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Effects of succinic acid 2,2-dimethylhydrazide and succinic acid on some physiological processes of Phaseolus vulgaris L

James G. Staley

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To the Graduate Council:

I am submitting herewith a dissertation written by James G. Staley entitled "Effects of succinic acid 2,2-dimethylhydrazide and succinic acid on some physiological processes of Phaseolus vulgaris L." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Plant, Soil and Environmental Sciences.

B. S. Pickett, Major Professor

We have read this dissertation and recommend its acceptance:

Homer D. Swingle, David L. Coffey, Gordon E. Hunt, Henry A. Freibourg

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
July 31, 1970

To the Graduate Council:

I am submitting herewith a dissertation written by James G. Staley entitled "Effects of Succinic Acid 2,2-Dimethylhydrazide and Succinic Acid on Some Physiological Processes of Phaseolus vulgaris L." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Agricultural Plant and Soil Science.

Major Professor

We have read this dissertation and recommend its acceptance:

[Signatures]

Accepted for the Council:

[Vice Chancellor for Graduate Studies and Research]
EFFECTS OF SUCCINIC ACID 2,2-DIMETHYLHYDRAZIDE AND SUCCINIC ACID ON SOME PHYSIOLOGICAL PROCESSES OF PHASEOLUS VULGARIS L.

A Dissertation
Presented to
the Graduate Council of
The University of Tennessee

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

by
James G. Staley
August 1970
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The author is deeply indebted to his wife, Bobbie, for her patience, support and encouragement during the period of graduate study.
The purpose of this investigation was to determine if succinic acid, 2, 2-dimethylhydrazide (Alar), a growth retardant, hydrolyzes within the bean (Phaseolus vulgaris L. cultivar Tendercrop) plant.

The experimental data for the investigation were obtained by measuring various physiological processes at two growth stages comparing Alar-treated plants to plants treated with succinic acid. The areas of interest were plant fresh and dry weight, chlorophyll content, some hexose sugars, sucrose and starch synthesis.

Designated plants were treated with 0.15 per cent Alar and 0.11 per cent succinic acid. A total of three applications were applied at three-day intervals as sprays to the foliage of one month old plants growing in the field. Plants were collected during the blooming stage and fruiting stage. Leaflets, stems and pods were analyzed separately.

It was found that these chemicals affected some of these plant processes. Both increased D-sucrose in the leaflets and stems, whereas they increased the hexoses (alpha D-glucose, beta D-glucose and beta D-fructose) in the pods. Starch was increased in the leaflets with the Alar treatment. In most cases, increases were greater with the succinic acid treatment than with the Alar treatment.

It was found also that chemical effect was not the same for both growth stages. The Alar treatment effect was usually greater at the fruiting stage than the blooming stage, whereas this effect was not as noticable with the succinic acid treatment. This seems to indicate the Alar molecule was slowly hydrolyzed into the hydrazine moiety and succinic acid.
In conclusion, these data seem to suggest the Alar molecule slowly hydrolyzes within the bean plant. Also, the results obtained from the sugar analysis seem to suggest that succinic acid might be utilized as a growth regulator in increasing sugar content of plants.
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CHAPTER I

INTRODUCTION

For the last half century, agricultural scientists have been experimenting with organic chemicals known as "growth regulators." The story of growth regulators is one of the interesting chapters in science. Like most discoveries and developments, it came as a gradual unfolding to a great number of individuals over a period of time, culminating in a rapid surge of activity which began in the 1930's (52).

The general trend has been for a vast amount of research to be conducted on a particular group of growth regulators. After some period of time, someone reports on a different type of growth regulator and the emphasis switches to that area. Such has been the case with the substituted succinamic acids.

Since the release of Alar, a substituted succinamic acid designated succinic acid 2,2-dimethylhydrazide, a tremendous amount of research has been done over a wide range of plant species. Most research using Alar has been of the applied nature with only a small number of scientists reporting from a basic approach.

