Effects of Ethephon on the Pattern of Flowering and Fruit Set of Summer Squash

Carl Earnest Sams

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William A. Krueger, Major Professor

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Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
To the Graduate Council:

I am submitting herewith a thesis written by Carl Earnest Sams entitled "Effects of Ethephon on the Pattern of Flowering and Fruit Set of Summer Squash." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant and Soil Science.

William A. Krueger
Major Professor

We have read this thesis and recommend its acceptance:

David L. Coffey
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Accepted for the Council:

Henry A. Smith
Vice Chancellor
Graduate Studies and Research
EFFECTS OF ETHEPHON ON THE PATTERN OF FLOWERING
AND FRUIT SET OF SUMMER SQUASH

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Carl Earnest Sams
August 1976
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ABSTRACT

The plant growth regulator 2-chloroethylphosphonic acid (ethephon) was applied to summer squash to determine its effects on the flowering pattern. Treatments with all pistillate flowers pruned at anthesis, with and without ethephon applications were also compared to control plants to investigate the factors regulating flowering and fruit set of summer squash.

It was found that plants in the pruned treatments had a greater carbohydrate accumulation than plants in the non-pruned treatments, indicating that carbohydrate accumulation may be inversely associated with fruit development. Ethephon treatments produced more pistillate flowers per plant but also had a large number of aborted flowers. Thus, the potential of ethephon to increase yield has not been fully realized because the plant cannot support the added fruit.

Another observation noted was the production of hermaphroditic flowers on a monoecious summer squash cultivar. These flowers were produced when the effect of the ethephon on the plant was diminishing. Also, flowering and yield exhibited a cyclic pattern throughout the season, but no correlation was found to exist between flowering and/or yield and any plant nutrient content (carbohydrate, nitrate, calcium, magnesium, phosphorus, potassium, and nitrogen).
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CHAPTER I

INTRODUCTION

Summer squash often exhibits a wide fluctuation in daily yield throughout the growing season, and reasons for this variation are not evident at present. Ethephon has been shown to greatly increase early yield in some cultivars without increasing the total seasonal yield. Researchers have found that this growth regulator shifts sex expression balance toward femaleness. Thus, ethephon changes the flowering pattern of summer squash, causing more pistillate flowers to be produced in the early part of the season. However, many of these extra pistillate flowers are aborted, and it has been proposed that the yield response to ethephon would be much greater if this occurrence could be prevented.

It is thought that the onset of fruit development stops flower production (perhaps by acting as a "metabolic sink" and altering the nutritional status and hormone balance of the plant). Another conjecture is that carbohydrate accumulation might control flowering and the development of fruit might decrease the level of carbohydrate within a plant.

The objectives of this study were to investigate the factors associated with floral initiation and fruit set in summer squash and to determine the effects of ethephon on
these factors. Also an attempt was made to correlate several nutritional variables in the plant with floral initiation and/or fruit set and to examine the effects of ethephon on these variables.
CHAPTER II

LITERATURE REVIEW

Factors Controlling Floral Initiation and Fruit Set

In 1918, Kraus and Kraybill (30) published what was to become a classical theory on the relationship between the ratio of carbohydrate to nitrogen and the reproductive status of plants. They drew the following general conclusions about the relationship between the concentration of nitrates, carbohydrates, and moisture within a plant, and the resulting physiological responses of the plant.

1. If carbohydrates are limited, even with an abundance of moisture and mineral nutrients, vegetation is weakened, and plants are not reproductive.

2. An abundance of moisture and mineral nutrients, particularly nitrates, coupled with an available carbohydrate supply, causes increased vegetation, barrenness, and sterility.

3. A relative decrease of nitrates in proportion to carbohydrates causes an accumulation of the latter, fruitfulness, fertility, and less vegetative growth.

4. A further reduction of nitrates, accompanied by an increase of carbohydrates, causes a suppression of both vegetative and reproductive growth.
The authors were dealing specifically with tomatoes, but a similar relationship was suggested to exist for other plant species in general.

A few years later, Harvey and Murneek (25) described the carbohydrate to nitrogen ratio as it affects the behavior of apple spurs. They noted that the carbohydrate to nitrogen ratio, if given a general interpretation, might be classified along with other important controlling factors of plant development. However, a strict mathematical interpretation was not justified by the results of their study.

Hooker (27) reported that increased starch in non-barring apple spurs was associated with the initiation of flower buds. This relationship was confirmed by the studies of Harley et al. (23). However, Harley found an inverse relationship for soluble sugars, with greater amounts of these sugars being found in fruit-bearing than in non-bearing spurs. In a similar report, Priestly (42) stated that there was no positive relationship between total carbohydrates and flowering.

The different results reported by various authors, which have lead to controversial conclusions, may be due in part to the different methods used and the type of carbohydrate measured.

The importance of carbohydrate level in cold requiring plants has been indirectly implied by many authors (19, 36, 43). However, none of these researchers demonstrated a
Sadik and Ozbun (45) reported that cauliflower plants induced to flower under 16 hours of light and at 5°C for two weeks had a significant increase in sugar and starch content when compared to vegetative plants grown at 26°C. They also reported that in the absence of light, or given high temperature (33°C) with light, the plants failed to flower, and carbohydrate content was reduced. In a similar experiment with broccoli, Fontes and Ozbun (16) found that under some conditions the level of carbohydrate found in the shoot tip was correlated with floral induction. However, if plants were grown continuously at a warm temperature, a high level of carbohydrate did not insure flowering. They suggested that the association between carbohydrate accumulation and floral induction is coincidental, and that carbohydrate accumulation is not directly responsible for floral induction.

