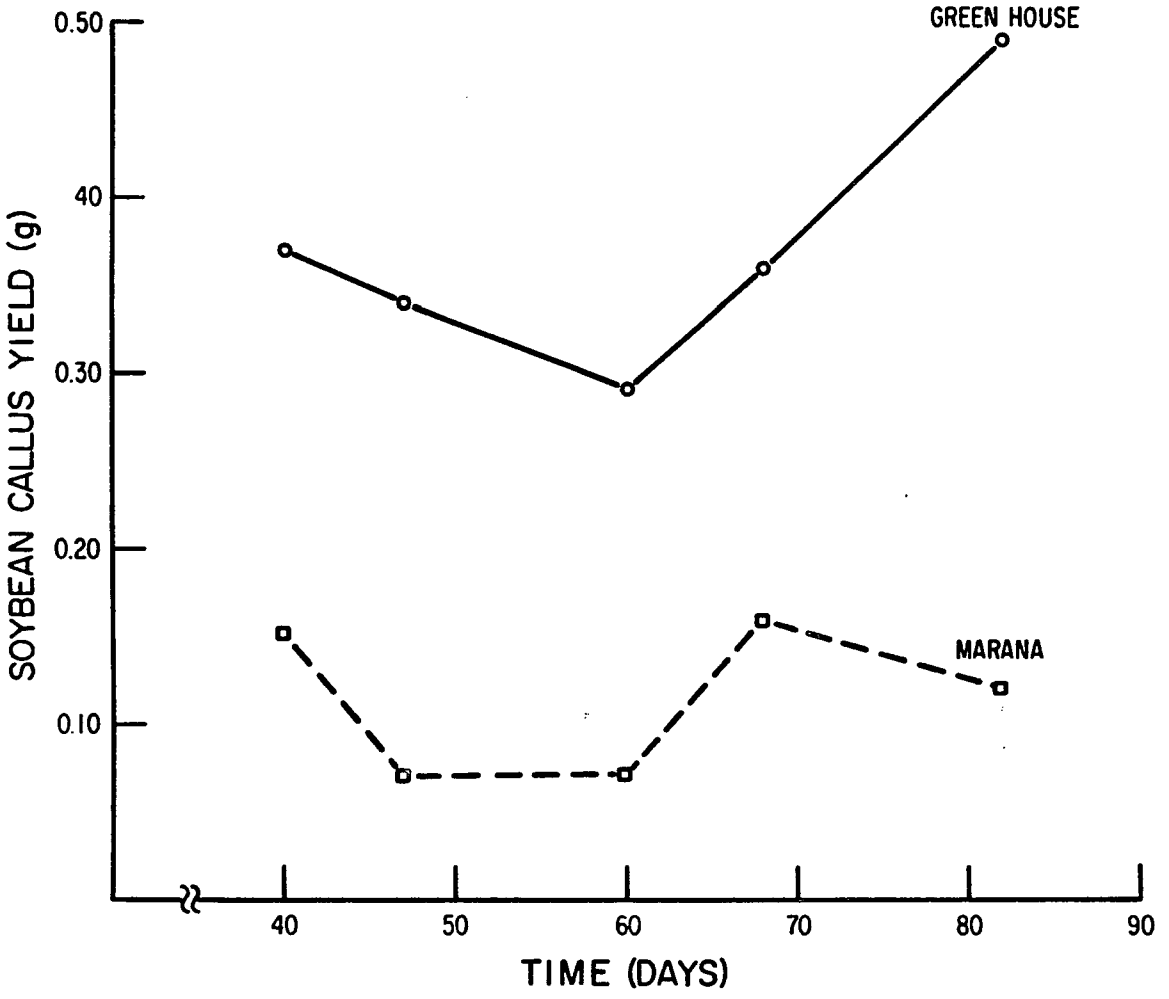

Figure 7. Cytokinins levels in root exudates of tomato plants at different stages of growth.

anthesis, this low level was accompanied by flower drop, bud malformation or abortive ovaries under high temperature conditions.

These results disagree with the hypothesis of Henson and Wareing (1974) who proposed that a decrease in the cytokinin level in Xanthium might be a necessary prerequisite for some part of the sequence of events leading to flowering.

Under greenhouse conditions, a similar trend in the translocation of cytokinins was obtained, but the amount detected was 4 to 5 times higher. A similar decrease in the total cytokinin of sunflower (Sitton et al., 1967) and in tomatoes (Davey and Van Staden, 1976) was reported when flower heads enlarged.

An important implication of this finding is that heat stress also appears to affect the level of hormone in the root and their export to the shoot. This adds up another environmental situation, to the previously known cases of flooding (Reid and Railton, 1974); water stress (Mizrahi and Richmond, 1972) and salinity stress (Itai et al., 1973) which change the hormone flow from the root to the shoot. The experimental imposition of heat stress on the root system leading to reduced hormone production, confirms the view that cytokinins are transported to the shoot mainly through the transpiration stream (Van Staden, 1981).

Partitioning of Dry Matter

Partitioning of dry matter in shoot and root of tomato plants grown in the greenhouse is shown in Table 10. The number of leaves preceding the formation of the inflorescences on the main stem, is an

Table 10. Partitioning of dry matter under greenhouse conditions.