Discrepancies have been reported among those scientists studying from a basic approach as to the mode of action of Alar. Some data indicated the Alar molecule hydrolyzed whereas, other data indicated the molecule did not hydrolyze. It seems that if the Alar molecule hydrolyzes yielding 1, 1-dimethylhydrazine and succinic acid, the succinate moiety as suggested should have some effect on the plant.
To the author's knowledge there has been no research reported studying the succinic acid moiety of Alar. Therefore, it became the purpose of this study to determine if the Alar molecule hydrolyzes by discovering whether the succinic acid moiety has an effect on plants in terms of chlorophyll development in leaves, alcohol soluble sugars, starch, fresh weight, dry weight, and percentage dry matter of leaves, stems, and fruit. This was to be accomplished by comparing Alar-treated plants to plants treated with succinic acid at equivalent concentration.

*Phaseolus vulgaris* L. cultivar Tendercrop was selected for this study since results could be obtained over a short period of time.

The areas of chemical determinations were restricted to chlorophyll, alcohol soluble sugars, and starch, since succinic acid is involved either directly or indirectly with these plant products. It seems reasonable that a change should occur in one or more of these areas with succinic acid treatment. If the Alar molecule splits, then it also seems reasonable that a change should occur similar to that of the succinic acid treatment.
CHAPTER II

REVIEW OF LITERATURE

I. HISTORY OF ALAR DEVELOPMENT

Some of the most exciting potential for the future application of basic plant physiology to horticulture involves growth regulators. Many compounds are already in regular use. New ideas about application of these materials are demanding better understanding of their metabolism and mode of action. This demand will be even greater as more compounds are discovered that will have new and different influences on plant growth and morphogenesis.

A number of growth inhibitors are utilized in ornamental horticulture because they will retard the height and spread of plants. The result is a more compact plant. Important current compounds include Alar, Maleic Hydrazide, and Phosphon (tributyl-2, 4-chlorobenzyl-phosphonium chloride). These growth retardants appear to be valuable in other aspects of horticulture, such as in fruit production, where they may retard preharvest drop, encourage blossom set, and promote fruit color.

The widest spectrum of activity of chemical growth retardants belongs to a selected hydrazide of succinic acid, called B995 or Alar. Chemically its name is succinic acid 2,2-dimethylhydrazide. The dosage required to regulate growth is one of the highest of growth retardants (10).
Cathey (9) reported that B995 is unique in its chemical structure as a growth retardant. It does not contain a benzene ring, quaternary ammonium or phosphonium cation, or substituents that are of small size, nucleophilic, and non-ionizable. All the previous compounds that were active contained one or more of these radicals. B995 is a free, ionizable acid with the C-C-N-N system as found in beta hydroxyethyl hydrazine.

Riddell et al. (40) reported in 1962 that application of sprays at 5 per cent of substituted maleamic and succinamic acids to foliage retarded the growth of legumes, vine crops, potatoes, and ornamental plants. One of the most active compounds was N-dimethylamino succinamic acid, designed B995.

In 1964, a plant growth retardant formulation named Alar-50, identical to B995, was released by the Chemical Division of United States Rubber Company. In 1965, this company released Alar-85, a new formulation (53).

II. BREAKDOWN AND MOVEMENT OF ALAR

Research workers (38, 33) have reported that the Alar molecule tends to hydrolyze slowly over a long period of time, yielding succinic acid and the hydrazine moiety, while others (32, 42) have reported the Alar molecule resistant to breakdown.
Work by Reed, Moore, and Anderson (38) showed that inhibition of shoot elongation in dwarf and tall peas by B995 could be correlated with inhibition of tryptamine-2-C\(^{14}\) oxidation to indole-acetaldehyde-2-C\(^{14}\) in homogenates prepared from epicotyls of young B995 treated plants. The growth-retarding action of B995 was attributed to the formation of 1,1-dimethylhydrazine which strongly inhibited tryptamine oxidation. They suggested the chemical B995 hydrolyzed yielding succinic acid and 1,1-dimethylhydrazine.