Lewis et al. (33), working with mandarin oranges, found that treatment with NAA (α-napthalene-acetic acid) changed the production cycle without affecting carbohydrates. They concluded that the mechanism controlling alternate bearing appeared to be related to an NAA sensitive regulatory mechanism and not to carbohydrate content. In a comparable experiment, Jones et al. (29) found that carbohydrate accumulation was inversely related to the fruit load on Valencia orange trees at the time of sampling, but directly related to the amount of fruit produced from the flowering
which followed the time of the leaf sample. Late harvest reduced fruit production in the following year but did not significantly alter carbohydrate accumulation. They assumed that the carbohydrate present at flowering was exhausted before the "June Drop" period. Thus, the usable carbohydrate during the June fruit drop depended on current photosynthate. Old fruit left on the tree resulted in less carbohydrate and less fruit set for the new crop on late harvested trees.

Hoffman (26) studied the effects of carbohydrate and nitrogen deficiency on the flowering and fruit set of muskmelon, pepper, and lima bean. He found that carbohydrate deficiency was detrimental to the development of the male gametophytes of these three species, but nitrogen deficiency affected the development of the female gametophyte more severely than did carbohydrate deficiency.

In a review on the floral stimuli in plants, Grainger (19) suggested two conditions necessary for flower initiation.

1. A sufficient number of leaf initials for the species or cultivar must be developed.

2. A sufficiently high value (for the species) of the combined percentage of total carbohydrate + ash must be present in the shoot.

He suggested that the attainment of a sufficient number of leaf initials requires internal control and may be under hormonal influence.
Grochowska (20) noted a decrease in the starch content of fruit bearing apple spurs at the end of June. Application of Aminozide (succinic acid-2,2-dimethylhydrazide) caused a cessation of growth, accompanied by a significant increase in the spur starch content, even though fruits were present. The flowering of the treated trees was doubled. Gibberellic acid applied to off-year trees reduced the starch level in their spurs. The author suggested that the starch content of apple spurs is lowered by hormones supplied by developing seeds. She concluded that a high content of starch cannot be regarded as a direct cause of flower initiation, but it is an excellent indicator of the direction of metabolic processes resulting from low amounts of auxin and gibberellin in the surrounding tissues.

The conflict of opinion which developed rather early regarding the role of carbohydrates in floral initiation and fruit set has continued and has yet to be thoroughly resolved. In the past two decades there have been many research efforts pertaining to the role of growth regulators in floral initiation and fruit set. Because of these research efforts, the concept of hormonal control of floral initiation and fruit set is now being explored by many scientists.

In a review on the physiology of fruit growth, Nitsch (40) emphasized that, while genetic factors play a role in determining sex in plants, environmental conditions can modify the genetic regulation of the formation of pistils
and, thus, fruits. He noted that with squash, when daylength is short, and night temperature is low, the formation of pistillate flowers is promoted. He also noted that NAA stimulated production of female flowers in cucurbits at sites where male flowers are normally produced, indicating that auxin metabolism might be involved in the effect of low night temperatures on development of the pistil. Nitsch further stated that fruit setting and growth were obviously under a control mechanism, and presented evidence that auxin originating in the developing seed was the key factor involved.

The significance of auxin in fruit setting has been recognized for almost thirty years. The presence of auxin in pollen; its production in the style and ovary during pollen tube growth and fertilization; and the resultant stimulation in the growth of the ovary are documented facts (39, 51).

In a review on substances in fruit setting and development, Crane (12) stated that the developing seed appeared to be a center of auxin production and, perhaps, of substances similar to gibberellin and cytokinin. He pointed out, however, that no firm relationship has been proven to exist between the levels of any of these substances in seeds and fruit growth.

In a plant, vegetative and reproductive development may proceed concurrently. However, at each shoot apical meristem,
a transition occurs from leaf production to flower production at the time of flower initiation. Therefore, vegetative and reproductive development represent alternative courses of differentiation of apical meristematic tissues. Sachs et al. (44), in a review on the control of vegetative and reproductive development, presented evidence that one hormone, florigen, is produced in leaves and translocated to apical meristematic tissues, where it causes floral initiation. The exact nature and mode of action of florigen remains a mystery, and there is now evidence that several other hormones, including gibberellic acid, are involved in flower initiation.

Promotion of flower initiation by gibberellic acid in many long day plants and several other caulescent species is well documented (31). The mode of action of gibberellins as flower inducing substances is not known, but there is evidence that they are required by the leaves for the synthesis of florigen (4, 56). Abscisin is a naturally occurring growth regulator with which much work has been done recently. There is evidence that it may act by blocking the action of gibberellin (11), and that a balance between growth substances may be as important in controlling reproductive development as it is in other processes. Sachs and Hackett (44) have suggested that a balance between growth regulators may control carbohydrate levels in plants, thus, regulating reproductive and vegetative development. They suggested that in some plants any compound which inhibits stem
elongation, but does not inhibit apical meristematic activity in auxillary buds, may promote flowering because of increased carbohydrate levels. Another supposition is that carbohydrate levels might be altered to effect flowering with little concomitant effect on vegetative growth.

Flowering and Fruiting Patterns of Summer Squash

The term "summer squash" is used to identify several related cultivars in the botanical classification Curcurbita pepo. Squash flowers are yellow, imperfect, and are normally born singly in the axils of the leaves. Female flowers are open for several hours, and stigmas are receptive to pollen only during this short period (47).