	Age of the Plant (Days)				
	40	47	60	68	82
No. leaves to 1st inflorescence	7.40	8.0	7.2	7.6	7.4
" " " 2nd "	9.20	9.8	8.6	8.8	9.2
" " " 3rd "	10.6	10.8	10.0	10.0	10.6
Fresh wt. stems (g)	116.30 a[z]	136.52 b	156.48 c	163.90 d	204.78 e
Dry wt. stems (g)	16.94 a	22.06 b	24.32 bc	26.90 bc	34.16 d
Length of stem (cm)	61.98 a	88.10 b	105.00 c	110.00 d	127.74 e
Total no. leaves	21.20 a	22.40 b	27.40 c	34.60 d	42.20 e
Fresh wt. leaves (g)	159.04 a	164.32 b	192.60 c	202.10 d	258.52 e
Dry wt. leaves (g)	24.14 a	24.96 a	29.72 b	31.12 b	38.56 c
No. of branches	3.00 a	5.40 ab	6.80 ab	7.00 ab	7.80 ab
Fresh wt. roots (g)	22.42 a	26.80 a	28.88 ab	29.40 ab	33.26 c
Dry wt. roots (g)	6.22 a	6.86 a	7.02 a	7.84 a	9.10 a
Fresh wt. inflorescence (g)	1.68 a	2.44 a	2.66 a	9.58 b	10.48 b
Dry wt. inflorescence (g)	0.20 a	0.34 a	0.47 a	1.83 a	2.16 a

[z]Means followed by the same letter within the same parameter (row) are not significantly different at the 5 percent level according to Student-Newman-Keuls procedure.

indication of earliness to flower and set fruits. Under the greenhouse conditions, a fewer number of leaves was obtained prior to flowering. The dry and fresh weights of the stem show significant increases in accumulation of dry matter during the last six weeks of growth. The total number of leaves and their fresh and dry weights showed a significant increase throughout the period of growth. Root growth showed a very slow rate of increase in comparison to the aerial parts. The fresh and dry weights of the inflorescences showed a rapid increase after 68 days.

The partitioning of dry matter between different plant fractions under high temperature, is shown in Table 11. In contrast to the greenhouse, plants growing under high temperature produced more leaves prior to the onset of flowering. This indicated that, under high temperatures, tomatoes plants have a tendency to grow more vegetatively.

In comparing the dry matter partitioning in the different plant parts, it can be seen that there was a higher growth rate under the greenhouse conditions until the plants were 68 days old. Thereafter, plants under high temperature were growing at a much faster rate.

The relationship between vegetative growth and the extent of flowering as determined by the dry weights of all the flowers on the plant is shown in Table 12 and Figure 8. It is clear that, tomato plants in the greenhouse are producing more flowers at any time during their growth and development, whereas the plants under high temperatures produced very few flowers, but experienced more dry weight in their shoots especially after they attained 68 days old and thereon. It is interesting to note that under high temperature, the number of leaves

Table 11. Partitioning of dry matter under high temperature (Marana).

	Age of the Plant (Days)				
	40	47	60	68	82
No. leaves 1st inflorescence	13.20	13.60	12.20	12.20	11.60
" " 2nd "	-	-	14.20	13.80	13.20
" " 3rd "	-	-	15.60	15.20	14.80
Fresh wt. stems (g)	20.14 a	35.14 b	80.18 c	190.76 d	262.80 e
Dry wt stems (g)	6.12 a	8.22 a	14.50 b	27.24 c	41.34 d
Length stems (cm)	32.34 a	39.76 b	54.64 c	65.80 d	70.60 e
Total no. leaves	21.20 a	29.80 b	36.00 c	64.60 d	80.80 e
Fresh wt. leaves (g)	39.68 a	64.04 b	127.90 c	252.06 d	335.44 e
Dry wt leaves (g)	10.40 a	14.20 b	23.02 c	46.08 d	66.24 e
No. branches	2.20 a	5.80 b	7.40 b	7.60 b	13.20 c
Fresh wt. roots (g)	12.98 a	20.38 b	22.60 c	24.30 c	48.20 d
Dry wt roots (g)	4.78 a	6.78 ab	8.74 ab	9.50 b	10.66 b
Fresh wt. inflorescence (g)	0.13 a	0.18 a	0.73 a	4.16 b	5.56 b
Dry wt inflorescence (g)	0.03 a	0.05 a	0.12 a	0.58 a	0.87 a

[z] Means followed by the same letter within the same parameter (row) are not significantly different at the 5 percent level according to Student-Newman-Keuls procedure.

Table 12. Relation between vegetative growth and flowering.

	Age of Plant (Days)				
	40	47	60	68	82
Greenhouse					
Dry wt inflorescence (g)	0.20a[z]	0.34a	0.47a	1.83b	2.16b
Total no. of leaves	21.20a	22.40a	27.40b	34.60c	42.20d
Dry wt shoot (g)	41.08a	47.02b	54.04c	58.02d	72.72e
Marana					
Dry wt inflorescence (g)	0.03a	0.05a	0.12a	0.58a	0.87a
Total no. of leaves	21.20a	29.80b	36.00c	64.60d	80.80e
Dry wt shoot (g)	16.52a	22.42b	37.52c	73.32d	107.58e

[z]Means followed by the same letter within the same parameter (row) are not significantly different at the 5 percent level according to Student-Newman-Keuls procedure.