Martin and Williams (33) working with Alar, labeled in the dimazine and succinic acid moisties via trunk injections and root dips of apple trees, reported that it moved freely into all areas of the plant. They inferred that breakdown occurred slowly throughout the growing period. Similar decomposition rates were found for both labels.

Ryugo (41), working with sweet cherries, found Alar present in new leaves in the spring following a late fall application of Alar. The residue level in green fruits decreased initially but gradually increased in the ripening fruit, indicating that movement into the fruit exceeded the rate of its catabolism.
Edgerton and Greenhalgh (13) applied sprays of $^{14}$C labeled N-dimethyamino succinamic acid to the young fruit and leaves of 'McIntosh' apple trees. With frequent sampling they found the levels of radioactivity in alcoholic extracts of receptacle and seeds reached maximum values in about three weeks. The compound was accumulated in flower buds, vegetative buds, cluster bases, one-year-old bark and one-year-old xylem.

Kilby et al. (26) treated the lower epidermis of the leaves of young tung trees (Aleurites fordii Hemsl) with $^{14}$C labeled Alar. They found that after one hour the labeled compound had been absorbed and translocated to the petiole. Alar was found in all parts of the plant 24 hours after treatment. Four days after treatment, peak accumulation was reached throughout the plant with the terminal growing point showing the greatest accumulation.

Undurraga and Ryugo (52) in making autoradiographs of girdled and non-girdled almond seedlings treated with $^{14}$C Alar, revealed that the growth retardant moved readily from the phloem to the xylem. Autoradiographs of comparable seedlings treated with $^{14}$C sucrose, with and without Alar pretreatment, showed that Alar induced greater leakage of radioactive material from the symplast to the apoplast, indicating that the material increased membrane permeability. Since Alar has been reported to reduce respiration rates of lettuce (23) and blueberry (38) and was found to reduce the respiration rate of peas by these authors, Alar may account for the enhanced diffusion of cell contents. This is possible since respiratory energy is known to be a requirement for the accumulation and retention of solutes in the vacuole.
This effect of Alar on membrane permeability was examined by immersing dormant stems, leaf discs, and beet root tissue in solutions of Alar and water. In all tissues, the compound induced greater leakage of cellular contents than did water. Since Alar depresses respiration, then it seems likely that the smaller amount of energy available in the presence of Alar may be responsible for the leakage of solutes from the vacuole.

Work by Martin et al. (32) with C\textsuperscript{14} labeled B995 showed the growth retardant was quite mobile and stable in apple seedlings. The chemical analysis of the organic constituents with column and paper chromatography showed that even after long periods of metabolism the majority of the previously injected C\textsuperscript{14} labeled B995 remained intact.

Ryugo and Sachs (42) working with \textit{in vitro} studies of Alar, reported that commercial proteolytic enzyme preparation and enzymes prepared from Alar sensitive plants were not capable of breaking the C-N bond in Alar. Their data showed that Alar did not hydrolyze to succinate and unsymmetrical dimethylhydrazine to a detectable degree.

The research reported to date on Alar breakdown shows that discrepancies still remain as to whether or not it hydrolyzes. If it hydrolyzes within the cells, it seems likely that the succinate should cause some physiological plant response since it is associated with plant processes such as chlorophyll development (11) and respiration (6).
III. SUCCINIC ACID

Succinic acid (L. succinum, amber), HOOC(CH₂)₂COOH, was obtained originally from the distillation of amber, a hard fossil resin. It has also been obtained from lignite and occurs in small quantity in plants. It is produced commercially by the reduction of maleic or fumaric acids (46).

Every living cell probably contains catalytic amounts of succinic acid (46). It is the most plentiful acid in shoot of Medicago sativa and an important constituent of the stem tissue of Vinca rosea (36).

Succinic acid is used within the plant in the biosynthesis of cyclic pyrrole structures utilized in making the chlorophyll molecule (11).