Many authors have associated photoperiod and temperature with the sex expression of cucurbits. Danielson (15) suggested that the photoperiodic reaction of the stem resulting in stem elongation is somehow physiologically related to, if not dependent on, the onset of flowering. He found that maximum staminate flower production occurred in the eight hour day treatment and that maximum stem elongation also occurred under these conditions. Currence (14) reported a nodal sequence of flower type in the cucumber. He noted that sex expression appears to undergo a gradual change from the strongly staminate to the strongly pistillate condition as the plant develops. The first nodes bear only male flowers, while female flowers appear later in the plant's
development. He also found that near the twentieth node, the male and female flowers developed in almost equal proportions. It was further implied that lateral nodes develop a greater number of pistillate flowers than do main stems. Nitsch et al. (41) reported the results of an experiment on the development of sex expression in cucurbit flowers. They stated that from the first leaf, the type of flower that develops is as follows: (1) underdeveloped male, (2) normal male, (3) normal female, (4) inhibited male, (5) giant female, and (6) parthenocarpic female. They further suggested that high temperatures and long days encourage male flowers, whereas low temperatures and short days encourage female flowers. Climatic factors were said to modify the length, but not the order, of each phase.

Hall (22) reported that peak flower bud production occurred 15 days earlier in plants exposed to eight-hour day length, and about 50 percent more flowers were formed in short day treated plants than in long day treated plants. Plants highly fertilized with nitrogen produced more pistillate flowers in both photoperiods.

In the flower bud of cucurbits, ovary growth occurs mainly through cell division (40). Cell division gradually ceases at the time of anthesis, while cell enlargement begins and becomes responsible for the latter part of fruit enlargement. When a fruit is set and begins growth, it
becomes an active metabolic sink into which nutrients flow, frequently curtailing vegetative growth.

**Historical Aspects of Ethylene Research**

The first researcher to report that ethylene regulated the growth and development of plants was Dimitry Nikolayevich Neljubov (1) in 1901. He observed that illuminating gas caused pea seedlings to grow horizontally. Investigating the components of the illuminating gas, he found that ethylene was the most active component. Crocker et al. (13) and, later, Harvey (24) confirmed that ethylene was the active ingredient in illuminating gas and smoke.

The initial suggestion that plants produced ethylene was made in 1910 by Cousins, who reported that oranges produced a gas that promoted the ripening of bananas (1). Later, Gane proved chemically that plants produced ethylene. Thus, he provided the remaining evidence that ethylene was indeed a plant hormone (1).

Ethylene is a C₂ hydrocarbon gas with a double bond and a molecular weight of 28.05. It is a colorless gas, with a sweet odor resembling that of ether. Ethylene is about five times as soluble in water as is oxygen, and, at 25°C in a concentration of 1 ppm, the molarity of ethylene in water is $4.43 \times 10^{-9}$.

AM-CHEM Products Inc., Ambler, Pennsylvania, marketed ethephon (Ethrel) in 1967. This was the first product
marketed which allowed the practical application of an ethylene releasing compound in field situations. It acts by releasing ethylene as a decomposition product in living plant tissue (53,55). According to Yang (55), a \(-\text{CH}_2\text{-CH}_2-\) grouping in the center of the molecule, with one end an electron-withdrawing center and the other an electron donor, is capable of producing ethylene. The mechanism of ethylene production is thought to occur as illustrated by the following scheme.

\[
\begin{align*}
\text{Cl-CH}_2\text{-CH}_2\text{-P-0}^- + \text{OH}^- & \rightarrow \\
\text{Cl-CH}_2\text{-CH}_2\text{-P-0}^- & \rightarrow \\
\text{Cl}^- + \text{CH}_2 = \text{CH}_2 + \text{HPO}_4^{2-}
\end{align*}
\]

Warner and Leopold (52) were the first to describe the use of ethephon as a plant growth regulator. Ethephon is stable in the acid form but breaks down to form ethylene at a pH of 3.5 and above. The rate of ethylene formation increases as the pH is raised (2). Most of the ethephon applied to plants is eventually converted to ethylene. Since ethylene is a known plant hormone that affects many plant processes (54),
including fruit development, the use of ethephon as an ethylene source has become widespread in research and crop production. Ethylene has been implicated as being involved in the transcription and translation of the genetic code from DNA to RNA to protein (10). The gas interacts with auxin in affecting cell growth and division, and has been said to inhibit auxin synthesis by interfering with tryptophan to auxin conversion (50). Fuchs and Lieberman (18) have reported that an interaction between auxin and kinetin regulates ethylene production by affecting systems of protein synthesis. Lau (32) reported that a synergistic stimulation of ethylene production by kinetin and calcium exists in some tissues. These findings are constantly being supplemented with new research indicating that ethylene is involved in many hormonal interactions which regulate various plant processes.

Effects of Ethylene on Flowering of Cucurbits

Greenhouse operators in the 1860's noticed the promotion, by smoke, of female flowers in cucurbits, and ethylene was implicated as the active agent in smoke causing this sex reversal. Russian workers found that the best time to smoke plants to increase yield was in the three leaf stage (1). Most subsequent work on the sex reversal of flowers has been done with ethephon. McMurray and Miller (37) reported that ethephon treated plants produced only female flowers on
monoecious cucumbers (commonly exhibiting both pistillate and staminate flowers) for as many as 19 nodes. The effects of ethephon seemed to be additive, since multiple applications of lower rates had the same effect as single applications of higher rates. Other investigators have reported similar findings (17, 28, 34). Best (5) indicated that a 150 ppm concentration was optimum for increasing both early and season total summer squash yields.