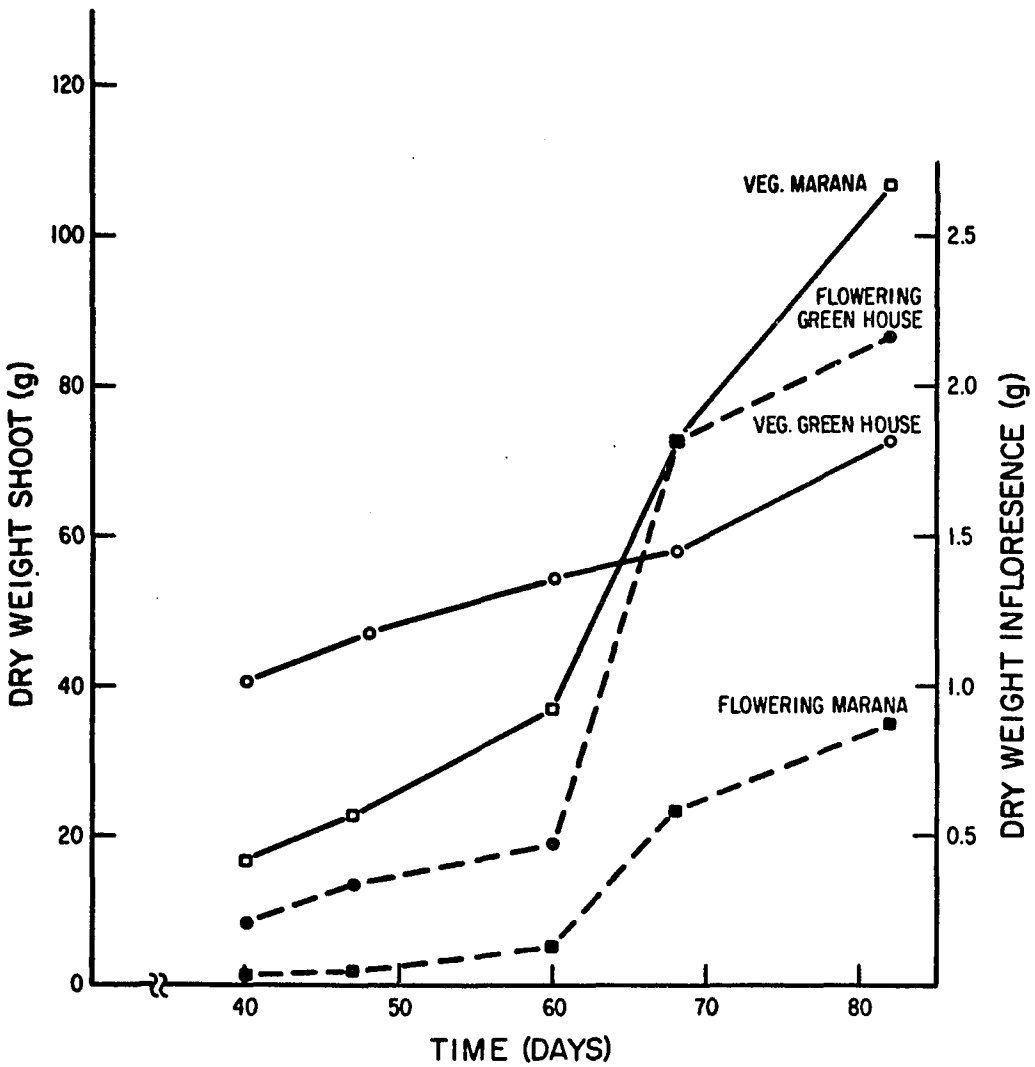

Figure 8. Relationship between vegetative growth and flowering.

was almost twice as much in the field as in the greenhouse when the plants were 68 or 82 days old. The dry weight of the inflorescence in the greenhouse was seven times higher than under high temperatures. During the period 40-47 days, this was followed by 3 to 4 times more inflorescences during the period 60-82 days, respectively.

In the reported experiment of dry matter partitioning; tomato plants in the greenhouse environment produced flowers at lower nodes than at high temperatures. This suggested that, the delay in flowering under high temperature is due to a higher rate of vegetative growth in terms of production of more leaves and increase in the weight of stems. Apparently there was competition between formation of new shoot growth and the tendency of the plant to flower. Although, photosynthesis was not directly measured, the rate of dry matter production indicated that assimilates were not a limiting factor in flowering, but rather the distribution of assimilates between new leaves and flower buds. This is in agreement with Hussey (1963) who showed that there is competition for available assimilates between the developing leaves and the apex. While Calvert (1969) found that under low temperature the apex enlarges rapidly, but the rate of leaf production is low.

Contrary to the report of Abdalla and Verkerk (1970) and Satti (1975), that high temperature conditions cause tomato vine to be small with elongated branches; the cultivar tested in the present study was vigorous growing with thick stems and branches with new growth and thick leaves.