IV. ALAR AND CHLOROPHYLL DEVELOPMENT

Several research workers (18, 8, 1) have reported darker green and thicker leaves following the use of Alar. Knavel (27) reported that Alar treatment of young tomato plants resulted in darker green plants than untreated plants. Alar-treated tomato plants contained an average of 3.952 mg of total chlorophyll per liter, whereas control plants contained an average of 2.547 mg per liter.

Knavel further found that leaf sections of treated tomato plants contained an average of 7.7 palisade parenchyma cells, while control plants contained only 6.0 palisade parenchyma cells per square mm. There were more intercellular spaces in control leaves in both spongy mesophyll and palisade parenchyma than in treated plants.
Halfacre et al. (20) working with one-year-old apple plants found that chlorophyll per unit area of leaf fresh tissue was increased by Alar treatment with a trend towards an increase in chlorophyll on a weight basis in the treated plants. They suggested that the increase in chlorophyll may have been a result of accelerated or prolonged synthesis, or a delay in breakdown.

V. ENERGY AND SUCCINIC ACID

Succinic acid in plants is also a participant in the Krebs cycle or tricarboxylic acid cycle, the cycle of reactions which accounts for the oxidation of pyruvic acid to CO$_2$ and water.

The free-energy change in the complete oxidation of glucose to CO$_2$ and water has been calculated as $-686$ kcal per mole (11). Thus, if added succinic acid could enter the Krebs cycle it might be possible to slow the cycle. This possibly might allow sugars to accumulate, thus decreasing part of this energy change. This would tend to agree with the suggestion of Undurraga and Ryugo (52) that cell wall permeability increases when there is less energy available.

VI. FLOWERING AND FRUITING

Jones et al. (25) reported that carbohydrate reserves appear to influence fruiting. They found that carbohydrate accumulation in 'Valencia' Orange leaves was related to the fruit set on the tree. Less carbohydrate was found in the leaves when the trees were carrying fruit loads.
Batjer et al. (4) reported marked increases in the amount of bloom on apples, pears, and sweet cherries treated with B995. Looney et al. (29) reported not only an increase in bloom on Alar-treated apple trees but also an increase in number of fruits.

Read and Fieldhouse (37) and Bergman (5) have reported increased fruit yield following Alar treatment of tomato plants. Their research demonstrated potential benefits to commercial producers in processing tomatoes.

Other research workers (14, 21, 43, 51) have reported increases in flower buds and fruit set of Alar-treated plants.

Hilgeman et al. (22) in studying time of harvest as related to leaf carbohydrate content and subsequent set of fruit, found that as harvest dates of mature 'Valencia' oranges were advanced by monthly increments from March to July, fruit set of the following crop was successively reduced. Carbohydrate content in leaves also was reduced with successive harvests. The higher carbohydrate content in leaves at earlier harvest was a factor apparently related to increased set of young fruit.

Sachs and Hackett (43) reported that sugar concentration appeared to play a major role in morphogenesis. They showed that the level of sucrose in the medium, even when light intensity was high, was of great importance in regulating reproductive development. There appeared to be optimal levels of carbohydrates for reproductive development.

Carbohydrates are of special significance in plants since they represent food reserves and are part of the structural framework of each cell. They comprise 50 to 70 per cent of the total dry weight
of most species (44). Shallenberger (45) has shown that the major sugars in the snap bean pod, on a fresh weight basis, are composed of 1 per cent glucose, 1 per cent fructose, and 0.3 per cent sucrose.

Mack and Singh (30) reported that the percentage set of blossoms, the number and the weight of pods of bush snap beans were reduced when plants were subjected to high maximum temperatures during bloom. Also, the carbohydrate content decreased in the leaves, and starch decreased more than sugar content. Hence, any factor or factors affecting carbohydrates in plants, whether directly or indirectly, may impose physiological or morphological changes on the plant.