The time of ethephon application influences the formation of female flowers. Applications by seed soaking or in the cotyledon stage are ineffective (8, 28). Iwahori et al. (28) indicated that the most effective time of ethephon application seems to be in the three leaf stage. The later the time of application past the three leaf stage, the more distal the node at which the first female flower appears. However, the total number of female flowers is the same, regardless of application time. Abeles (1) reported that the abortion of flowers at the lower nodes is probably due to the ability of ethylene to hasten floral senescence. He further stated that plants at the first through third leaf stage have already differentiated flower primordia up to the ninth through fifteenth nodes and that at the third leaf stage, the sex of flowers has already been determined up to the seventh node. The conclusion drawn was that ethylene action is probably directed toward determining the sex in preformed floral primordia which have not advanced to the point of
sexual differentiation. Thus, the gas apparently has no effect on either sexually differentiated primordia or primordia that have not been initiated.

Iwahori et al. (28) found that ethephon promoted femaleness and that gibberellins promoted maleness. The authors assumed that there were different sites of action because there was not a significant interaction.

There have been implications that the time of ethylene applications affects the number of parthenocarpic fruit set (5). The hypothesis has been made that auxin transport inhibitors induce parthenocarpy in cucurbits by blocking the natural flow of auxin from the ovary, thereby resulting in an auxin accumulation sufficient to create a metabolic sink (6). Lyons (35) stated that ethylene may inhibit the polar transport of auxin by gravity. These observations indicate that ethylene may be involved in some types of parthenocarpy by blocking auxin transport from the ovary.
CHAPTER III
MATERIALS AND METHODS

The primary objectives of this experiment were to discover the controlling factors in the frequency of fruit set in summer squash and to determine the effects of ethephon on these factors. The experiment was conducted in the summer of 1975 at The University of Tennessee Plant and Soil Sciences Field Laboratory at Knoxville and was duplicated, with minor variations, at the Plateau Experiment Station near Crossville, Tenn.

General Information

Plants of Early Prolific, a summer squash cultivar, were planted in a randomized complete block design with six replications of the following four treatments: (1) control, (2) all pistillate flowers pruned at anthesis, (3) 150 ppm ethephon applied at the three leaf stage, and (4) 150 ppm ethephon applied at the three leaf stage and all pistillate flowers pruned at anthesis. Each treatment consisted of three rows of 13 plants each, with plants 18 inches apart in rows, and 3.5 feet between rows. Yield data and flower counts were taken on the center row of each treatment, and leaf samples were taken from the two outside rows. The experimental design was the same at both locations. The
experiment at Knoxville was planted on a Huntington silt loam and the Crossville experiment on a Hartsells sandy loam.

**Cultural Practices**

Cultural practices were kept as uniform as possible at both locations throughout the experiment. Mechanical cultivation was used for weed control. Insect and disease control consisted of using recommended pesticides (3, 21) as needed. Fertilization was based on soil test. Supplemental irrigation was provided at Knoxville but not at Crossville.

**Field Data Collection**

Pistillate and staminate flower counts were taken daily for a three and a half week period. Flowers were counted only when they reached anthesis, and in the pruned treatments the pruning of pistillate flowers was done at anthesis. The fruit was harvested two to three times a week during this same period, and the number and weight of fruit was recorded. Leaf samples were taken three times a week. These composite samples consisted of five leaves, including petioles, taken from five different plants at random. The fourth leaf from the terminal growing point was used (7). Samples were sealed in polyethylene bags, placed on dry ice immediately, and stored at -20°C until sample preparation was performed. Sample preparation consisted of freeze-drying, grinding to 20-mesh, and returning to -20°C for storage until analysis was performed.
Nitrate-Nitrogen Determination

An Orion Model 92-07 nitrate ion activity electrode and a single junction reference electrode, Orion Model 90-01, filled with saturated KC1 were used with an Orion Model 701 digital pH meter having an expanded millivolt scale and direct display for monovalent anion electrodes. A standard calibration curve was constructed using 10 to 100 mg NO3-N standards derived from KNO3 in a 0.01 N KH2PO4 buffering solution. De-ionized water was used as the extracting solution. The analysis procedures employed were those described by Brown (9).

Total Nonstructural Carbohydrate Determination

The anthrone method (38) was used to make a quantitative determination of total nonstructural carbohydrates. Carbohydrates were extracted with 0.02 N H2SO4 in a boiling water bath for one hour and filtered through Whatman's No. 2 filter paper. The standard curve was constructed using 0 to 0.1 mg/ml glucose solutions. The instrument used was a Perkin-Elmer Model 202 Ultraviolet-Visible spectrophotometer.

Ammonia Nitrogen Determination

A 0.2 g dry weight sample was digested in concentrated sulfuric acid and 35 percent hydrogen peroxide, diluted in de-ionized water, and analyzed with a Technicon Autoanalyzer. Nitrogen was determined using the phenolhypochlorite color
reaction as described by Thomas et al. (49). Results are reported as percent N on a dry weight basis.

**Potassium, Calcium, Phosphorus, and Magnesium Determination**

A 0.5 g sample was dry ashed at 500°C for four hours. The sample was then dissolved in 10 ml of 3 N HCl, filtered through Whatman's No. 40 filter paper, and diluted with de-ionized water. The K and Ca were determined using a Technicon III dual-channel flame photometer. The Mg was determined colorimetrically by the magnesium blue reaction, and P was determined by the ammonium vanadate reaction. These procedures were described by Steckel et al. (48). Results are reported as percent K, P, Mg, or Ca on a dry weight basis.