In the light of the above mentioned facts it is clear that assimilates in slow flowering and fruiting plants were translocated to

newly formed leaves and branches. The relatively stable rate of tomato photosynthesis (Starck, 1978) may be connected with continuous growth of vegetative organs, even during rapid flowering and fruiting of plants growing in the greenhouse. Tanaka (1974b) postulated that, tomato leaf is the most important acceptor of its own assimilates. A similar relation between vegetative growth and flowering was observed in watermelons (Buttrose, 1978). The demand for assimilates under high temperature was not affected by poor flowering and fruiting under these conditions, but mobilization of reserved dry matter was occupied in vegetative growth.

These results suggest the hypothesis that assimilates partitioning may be influenced by cytokinins in the developing flower buds and since under high temperature, the levels of cytokinins were found to be 4 to 5 times lower than under normal growing conditions, flowering is thus retarded evidently because of hormonal imbalance. A mechanism possibly involving hormonal control was also suggested by Bidwell (1974) and Geiger (1976). Hoad (1977) reported that in grapes deprived of fruits, there is an increase of cytokinins and a decrease of gibberellins in vine leaves. The relatively higher level of cytokinins in leaves help to explain the fast rate of vegetative organs and their mobilization of assimilates and at the same time resulting in poor flower formation.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

The problem we are concerned with in this study is poor flowering and fruit set of tomatoes grown under high temperatures. The research was undertaken to develop a better understanding of the physiology of the tomato plant under these conditions. We were looking especially at the role of the growth regulators BA and GA4/7 and carbohydrates distribution in the plant.

Flowering and fruiting in tomato were increased substantially by using applications of BA and GA4/7. It seems that these growth regulators might enhance the translocation of a floral stimulus together with mobilization of nutrients from vegetative organs. The endogenous levels of cytokinins in root exudates were lower under high temperatures in the field than in the greenhouse. This possibly accounts for the stunted and poor development of the inflorescence and ultimate failure of fruit set. The study has shown clearly that vegetative growth is not a limiting factor in flowering and fruit set but rather that the distribution and allocation between developing leaves and flower buds is important.

The results of this study has indicated clearly an important role for BA and GA4/7 in flowering and fruit set of tomatoes. An increase in the number of flowers and percent of fruit set when growth regulators are added, is considerable evidence denoting that these

materials are in short supply under high temperature. Although more evidence of the endogenous levels of the hormones in poorly developing inflorescences is needed, there can be no question that BA and GA4/7 are involved in improving flowering. The importance of an endogenous supply of cytokinins was realized, when we found that low amounts of cytokinin were translocated in root exudates of tomato at the time of flower bud formation and anthesis under high temperatures. The author believes that modification of the microclimate conditions to lower the plant temperature, would tend to increase the endogenous levels of cytokinins.

The vegetative growth of tomato plants grown under high temperature was not impaired as previously reported in some cultivars. The data presented clearly demonstrates that the gain in fresh and dry matter of the shoot in Marana is twice as much in the greenhouse. This implies that the distribution of assimilates is an important factor contributing to poor flowering and fruit set under high temperatures. It is believed that higher levels of growth regulators in tomato inflorescences would enhance the flow of carbohydrates and other nutrients from leaves to developing flowers. If indeed, there is a rapid surge in formation of new leaves, this would indicate a constant demand for assimilates, resulting in competition between vegetative and reproductive organs. Such a competition could be harnessed by removal of newly developing leaves.

The findings of this study have brought forward considerable evidence in resolving the mechanism and the physiology of flowering and fruit set in summer grown tomatoes. The use of growth regulators in

promotion of flowering is a possibility in circumstances such as in these experiments.

At this juncture, it is suggested that further investigations should be conducted to: (1) determine the endogenous levels of hormones in inflorescences of tomato plants grown under optimum temperatures and (2) study the influence of removing developing leaves on subsequent growth and flowering under high temperatures.

CHAPTER 6

SUMMARY

High temperature inhibition of flowering and fruiting of tomatoes are not clearly understood, it seems probably that low levels of growth regulators or unfavorable distribution of carbohydrates might contribute to poor flower development.

Tomato plants (cv 'Walter') were grown in the greenhouse and in the field at Marana from July to November in 1979 and 1980. Aqueous solutions of 25 ppm GA4/7 and/or 10 ppm BA were applied to inflorescences at various stages of development. Root exudate was collected for 6 hr using sterilized rubber tubes. Samples were stored at -20C until cytokinin bioassay was conducted according to Miller's procedure. Growth and development data were taken to evaluate inflorescence earliness and onset of flowering. The number of flowers and number and weight of fruits from each of the first 3 inflorescences developed on the main stem were recorded. Cut shoots were used for fresh and dry weight measurements of leaf, stem and flowers. Quantitative estimation of total soluble sugars and starch was done from freeze dried inflorescences.

The number of flowers on the first 3 inflorescences of tomato plants was significantly increased when BA alone or BA in combination with GA4/7 was used. These growth regulators produced a substantial

and significant increase in fruit set and yield of tomatoes in both greenhouse and field experiments.