VII. OTHER ASPECTS OF ALAR

Other aspects of Alar effect on plants have been reported. Culbert (12) found that a single application of 0.5 per cent B995 to potted chrysanthemum plants not only resulted in shorter internodes, but also prolonged the life of the flowers when compared with untreated plants. This might perhaps suggest reduced respiration rate. Flower life was extended further by applying a second spray about 5 weeks after the first at a concentration of 0.25 per cent.

Halevy and Wittwer (17) reported an increase in life of cut carnations from the use of an 18-hour stem base immersion in B995. Larsen and Scholes (28) also reported increased vase life of cut carnations as a result of using Alar. They proposed that Alar may have had several effects on the increased vase life, indicating slower metabolism.

Alar also has been shown to decrease fruit drop (15, 3) and delay senescence (19).
VIII. GAS CHROMATOGRAPHY FOR DETERMINING SUGARS

Wiley et al. (54) separated sugars of apple cell walls by using gas liquid chromatography. Trimethylsilyl (TMS) derivatives were made by the direct addition of dry pyridine, hexamethyldisilazane, and trimethylchlorosilane. They were one of the first groups of horticulturists to report a gas chromatography procedure for sugar analysis.

Since the work of James and Martin (24) in 1952, gas chromatography has been increasing in use as an important analytical tool. Stevens (48) has reported recently that gas chromatography should be a useful tool for horticulturists in analytical research for obtaining accuracy.

Fretz et al. (16) reported a gas chromatographic procedure for determining soluble carbohydrates extracted from leaf tissue of Ilex opaca. They found the procedure to be a sensitive and reliable technique for carbohydrate analysis.
CHAPTER III

MATERIALS AND METHODS

The field portion of this study was conducted on Morgan Farm of the University of Tennessee, Knoxville, Tennessee. Tender Crop bush snap beans, a cultivar of *Pasheolus vulgaris*, were seeded on June 21, 1968. Seeds were sown in three foot rows and plants thinned to four inches apart in the row. A randomized complete block design of three treatments replicated four times was used. The plot was on Etowah silty clay loam and recommended production practices were used.

Three applications of a 0.15 per cent aqueous solution of Alar-85 (succinic acid 2, 2-dimethylhydrazide) and of an 0.11 per cent succinic acid solution were sprayed on designated plants to run-off at three day intervals starting on July 28, 1968. Sprayings were performed in the mornings. No rainfall occurred within 24 hours after the applications.

I. FRESH AND DRY WEIGHTS

The first sampling was performed during the blooming stage on August 8, 1968. Six plants per treatment per replication were collected at random and the fresh weights of leaflets and stems, including petioles were recorded separately. The leaflets and stems were oven-dried at 70°C and dry weights recorded. The dried samples were ground in a Wiley Mill and stored in stoppered bottles.

The second sampling was performed at the fruiting stage (pod formation) on August 15, 1968. Again six plants per treatments per
replication were collected at random and fresh weights of the leaflets, stems, with petioles, and pods recorded. The pods were those reaching 3 to 5 sieve size according to federal standards. These standards are based on pod diameter. Number 3 pods are 37-42/128 inch and number 5 pods are 54-68/128 inch. The leaflets and stems were oven-dried at 70°C, ground and stored. The pods were placed in a freezer until further use. On August 16, 1968, pods were collected again from six plants per treatment per replication, sieved (number 3 to 5) and fresh weight recorded. These were then oven-dried at 70°C and dry weight recorded.

II. CHLOROPHYLL DETERMINATIONS

Two one-gram samples per treatment per replication of dried leaflets were refluxed with 100 ml of 80 per cent acetone until the extracting fluid in the soxhlet remained colorless. The extracts were filtered using Whatman's No. 42 filter paper and the residues washed with 80 per cent acetone, filtered and brought to a 100 ml volume. Light absorbance of two aliquots per sample was read using a Beckman DU-2 Spectrophotometer set at 645 μm and again at 663 μm.