**Statistical Analysis**

Procedures of the Statistical Analysis System (46) were employed for this experiment. Data were analyzed in two separate procedures. First, an analysis of variance was performed on all season total data, and means were separated by Duncan's New Multiple Range Test. In the second procedure an analysis of variance was performed with the experiment analyzed as a split plot design, using sampling date as the subtreatment within the main treatments. This was done to detect changes in treatments at different dates and to determine if correlations exist between the measured variables at different dates.
CHAPTER IV

RESULTS AND DISCUSSION

As described in the statistical analysis section of the preceding chapter, the experimental results will be discussed in two separate sections. The effects of ethephon on flowering data, nutrient content, and various yield components for the total season will be discussed in the first section. In the second section special emphasis will be placed on the flowering and fruit setting pattern throughout the experiment, and an attempt will be made to correlate selected nutrient components with flowering and fruit set. The effects of ethephon on the flowering and fruit setting pattern will also be examined. To expedite discussion of experimental results, treatments will be referred to as follows: (1) control—a non-treated control, (2) pruned control—all pistillate flowers pruned at anthesis, (3) ethephon treatment—150 ppm ethephon applied at the three leaf stage, and (4) pruned ethephon—150 ppm ethephon applied at the three leaf stage and all pistillate flowers pruned at anthesis.

Effects of Ethephon on Flowering, Nutrient Content, and Yield For Total Season

Knoxville. There was no difference in the number of pistillate flowers per plant between the pruned control and
the pruned ethephon treatments. However, plants in both of these pruned treatments produced a significantly higher number of pistillate flowers than plants in the two non-pruned treatments. The ethephon treatment resulted in more pistillate flowers per plant than the control. This result is consistent with other reports (17, 37). However, plants in the ethephon treatment produced fewer pistillate flowers than plants in the pruned control and pruned ethephon treatments, as shown in Table 1. It has been suggested that flowering is promoted by increased carbohydrate levels (19) and that the fruit load on a plant is inversely related to carbohydrate accumulation (29). Thus, plants which had pistillate flowers pruned should maintain a higher level of carbohydrate accumulation and produce more flowers than plants on which fruits are allowed to develop. This finding is consistent with the fact that plants in the pruned treatments produced more flowers than plants in the non-pruned treatments.

Plants in the pruned control produced more staminate flowers than the control plants, and plants in both non-ethephon treatments produced more staminate flowers than plants in the two ethephon treatments. There was no difference in staminate flowers per plant between the ethephon treatment and the pruned ethephon treatment. These results indicate that ethephon application causes the production of more pistillate flowers and fewer staminate
<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Pistillate Flowers</th>
<th>Staminate Flowers</th>
<th>Total Flowers</th>
<th>Aborted Flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knoxville</td>
<td>Control</td>
<td>8.99c*</td>
<td>49.87b</td>
<td>58.86b</td>
<td>4.18b</td>
</tr>
<tr>
<td></td>
<td>Pruned Control</td>
<td>32.00a</td>
<td>62.00a</td>
<td>94.00a</td>
<td>-----</td>
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<tr>
<td></td>
<td>Ethephon</td>
<td>15.56b</td>
<td>33.93c</td>
<td>49.49c</td>
<td>8.92a</td>
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<tr>
<td></td>
<td>Pruned Ethephon</td>
<td>30.62a</td>
<td>33.30c</td>
<td>63.92b</td>
<td>-----</td>
</tr>
<tr>
<td>Crossville</td>
<td>Control</td>
<td>6.86b</td>
<td>29.75a</td>
<td>36.61a</td>
<td>2.43a</td>
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<td>13.73a</td>
<td>27.41a</td>
<td>41.14a</td>
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<td>Ethephon</td>
<td>6.58b</td>
<td>18.03b</td>
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<td>Pruned Ethephon</td>
<td>15.54a</td>
<td>21.58b</td>
<td>37.12a</td>
<td>-----</td>
</tr>
</tbody>
</table>

*Means were separated by Duncan's New Multiple Range Test, 5 percent level. Means were separated independently for the Knoxville and Crossville data.
flowers. The added pistillate flowers are possibly produced by the sex reversal of undifferentiated flowers which would normally be staminate. A stimulation of sex reversal in cucurbit by ethephon has been suggested (1). A nodal sequence of flower development starting with predominately staminate flowers and gradually changing to pistillate flowers has also been reported (41). Figures 1-3 are photographs of the types of flowers observed in this experiment. Figure 1 is a characteristic pistillate flower showing normal development. Figure 2 is a cross-section of a normal staminate flower. A flower with both staminate and pistillate characteristics is shown in Figure 3. Anthers are present, in addition to a somewhat misshapen stigma and ovary. These hermaphroditic flowers are not normally found in cucurbits (41). They seem to appear when the effect of ethephon on sex reversal is diminishing, and staminate flowers start appearing. Perhaps at this stage of development the ethephon still has enough influence on sex determination to stimulate pistillate flower production but is not concentrated enough to suppress development of staminate characteristics. This occurrence is consistent with the report that low concentrations of ethephon produced hermaphroditic flowers on male plants of *Canabis sativa* (1).

The ethephon treated plants produced more pistillate flowers than the control plants, but they also had a greater number of pistillate flowers aborted. This result is
Figure 1. A normal pistillate flower of summer squash.
Figure 2. A normal staminate flower of summer squash.
Figure 3. Hermaphroditic flower of summer squash with misshapen stigma and ovary.
consistent with the report that once a plant has set a threshold level of fruit, and its carbohydrate reserves are exhausted, the remaining flowers are aborted (29). A plant's photosynthetic capacity may thus limit the potential of ethephon to increase early yield because the plant cannot support the added fruit.