Soluble sugars and starch content of inflorescences treated with BA and GA4/7 showed a steady increase until the time of anthesis. In the post-anthesis stage, BA treated inflorescences had the highest levels of soluble sugars, whereas the control levels declined very rapidly, under the greenhouse conditions. Under Marana conditions, there was a remarkable response to hormonal applications when compared with the control. The levels of soluble sugars in BA treatment was similar to that obtained in the greenhouse experiment but the starch content was significantly lower at all stages during inflorescence development. The low carbohydrate content of the high temperature field grown plants resulted in a much reduced inflorescence size.

Root exudation steadily increased with dry weight of the plant until shortly after anthesis and then declined rapidly as fruits developed. Under high temperatures, root exudate flowed at an increasing rate until termination of the experiment.

The quantitative bioassay of cytokinins in root exudate of tomato plants grown in the greenhouse or at Marana revealed important changes during growth and development. A high level of cytokinins was detected during growth and development. A high level of cytokinins buds developed and attained its lowest level at the time of anthesis. In the greenhouse, this stage was followed by continuous gradual cytokinin increase as fruits developed. In contrast, under high temperature, a slight increase was detected and then the level dropped. This decrease in cytokinins level was accompanied by bud malformation, abortive ovaries

and flower crop. In general, the amounts of translocated cytokinins obtained under the greenhouse conditions were 4 to 5 times higher than under high temperatures in the field.

In contrast to the greenhouse, field grown plants produced more leaves prior to the onset of flowering, an indication of high temperature influence on vegetative growth. When comparing dry matter partitioning to different plant parts, there was a fast rate of vegetative growth under the greenhouse until 68 days old, thereafter plants under high temperatures grew faster. It was evident, from this study, that plants in the greenhouse produced more flowers at all times during their growth and development. Plants under high temperatures produced twice the weight of leaves and fewer inflorescence. This demonstrates competition between vegetative growth and flowering due to allocation of assimilates towards the vegetative organs.

In hot arid climates, tomato growth and development is profoundly altered. The manifestation of abnormal growth depends on the adaptability of the cultivar to tolerate the adverse conditions. Nonetheless, the bulk of tomato genotypes released have shown poor performance under high temperature. An understanding of the physiological mechanism for high temperature tolerance may enhance production of tomatoes in arid conditions.

Results presented in this study suggest the use of benzyladenine and gibberellins in tomato plants for promotion of flowering and fruit set. Flowering was promoted possibly due to enhanced export of assimilates from leaves or by enhanced floral stimulus translocation.

The data presented in this investigation revealed that the endogenous levels of cytokinins under high temperatures were low, particularly at the time of flowering. However, the question of the mechanism of flower promotion remains to be investigated. The results point towards an apparent competition between developing leaves and inflorescences under high temperatures, hence the importance of manipulating assimilate sources and sinks to insure a desirable translocation in the direction of flower development.

APPENDIX A

TEMPERATURE RECORDS

Table A.1. Daily maximum and minimum temperature at Marana (August, 1979).

Date	°C Max.	Min.	Date	°C Max.	Min.
8/1/79	37	22.8	8/16/79	38.9	17.8
8/2	37	23.3	8/17	40	18.9
8/3	37.8	22.2	8/18	41.1	19.4
8/4	37.8	25.6	8/19	40.5	22.2
8/5	37	26.7	8/20	42.2	18.9
8/6	32.2	21.1	8/21	41.1	21.2
8/7	33.3	20	8/22	38.9	23.3
8/8	37	16.7	8/23	37.8	20
8/9	33.9	18.3	8/24	37.8	21.2
8/10	32.2	17.2	8/25	38.9	20
8/11	33	15.6	8/26	37.8	18.9
8/12	33.9	18.3	8/27	38.9	21.2
8/13	36.1	17.8	8/28	40	23.3
8/14	37.8	15.6	8/29	41.7	22.2
8/15	37.8	15.6	8/30	41.1	22.2
			8/31	37.8	22.2

Table A.2. Daily maximum and minimum temperature at Marana (September, 1979).

Date	°C Max.	Min.	Date	°C Max.	Min.
9/1/79	40.5	23.3	9/16/79	38.9	20
9/2	38.9	24.4	9/17	40	20
9/3	37.8	24.4	9/18	40.5	20.5
9/4	40	21.1	9/19	39.4	23.3
9/5	40	21.1	9/20	38.9	22.2
9/6	37.8	22.2	9/21	38.3	20
9/7	36.1	21.1	9/22	38.9	20
9/8	33.3	21.1	9/23	36.7	19.4
9/9	36.7	20	9/24	36.1	20
9/10	37.8	21.1	9/25	36.1	18.9
9/11	32.2	23.3	9/26	36.7	17.8
9/12	34.4	17.8	9/27	37.8	17.8
9/13	36.7	20	9/28	36.7	18.3
9/14	37.8	20	9/29	34.4	17.8
9/15	37.8	18.9	9/30	37.8	16.7

Table A.3. Daily maximum and minimum temperature at Marana (October, 1979).