Total chlorophyll, chlorophyll a, chlorophyll b, and a:b ratio were then determined from the absorbance values as described by Mackinney (31) and Bruinsma (7).

III. ALCOHOL SOLUBLE SUGAR DETERMINATIONS

Sugar determinations were made by modification of the procedures described by Sweely et al. (49) and Williams (55). Two one-gram samples
per treatment per replication of leaflets and stems were refluxed for one hour in soxhlets with 100 ml of 80 per cent ethanol and then evaporated to 50 ml.

Two one-gram samples per treatment per replication of pods were ground with 100 ml of 80 per cent ethanol in a blender for 3 minutes. The slurry was filtered, washed and evaporated to 50 ml.

Two 2 ml aliquots were pipetted from each extract into small glass vials and oven-dried (forced air oven) at 40°C. After complete drying (overnight), 0.5 ml of triethylsilyl (TMS) was added to each vial. The vials were stoppered, shaken and allowed to stand at room temperature (21°C) overnight.

Two 1 ul aliquots of each sample were injected with a graduated microliter syringe into a Perkin-Elmer 881 Gas Chromatograph using a flame detector. A Texas Instrument Company recording potentiometer operating at a chart speed of 38.10 centimeters per hour provided the print-out.

A six-foot chromatograph column containing a 10 per cent SE-30 (diethylsilyl ether) stationary phase on Chromosorb W (10/100 mesh), DMCS treated solid support was used to separate the sugars.

Injections were performed at an injection port temperature of 300°C, a detector temperature of 225°C and a column temperature of 195°C for alpha and beta D-glucose and beta D-fructose. A 250°C column temperature was used for D-sucrose.

Helium (carrier gas) pressure was set at 0.35 kg/cm² (50 psi) with a flow rate of 70 ml per minute. Air pressure was set at 0.35 kg/cm² (50 psi) with a hydrogen pressure of 0.11 kg/cm² (16 psi).
The standards, alpha and beta D-glucose, beta D-fructose and D-sucrose were taken from a Sugar Sil Kit received from Pierce Chemical Company of Rockford, Illinois. Several 0.15 ul samples of each standard were injected into the gas chromatograph.

The base at one-half the height times the height calculation was used in determining the area under the curve for standards and unknowns.

Most recently McDonald and Newson (34) reported, working with gas chromatography of sweet potato sugars, some discrepancies in other gas chromatography procedures. These discrepancies were also found by this author in preliminary work.

In determining the per cent sugars of the unknowns, the following calculations were used:

\[
\frac{\text{Amount of Known}}{\text{Area Under Curve (Known)}} \times \frac{\text{X}}{\text{Area Under Curve (Unknown)}} = \frac{\text{X}}{100}
\]

where \( X = \) Amount of Sugar in Unknown

\[
\frac{\text{Amount of Sugar in Unknown}}{\text{Amount of Unknown}} = \frac{\text{X} \times 100}{\text{Per Cent Sugar in Sample}}
\]

IV. STARCH DETERMINATIONS

Starch determination was made using modifications of the procedures described by the AOAC (2), Nelson (35), and Somogyi (47). One-gram samples of leaflets and stems were immersed in hot ethanol for 10 minutes with frequent stirring to dissolve out the alcohol-soluble sugars. The mixtures were filtered through Whatman No. 42 filter paper and the residues oven-dried at 60°C for 24 hours.
A 250 mg aliquot from each dried residue was placed in a 250 ml Erlenmeyer flask with 10 ml of distilled water and refluxed in a boiling water bath for 30 minutes. Twenty-five ml Erlenmeyer flasks were used as condensers. The samples were cooled to room temperature and prepared for incubation. Incubation was accomplished in a 10 ml solution buffered to pH 4.2 with 0.2N acetic acid and 0.2N sodium acetate with 10 ml 'Clarase 900' solution, a form of takadiastase prepared by the Miles Laboratory of Elkhard, Indiana. The takadiastase was made up at the rate of 5 g per liter. One gram of powdered thymol per liter of solution was added to the stock enzyme and buffer solutions to serve as an antibacterial agent. The samples were then incubated for 44 hours at 37°C.