Plants in the pruned control had more total flowers than those in the control, and the pruned ethephon treated plants had more total flowers than those in the ethephon treatment. The increased number of flowers in the two pruned treatments is primarily due to the increased number of pistillate flowers.

The ethephon treatment resulted in more fruit per plant and a greater weight of fruit per plant than the control. However, as Table 2 shows, there was no difference in fruit size between plants in the ethephon treatment and the control. Other reports have indicated that ethephon did not increase total season yields (5, 17). These authors, however, took yield data for a four to six week period, and in this experiment yield data was only taken for a three week period. These results are similar to results found by other authors during a three week harvest interval.

As shown in Table 3, plants in the pruned control had a greater percentage of total carbohydrates than those in the ethephon and pruned ethephon treatments. Although there was no significant difference in total carbohydrate between
<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Number of Fruit</th>
<th>Weight of Fruit (kilograms)</th>
<th>Average Fruit Size (kilograms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knoxville</td>
<td>Control</td>
<td>4.80b*</td>
<td>1.00b</td>
<td>0.20a</td>
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<td>Pruned Control</td>
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<td>----</td>
<td>----</td>
</tr>
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<td>0.20a</td>
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<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Crossville</td>
<td>Control</td>
<td>4.43a</td>
<td>0.85a</td>
<td>0.19a</td>
</tr>
<tr>
<td></td>
<td>Pruned Control</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Ethephon</td>
<td>3.27b</td>
<td>0.56b</td>
<td>0.17b</td>
</tr>
<tr>
<td></td>
<td>Pruned Ethephon</td>
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<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

*Means were separated by Duncan's New Multiple Range Test, 5 percent level. Means were separated independently for the Knoxville and Crossville data.
TABLE 3

SEASON AVERAGE NUTRIENT CONTENT FOR SUMMER SQUASH ON A DRY WEIGHT BASIS

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Percent Carbohydrate</th>
<th>ppm Nitrate-Nitrogen</th>
<th>Percent Nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knoxville</td>
<td>Control</td>
<td>8.37c*</td>
<td>40.61a</td>
<td>3.63a</td>
</tr>
<tr>
<td></td>
<td>Pruned Control</td>
<td>10.40a</td>
<td>41.41a</td>
<td>3.60a</td>
</tr>
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<td></td>
<td>Ethephon</td>
<td>7.39d</td>
<td>39.78a</td>
<td>3.66a</td>
</tr>
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<td></td>
<td>Pruned Ethephon</td>
<td>9.34b</td>
<td>38.15a</td>
<td>3.51a</td>
</tr>
<tr>
<td>Crossville</td>
<td>Control</td>
<td>9.12ab</td>
<td>53.51a</td>
<td>4.36a</td>
</tr>
<tr>
<td></td>
<td>Pruned Control</td>
<td>9.72a</td>
<td>55.24a</td>
<td>4.19a</td>
</tr>
<tr>
<td></td>
<td>Ethephon</td>
<td>8.07b</td>
<td>60.35a</td>
<td>4.42a</td>
</tr>
<tr>
<td></td>
<td>Pruned Ethephon</td>
<td>9.46a</td>
<td>60.33a</td>
<td>4.40a</td>
</tr>
</tbody>
</table>

*Means were separated by Duncan's New Multiple Range Test, 5 percent level. Means were separated independently for the Knoxville and Crossville data.
plants in the two ethephon treatments, the percent total carbohydrate was in fact higher in the pruned ethephon than in the ethephon treatment. Thus, plants in the pruned treatments had more carbohydrate accumulation than those in the non-pruned treatments. This finding is consistent with other reports that fruit acts as a metabolic sink into which nutrients flow, causing reduced nutrient accumulation in foliage (12, 40).

There were no differences detected in either nitrate-nitrogen or total nitrogen content among any of the treatments. Neither fruiting habit nor ethephon treatment affected the total season average content of these nutrients.

The ethephon treated plants had a higher season average calcium content than those in the pruned ethephon treatment, as shown in Table 4, and the control plants had a higher average calcium content than those in the pruned control. However, there was no statistically significant difference in calcium content noted in other comparisons of the treatments. Plants in the fruit bearing treatments had a higher calcium content than those in the pruned treatments. The requirement for calcium by fruits to form pectic substances may stimulate greater calcium uptake. However, there is no documented explanation for this observation.

Detectable differences were not observed among plants of any of the treatments for average percent magnesium,
TABLE 4

SEASON AVERAGE MINERAL CONTENT FOR SUMMER SQUASH ON A DRY WEIGHT BASIS

<table>
<thead>
<tr>
<th>Location</th>
<th>Treatment</th>
<th>Percent Magnesium</th>
<th>Percent Calcium</th>
<th>Percent Phosphorus</th>
<th>Percent Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knoxville</td>
<td>Control</td>
<td>1.42a*</td>
<td>6.07b</td>
<td>.23a</td>
<td>4.41a</td>
</tr>
<tr>
<td></td>
<td>Pruned Control</td>
<td>1.36a</td>
<td>5.60b</td>
<td>.24a</td>
<td>4.14b</td>
</tr>
<tr>
<td></td>
<td>Ethephon</td>
<td>1.42a</td>
<td>6.57a</td>
<td>.24a</td>
<td>4.55a</td>
</tr>
<tr>
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<td>Pruned Ethephon</td>
<td>1.36a</td>
<td>6.05b</td>
<td>.23a</td>
<td>4.46a</td>
</tr>
</tbody>
</table>

*Means were separated by Duncan's New Multiple Range Test, 5 percent level.
phosphorus, or potassium. Average percentage of these minerals was not affected by fruit set or ethephon treatment.