Date	°C Max.	Min.	Date	°C Max.	Min.
10/1/79	38.3	17.7	10/16/79	32.2	10
10/2	36.7	16.6	10/17	32.8	10.5
10/3	35.6	15.5	10/18	33.8	10.5
10/4	37.2	16.1	10/19	31.1	10
10/5	37.2	15.5	10/20	29.4	10
10/6	33.9	14.4	10/21	30	10
10/7	32.2	14.4	10/22	27.8	10
10/8	32.2	12.2	10/23	18.9	1.6
10/9	32.2	12	10/24	23.3	4.4
10/10	32.2	12.2	10/25	23.3	3.3
10/11	32.2	14.4	10/26	24.4	2.8
10/12	31.1	14.4	10/27	30	4.4
10/13	30.6	12.2	10/28	23.3	8.9
10/14	31.1	14.4	10/29	24.4	4.4
10/15	28.9	15	10/30	20	4
			10/31	24	6

Table A.4. Daily maximum and minimum temperature at Marana (August, 1980).

Date	°C Max.	Min.	Date	°C Max.	Min.
8/1/80	39.4	21.1	8/16/80	38.3	13.9
8/2	38.3	21.1	8/17	38.9	15
8/3	36.7	21.1	8/18	38.3	15.5
8/4	37.8	21.7	8/19	36.1	16.6
8/5	38.9	20.5	8/20	36.1	14.4
8/6	41.1	20.5	8/21	38.9	16.1
8/7	42.8	25	8/22	38.9	18.3
8/8	42.2	21.7	8/23	39.4	18.3
8/9	40.5	22.2	8/24	33.3	16.6
8/10	40.5	22.2	8/25	36.7	13.9
8/11	42.2	21.7	8/26	38.3	16.6
8/12	39.4	20.5	8/27	38.9	19.4
8/13	33.9	17.2	8/28	39.4	18.8
8/14	36.1	17.7	8/29	39.4	21.1
8/15	42.2	16.6	8/30	38.3	13.9
			8/31	38.9	12.8

Table A.5. Daily maximum and minimum temperature at Marana (September, 1980).

Date	°C Max.	Min.	Date	°C Max.	Min.
9/1/80	37.7	18.3	9/16/80	37.7	13.9
9/2	38.8	17.7	9/17	40.5	16.1
9/3	36.7	20	9/18	41.6	14.4
9/4	32.2	17.7	9/19	39.4	15
9/5	37.7	21.1	9/20	38.8	12.2
9/6	35.5	21.1	9/21	37.7	10
9/7	38.3	18.3	9/22	36.7	11.1
9/8	35	17.2	9/23	36.1	11.7
9/9	36.7	17.7	9/24	36.1	15.5
9/10	35	14.4	9/25	36.7	15
9/11	36.7	12.2	9/26	32.2	16.6
9/12	36.7	13.3	9/27	34.4	16.6
9/13	35.5	15.5	9/28	36.6	14.4
9/14	36.7	16.6	9/29	35.5	16.6
9/15	36.7	13.3	9/30	38.8	14

LITERATURE CITED

Abdalla, A. A. and K. Verkerk. 1968. Growth, flowering and fruit set of the tomato at high temperature. Neth. J. Agr. Sci. 16: 71-76.

Abdalla, A. A. and K. Verkerk. 1970. Temperature and nitrogen nutrition in relation to flowering and fruiting in tomato. Neth. J. Agr. Sci. 18: 111-115.

Atkin, R. K., G. E. Barton and D. K. Robinson. 1973. Effect of root-growing temperature on growth substances in xylem exudate of Zea mays. J. Exp. Bot. 24: 475-487.

Aung, L. H. and J. M. Byrne. 1975. Changes in rooting substances of tomato explants. J. Amer. Soc. Hort. Sci. 100: 19-22.

Bidwell, R. G. S. 1974. A possible mechanism for the control of photoassimilate translocation. pp. 77-87. In: Proc. Res. Inst. Pomol. Transloc. 3rd Symp. on Accumulation and translocation, Proc. Res. Inst. of Pomol., Skierniewice.

Buttrose, M. S. and M. Sedgley. 1978. Some effects of light intensity, day length and temperature on growth of fruiting and non-fruiting watermelon. Ann. Bot. 42: 599-608.

Calvert, A. 1957. Effect of the early environment on the development of flowering in the tomato. I. Temperature. J. Hort. Sci. 32: 9-17.

Calvert, A. 1959. Effect of the early environment on the development of flowering in the tomato. II. Light and temperature interaction. J. Hort. Sci. 34: 154-162.

Calvert, A. 1969. Studies on the post-initiation development of flower buds of tomato. J. Hort Sci. 44: 117-126.

Charles, W. B. and R. E. Harris. 1972. Tomato fruitset at high and low temperature. Can. J. Pl. Sci. 52: 497-506.

Coyne, D. P. 1968. Differential response of stylar elongation in tomatoes to soil moisture levels. HortScience 3: 39.

Crane, J. C. 1964. Growth substances in fruit setting and development. Ann. Rev. Pl. Physiol. 15: 303-326.

Crane, J. C. 1969. The role of hormones in fruit set and development. HortScience. 4: 108-111.

Davey, J. E. and J. Van Staden. 1976. Cytokinin translocation: Changes in Zeatin and Zeatin-riboside levels in the root exudate of tomato plants during their development. Planta 130: 69-72.