After incubation, the samples were filtered through Whatman No. 42 filter paper, the residues washed with 5 ml of 0.7N HCl and the filtrate refluxed in a boiling water bath, as described previously, to complete hydrolysis. After refluxing, the samples were filtered again and brought to 250 ml volume with distilled water.

To determine reducing power, 2 ml of copper reagent (47) were added to 1 ml sample aliquots plus 1 ml of distilled water in 50 ml test tubes. The samples were then heated in a boiling water bath for 15 minutes using 25 mm funnels as reflux condensers. After heating, the samples were cooled to room temperature and 2 ml of arsenomolybdate (47) and 25 ml of distilled water added. Samples were placed in cuvettes and the absorbance read on a Beckman DU-2 Spectrophotometer at 510 mμ.
Using D-glucose in solutions containing from 0 to 0.30 mg/ml and plotting absorbance against concentration of glucose, a straight line curve was produced. The slope of the line served as the conversion factor. The sample absorbance times the slope of the line due to glucose concentrations gives the milligrams per milliliter of glucose present in the sample. The percentage of glucose was determined by the equation:

\[
\text{Per Cent Glucose} = \frac{\text{mg glucose/ml in sample}}{\text{mg dry matter/ml in sample}} \times 100
\]

The per cent starch was then calculated as follows:

\[
\text{Per Cent Starch} = \frac{\text{per cent glucose}}{111.1} \times 100
\]

The second formula is used since 100 parts of starch should theoretically yield 111.1 parts of glucose (39).

V. ANALYSES OF DATA

The data from these experiments were statistically analyzed using analysis of variance techniques. The \( P \leq 0.05 \) level of significance was used to determine significant differences. When significant differences were detected, means were compared using least significant difference. Analyses of the sugar data were carried out on an IBM 360/65 computer system at the University of Tennessee Computing Center, utilizing statistical library programs adapted to the parameters of these experiments.
The mathematical model utilized for this experiment was expressed as:

\[ AG, BG, FR, SU, T = A(I) + B(J) + AB(IJ) + C(K) \]
\[ + AC(IK) + BC(JK) + ABC(IJK) \]
\[ + ABCD(IJKL) + E. \]

The variances were pooled as follows:

\[ P, PLPTS(A=A), REPS(B=B), TMTS(C=C), PX(T(AC=AC), \]
\[ EXPERR(ABC=AB+BC+ABC), SAMP(ABCD=ABCD) \]
CHAPTER IV

RESULTS AND DISCUSSION

I. FRESH WEIGHT, DRY WEIGHT, AND PERCENTAGE DRY MATTER

Leaflets

As noted from Table I, fresh weight of bean leaflets was not significantly affected by chemical treatment or growth stage.

Comparing the dry weight figures in Table I, it may be noted that chemical treatment had no significant effect, whereas growth stage did affect the dry weight.

Those leaflets collected during the fruiting stage weighed more than those collected at the blooming stage on a dry weight basis. This seems to indicate that the plant leaflets were more succulent at the blooming stage than at the fruiting stage.

The trend is the same for percentage dry matter as with the dry weight. Only the growth stage had a significant effect. Those leaflets collected at the fruiting stage were higher in percentage dry matter than those collected at the blooming stage (Table I).

The chemical-growth interaction was not significant, indicating that plant leaflets responded to chemical treatment in much the same manner during both growth phases in terms of fresh weight, dry weight, and percentage dry matter.