**Crossville.** There was no difference in total pistillate flowers per plant between the pruned control and the pruned ethephon treatments (Table 1, p. 23). This result is consistent with the Knoxville data. Plants in the pruned treatments produced more pistillate flowers per plant than those in the control and the ethephon treatments. However, there was no difference in pistillate flowers per plant between the ethephon treatment and the control. This finding is inconsistent with the Knoxville data but consistent with other reports that the total number of pistillate flowers per plant is not changed by ethephon (1). The plants at Crossville were not at a uniform three leaf stage when the ethephon was applied, whereas the Knoxville plants were more uniform in their stage of development. Thus, the ethephon was more effective in producing pistillate flowers at Knoxville. Reports that the time of application is critical in ethephon stimulated sex reversal support this observation (8, 28).

There was no difference in the production of staminate flowers per plant between the control and the pruned control. However, plants in both of these treatments produced more staminate flowers than those in the ethephon treatments. This result is consistent with the Knoxville data.
The ethephon treated plants had fewer total flowers per plant than those in the other three treatments, and there was no difference in total flowers among the other treatments. As reported for the Knoxville data, plants in the pruned treatments had more total flowers than those in the non-pruned treatments due to an increased number of pistillate flowers.

Since there was no difference in pistillate flowers per plant between the control and ethephon treatment, no difference should be noted in aborted flowers per plant. As expected, there was no difference in aborted flowers per plant between these two treatments.

Plants in the control produced more fruit per plant and a greater weight of fruit per plant than those in the ethephon treatment. The control plants also had a larger average fruit size than the ethephon treated plants. This occurrence is not consistent with the Knoxville data as seen in Table 2 (p. 29). However, close examination of the data in Table 1 (p. 23) reveals an explanation of these results. There was not a statistically significant difference in pistillate flowers or aborted flowers per plant for the two treatments, but the control plants had more pistillate flowers and fewer aborted flowers than those in the ethephon treatment. A combination of these factors resulted in more fruit per plant in the control than in the ethephon treatment.

No difference was detected among plants in the control, pruned control, and pruned ethephon treatments in average
carbohydrate content, and there was no difference between the control and the ethephon treatment. But plants in the pruned control and the pruned ethephon treatments had a higher average percent carbohydrate content than those in the ethephon treatment. These results are similar to the Knoxville data since the pruned treatments had a higher average percentage of carbohydrates.

Differences were not detected among treatments in nitrate-nitrogen or total nitrogen concentration. Mineral analyses were not performed on the Crossville data.

**Effects of Ethephon on Flowering and Fruit Setting Pattern**

This phase of the experiment was analyzed as a split plot design, using sampling period as the sub-treatment within main treatments. This analysis was done to detect changes resulting from treatments at different dates and to test correlations between measured variables at different dates. An attempt was made to determine if flowering and fruit set followed a cyclic pattern, to correlate the measured nutrients with flowering and/or fruit set, and to test the effects of ethephon on factors related to flowering. Results for Knoxville and Crossville were identical for this phase of the experiment, and to avoid repetition the results will be discussed in terms of the Knoxville data only. The only variation between the two locations was that mineral analyses were not performed for the Crossville data.
There was a significant interaction between treatment and sampling period, and the number of pistillate flowers per plant was different for some of the treatments at different sampling periods as shown in Figure 4. This finding is consistent with the theory that flowering and fruit set in summer squash follow a cyclic pattern, with periods of increased flower production being followed by periods of decreased flower production. As illustrated in Figure 4, plants in the ethephon treatments produced more pistillate flowers per plant early in the flowering cycle; whereas, those in the non-ethephon treatments produced more flowers per plant later in the cycle.

The total number of flowers per plant was different among treatments, and there was a significant interaction between treatment and sampling period for total flowers per plant. Figure 5 illustrates that total flowers per plant followed a similar pattern as pistillate flowers per plant. The non-ethephon treated plants consistently produced more total flowers than those in the ethephon treatments after the first sampling period. This difference was due to more staminate flowers per plant produced by the non-ethephon treatments. This finding is consistent with reports that ethephon promotes pistillate flower production in cucurbits and inhibits staminate flower production (37, 28).

The number of fruit and weight of fruit per plant were different among treatments, and the interaction between
Figure 4. Number of pistillate flowers per summer squash plant by sampling period.
Figure 5. Total flowers per summer squash plant by sampling period.
treatment and sampling period was significant for both number and weight of fruit per plant. Figure 6 illustrates that plants in the ethephon treatment produced more fruit and a greater weight of fruit in the first sampling period than the control plants, but plants in the control produced more fruit and a greater weight of fruit than those in the ethephon treatment in several later sampling periods. This finding is consistent with reports that ethephon treatment increases early but not total yield (5). A cyclic pattern consisting of increased fruit production followed by a period of decreased fruit production can be observed for individual treatments in Figure 6. This observation is consistent with the theory that flowering and fruit set in summer squash follow a cyclic pattern.