Davey, J. E. and J. Van Staden. 1978a. Cytokinin activity in Lupinus albus I. Distribution in vegetative and flowering plants. Physiol. Plant. 43: 77-81.

Davey, J. E. and J. Van Staden. 1981. Cytokinin activity in Lupinus albus. V. Translocation and metabolism of (8-^{14}C) Zeatin applied to the xylem of fruiting plants. Physiol. Plant. 51: 45-48.

Ebell, L. H. 1969. Specific total starch determinations in conifer tissues with glucose oxidase. Phytochemistry 8: 25-36.

Fujishige, N. and T. Sugiyama. 1968. Effect of soil temperature on growth of seedlings of a few fruit vegetables. J. Jap. Soc. HortScience. 37: 211-226.

Geiger, D. R. 1976. Effects of translocation and assimilate demand on photosynthesis. Can. J. Bot. 54: 2337-2345.

Ginzburg, C. 1974. The effects of gibberellins and (2-chloroethyl)-trimethylammonium chloride on assimilate distribution in gladiolus in relation to corm growth. J. Exp. Bot. 89: 995-1003.

Goh, C. J. 1977. Regulation of floral initiation and development in an orchid hybrid. Ann. Bot. 41: 763-769.

Greene, D. W. 1980. Effect of silver nitrate, aminoethoxy-vinylglycine and gibberellins A4+7 plus 6-benzylaminopurine on fruit set and development of 'Delicious' apples. J. Amer. Soc. Hort.Sci. 105: 717-720.

Henson, I. E. and P. F. Wareing. 1974. Cytokinins in Xanthium strumarium: A rapid response to short day treatment. Physiol. Plant. 32: 185-187.

Hoad, G. V., B. R. Loveys and K. G. M. Skene. 1977. The effect of fruit removal on cytokinins and gibberellin-like substances in grape leaves. Planta 136: 25-30.

Howlett, F. S. 1939. The modification of flower structure by environment in varieties of Lycopersicon esculentum. J. Agr 58: 79-117.

Hsiao, T. 1973. Plant responses to water stress. Ann. Rev. Plant. Physiol. 24: 519-570.

Hussey, G. 1963. Growth and development in the young tomato. I. The effect of temperature and light intensity on growth of the shoot apex and leaf primordia. J. Exp. Bot. 14: 316.

Itai, C., A. Ben-Zioni and L. Orden. 1973. Correlative changes in endogenous hormone levels and shoot growth induced by heat treatment to root. Physiol. Plant. 29: 355-360.

Johnson, S. P. and W. C. Hall. 1953. Vegetative and fruiting responses of tomatoes to high temperature and light intensity. Bot. Gaz. 114: 449-460.

Johnson, S. P. and W. C. Hall. 1955. Further studies on vegetative and fruiting responses of tomatoes to high temperature and light intensity. Bot. Gaz. 117: 100-113.

Kende, H. and D. Sitton. 1967. The physiological influence of kinetin and gibberellin-like root hormones. Ann. N.Y. Acad. Sci. 144: 235-243.

Kinet, J. M. 1977a. Effect of light conditions on the development of the inflorescence in tomato. Scientia Hort. 6: 15-26.

Kinet, J. M. 1977b. Effect of defoliation and growth substances on the development of the inflorescence in tomato. Scientia Hort. 6: 27-35.

Kinet, J. M. 1978. Promotion of inflorescence development by growth substances treatments to tomato plants grown in insufficient light conditions. J. Amer. Soc. Hort. Sci. 103: 724-729.

Leopold, A. C. and F. I. Scott. 1952. Physiological factors in tomato fruit-set. Amer. J. Bot. 39: 310-317.

Lewis, D. 1953. Some factors affecting flower production in the tomato. J. Hort. Sci. 28: 207-219.

Linck, A. J. and C. A. Swanson. 1960. A study of several factors affecting the distribution of phosphorus-32 from the leaves of Pisum sativum. Plant and Soil. 12: 57-68.

Menary, R. C. and J. Van Staden. 1976. Effect of phosphorus nutrition and cytokinins on flowering in the tomato. Aust. J. Pl. Physiol. 3: 201-205.

Menhenett, R. and P. F. Wareig. 1975. Possible involvement of growth substances in the response of tomato plants to different soil temperatures. J. Hort.Sci. 50: 381-397.

Miller, C. O. 1968. Naturally occurring cytokinins. In Biochemistry and Physiology of plant growth substances, 33-45. Ed. F. Wightman and G. Setterfirld. Runge Press. Ottawa.

Mizrahi, Y. and A. E. Richmond. 1972. Hormonal modification of plant responses to water stress. Aust. J. Biol. Sci. 25: 437-442.

Mothes, K. and L. Engelbrecht. 1961. Kinetin induced transport of substances in excised leaves in the dark. Phytochemistry 1: 58-62.

Mullins, M. G. 1967. Morphogenetic effects of roots and of some synthetic cytokinins in Vitis vinifera J. Exp. Bot. 18: 206-214.

Niimi, Y. and H. Torikata. 1978. Changes in endogenous plant hormones in the xylem sap of grapevines during development. J. Jap. Soc. Hort.Sci. 47: 181-187.

Ogawa, Y. and R. W. King. 1979. Indirect action of Benzyladenine and other chemicals on flowering of Pharbitis nil chois. Plant Physiol. 63: 643-649.

Osborne, D. L. and F. W. Went. 1953. Climatic factors influencing parthenocarpy and normal fruit set in tomatoes. Bot. Gaz. 111: 312-322.

Railton, I. D. and D. M. Reid. 1973. Effects of benzyladenine on the growth of waterlogged tomato plants. Planta III: 261-266.

Reid, D. M. and I. D. Railton. 1974. The influence of benzyladenine on the growth and gibberellin content of shoots of waterlogged tomato plants. Plant Sci. Letters. 2: 151-156.

Richmond, A. E. and A. Lang. 1957. Effect of kinetin on protein content and survival of detached xanthium leaves. Science. 125: 650-651.

Rick, C. M. and W. H. Dempsey. 1969. Position of the stigma in relation to fruit setting of the tomato. Bot. Gaz. 130: 180-186.

Rylski, I. 1979. Effect of temperatures and growth regulators on fruit malformation in tomato. Scientia Hortic. 10: 27-35.

Saito, T. and H. Ito. 1962. Studies on growth and fruiting in the tomato. Effect of the early environment on growth and fruiting. J. Jap. Soc. Hort. Sci. 31: 303-307.

Satti, S. M. E. 1975. Temperature and nitrogen fertilization in relation to flowering and fruit set in tomatoes under hot arid conditions of Sudan. M.S. Thesis, University of Khartoum, Sudan.

Short, K. C. and J. G. Torrey. 1972. Cytokinins in seedling roots of pea. Plant Physiol. 49: 155-160.

Sitton, D., A. Richmond and Y. Vaadia. 1967. On the synthesis of gibberellins in roots. Phytochem. 6: 1101-1105.

Skene, K. G. M. 1972. Cytokinin in xylem sap of grape vine canes: changes in activity during cold storage. Planta 104: 89-92.

Skene, K. G. M. and A. J. Antcliff. 1972. A comparative study of cytokinin levels in bleeding sap of vitis vinifera and the two grapevine rootstocks, salt creek and 1613. J. Exp. Bot. 23: 283-93.

Skene, K. G. M. and G. H. Kerridge. 1967. Effect of root temperature on cytokinin activity in root exudate of Vitis vinifera. Plant Physiol. 42: 1131-1139.

Starck, Z., M. Kozinska and R. Szaniawski. 1978. Photosynthesis in tomato plants with modified source-sink relationship. In Photosynthesis and Plant Development, Symp., 233-241. Ed. R. Marcell. Kluwer Acad. Publ. Holland.

Summons, R. E., B. Entsch, C. W. Barker and D. S. Letham. 1979. Mass spectrometric analysis of cytokinins in plant tissue. FEBS Lett. 107: 21-25.

Tanaka, A., K. Fujita and M. Shioya. 1974b. Nutrio-physiological studies on the tomato plant. II. Translocation of photosynthates. Soil Sci. Plant Nutr. 20: 163-171.

Torrey, J. G. 1976. Root hormones and plant growth. Ann. Rev. Plant Physiol. 27: 435-459.

Tse, A. T. Y., A. Ramina, W. P. Hackett and R. M. Sachs. 1974. Enhanced inflorescence development in Bougainvillea 'San Diego Red' by removal of young leaves and cytokinin treatments. Plant Physiol. 54: 404-407.

Van Overbeck, J. 1966. Plant hormones and regulators. Science 152: 721-731.

Van Staden, J. and J. E. Davey. 1976. Translocation of cytokinins in xylem sap of herbaceous plants. Pflanzenphysiol. 77: 377-382.

Van Staden, J. and J. E. Davey. 1979. The synthesis transport and metabolism of endogenous cytokinins. Plant Cell Environ. 2: 92-106.

Van Staden, J. and J. E. Davey. 1981. Cytokinin activity in Lupinus albus. VI. Translocation and metabolism of zeatin applied to the leaves and fruits of fruiting plants. Physiol. Plant. 51: 49-52.

Watts, W. R. 1972a. Leaf extension in Zea mays. I. Leaf extension and water potential in relation to root zone and air temperatures. J. Exp. Bot. 23: 704-712.

Weaver, R. J., J. Van Overbeek and M. R. Pool. 1965. Induction of fruit-set in Vitis vinifera by a kinin. Nature 206: 925.

Went, F. W. 1928. Wuchsstoff und Wachstum. Rec. Trav. Bot. Neerland. 25: 1-116.

Went, F. W. 1943. Effect of the root system on tomato stem growth. Plant Physiol. 18: 51-65.

Went, F. W. 1957. The experimental control of plant growth. Chronica Botanica, Waltham, Mass.

Wittwer, S. H. and F. G. Teubner. 1956. Cold exposure of tomato seedlings and flower formation. Proc. Amer. Soc. Hort. Sci. 67: 369-376.

Yemm, E. W. and A. J. Willis. 1954. The estimation of carbohydrates in plant extracts by anthrone. Bioch. J. 57: 508-514.