The percent carbohydrate shown in Figure 7 and the percent calcium shown in Figure 8 were different among treatments, and the interaction between treatment and sampling date was significant for both. Grainger (19) has indicated that flowering is correlated with the added percentage of total carbohydrate + ash. Thus, the percent total of carbohydrate (CHO) was added to the percentage of Ca + Mg + K + P + N in the tissue and analyzed as one variable as shown in Figure 9. This combined percentage of CHO + Ca + Mg + K + P + N was different among treatments, and the interaction between treatment and sampling date was significant. This result was probably due to the influence of
Figure 6. Number and weight of fruit per summer squash plant by sampling period.
Figure 7. Percent carbohydrate of summer squash plants by sampling date.
Figure 8. Percent calcium of summer squash plants by sampling date.
Figure 9. Percent carbohydrate + Ca + Mg + P + K + N of summer squash plants by sampling date.
percent carbohydrate and percent calcium on the interaction, since carbohydrate and calcium accounted for a greater percentage of the dry weight, and both of these factors were different among treatments. There was no difference among treatments in nitrate-nitrogen, magnesium, phosphorus, potassium, or total nitrogen content, and there was no interaction between treatment and sampling date for any of these variables. Since these variables were not different among treatments, and interactions were not significant for treatments on different dates, no relationship to flowering and/or fruit set was indicated.

The analysis of variance for this phase of the experiment indicates that flowering and yield are different among treatments, and there is an interaction between treatment and sampling period for these variables. Carbohydrate, calcium, and CHO + Ca + Mg + K + P + N are also different among treatments, and these variables have a significant interaction between treatment and sampling date. Thus, it is possible to show a relationship between flowering and/or yield and carbohydrate, calcium, or CHO + Ca + Mg + P + K + N. An attempt was made to determine a mathematical relationship among any or all of these variables by comparing each to all other variables singly and in combinations. Flowering data were plotted against carbohydrate, calcium, and CHO + Ca + Mg + P + K + N. The procedure was repeated for yield data. The temperature data shown in Table 5 were also plotted
### TABLE 5

**DAILY TEMPERATURES FOR JULY 1975 IN DEGREES CENTIGRADE**

<table>
<thead>
<tr>
<th>Date</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Average</th>
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</thead>
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<td>20.0</td>
<td>25.9</td>
</tr>
<tr>
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<td>25.9</td>
</tr>
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<td>20.0</td>
<td>25.0</td>
</tr>
<tr>
<td>4</td>
<td>31.7</td>
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<td>26.4</td>
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<td>18.9</td>
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</tr>
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</tr>
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</tr>
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</table>
against flowering and yield. No consistent mathematical relationship was found to exist between any two of the tested variables or between any group of variables and flowering or yield.

A further attempt was made to determine if any of the variables tested were related to flowering and/or yield by testing for correlations between variables by treatment and sampling period. Correlations were determined by treatment and sampling period, between number of pistillate flowers per plant and percentage of all the tested nutrients (carbohydrate, nitrate, Mg, Ca, N, P, and K) singly and in combination. Correlations were also determined between pistillate flowers per plant and temperature and pistillate flowers per plant and CHO + Ca + Mg + P + K + N. And finally, correlations were determined between the number or weight of fruit per plant and measured nutritional variables or temperature by treatment and sampling period. No consistent pattern of correlation was found between any of the variables for any treatment. A few significant correlations did occur, but no variable was consistently correlated to flowering or yield for any treatment for all sampling periods. Significant correlations are assumed to be random, and no consistent correlation between flowering or yield and any tested variable is indicated by these results. This finding is inconsistent with some reports (30, 29, 19, 44) but consistent with others (20, 25, 16).
Correlations were done by grouping several days of flowering and/or yield data and comparing this average to a single sampling date for nutrient variables. This procedure was necessary because of the great number of samples which had to be analyzed. Perhaps if carbohydrates were compared to flowering data on a one-to-one basis and not to an average number of flowers per period, a more significant correlation might have been indicated. However, based on the results of this study no correlation is indicated between flowering or yield and any of the tested nutrients. Carbohydrate and other nutrient accumulations may be indicators of the direction of metabolic processes, as has been suggested (20), but they do not seem to be the direct cause of flowering in summer squash. Although there were differences among treatments for flowering and yield data and also for several nutrient percentages, there was no consistent correlation between flowering or yield and any of the nutrient concentrations for any treatment.
CHAPTER V

SUMMARY AND CONCLUSIONS

Results of the total season study indicate that there is a decrease in carbohydrate accumulation associated with fruit development in summer squash. Plants in the pruned treatments had a higher average percent carbohydrate than those in the non-pruned treatments. None of the other measured nutritional variables could be directly associated with flower initiation or fruit set. The ethephon treatment produced more pistillate flowers per plant, but it also had a greater number of aborted flowers per plant. This result is consistent with the theory that the onset of fruit development stops flower production and fruit set. A plant's photosynthetic capacity may not be sufficient to support developing fruit and continued flower production; therefore, the potential of ethephon to increase yields may be limited.

The production of hermaphroditic flowers on a normally monoecious summer squash cultivar seems to occur when the effect of ethephon on sex reversal is diminishing. Perhaps the reduced concentration of ethephon has enough influence on sex determination to induce pistillate flower production but does not suppress development of staminate characteristics.
Flowering and yield did exhibit a cyclic pattern throughout the season, as did carbohydrate accumulation. Pruning and ethephon treatment affected the pattern of flowering and fruit set and also affected the carbohydrate and calcium accumulation during the season. However, there was no direct relationship between carbohydrate content and/or flowering for any of the treatments. None of the other nutritional variables tested could be associated with flowering.

Based on the results of this study no correlation is indicated between flowering or yield and any of the tested nutrients. While carbohydrate and other nutrient accumulations may indicate the direction of metabolic processes, they do not seem to be directly associated with flowering in summer squash. Tissue samples used for carbohydrate analysis were analyzed on a per day basis. Perhaps if flowering data had been analyzed on a per day basis, rather than on a per period basis, a more significant correlation between carbohydrate and flowering might have been established.
LITERATURE CITED


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VITA

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