Stems (Containing Petioles)

As noted from Table II, fresh and dry weights of bean stems were significantly altered by chemical treatment, whereas no difference occurred due to growth stage.
### TABLE I
EFFECTS OF ALAR AND SUCCINIC ACID ON FRESH WEIGHT, DRY WEIGHT, AND PER CENT DRY MATTER OF BEAN LEAFLETS AT TWO GROWTH STAGES

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>Blooming Stage</th>
<th>Fruiting Stage</th>
<th>Chemical Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh Wt. (g)</td>
<td>Dry Wt. (g)</td>
<td>Per Cent Dry Matter</td>
</tr>
<tr>
<td>Check</td>
<td>23.00</td>
<td>4.82</td>
<td>20.92</td>
</tr>
<tr>
<td>Alar</td>
<td>22.71</td>
<td>4.94</td>
<td>21.71</td>
</tr>
<tr>
<td>Growth Stage Mean</td>
<td>23.94</td>
<td>5.12</td>
<td>21.53</td>
</tr>
</tbody>
</table>

Growth Stage LSD* N.S. 0.60 2.41

Chemical Treatment LSD

Chemical x Growth Stage LSD N.S. N.S. N.S.

*LSD ≤ .05.*
### TABLE II

**EFFECTS OF ALAR AND SUCCINIC ACID ON FRESH WEIGHT, DRY WEIGHT, AND PER CENT DRY MATTER OF BEAN STEMS AT TWO GROWTH STAGES**

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>Fresh Wt. (g)</th>
<th>Dry Wt. (g)</th>
<th>Per Cent Dry Matter</th>
<th>Fresh Wt. (g)</th>
<th>Dry Wt. (g)</th>
<th>Per Cent Dry Matter</th>
<th>Chemical Treatment Mean</th>
<th>Per Cent Dry Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alar</td>
<td>16.95</td>
<td>3.13</td>
<td>18.45</td>
<td>18.64</td>
<td>4.03</td>
<td>22.79</td>
<td>17.79</td>
<td>3.57</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>26.92</td>
<td>5.10</td>
<td>18.96</td>
<td>24.25</td>
<td>4.98</td>
<td>20.53</td>
<td>25.58</td>
<td>5.04</td>
</tr>
<tr>
<td>Growth Stage Mean</td>
<td>22.84</td>
<td>4.15</td>
<td>18.20</td>
<td>20.72</td>
<td>4.37</td>
<td>21.66</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Growth Stage LSD* N.S. N.S. 3.22

Chemical Treatment LSD 3.52 0.674 N.S.

Chemical x Growth Stage LSD N.S. N.S. N.S.

*LSD ≤ .05.
Fresh and dry weights from Alar-treated plants were less than untreated plants and those of succinic acid-treated plants were greater than check or Alar-treated plants. The low fresh and dry weights of the stems from Alar-treated plants would be expected since the chemical is a growth retardant.

No difference in fresh or dry weights of stems occurred between growth stages (Table II).

It may be noted that no significant difference in percentage dry weight of bean stems occurred due to chemical treatment (Table II) but the growth stage did significantly affect percentage dry matter.

The stems were higher in percentage dry matter at the fruiting stage than at the blooming stage. Differentiation of the stem would probably account for this difference.

The chemical-growth stage interaction was not significant, indicating that the plants responded to chemical treatment in much the same manner during both growth phases in terms of fresh weight, dry weight, and percentage dry matter of stems.

Pods

As noted from Table III, fresh weight, dry weight, percentage dry matter, and number of pods were not significantly influenced by chemical treatments.

However, a general trend occurred for an increase in pods with chemical treatment (Table III). It may be that this increase in fruit is related to increased sugars which would aid fruit set as reported in the literature (30, 43). This appears to be an area for further research.
### TABLE III

**EFFECTS OF ALAR AND SUCCINIC ACID ON NUMBER, FRESH WEIGHT, DRY WEIGHT, AND PER CENT DRY MATTER OF BEAN PODS PER PLANT**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh Wt. (g)</th>
<th>Dry Wt. (g)</th>
<th>Per Cent Dry Matter</th>
<th>Number of Pods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>45.31</td>
<td>4.91</td>
<td>10.88</td>
<td>9.12</td>
</tr>
<tr>
<td>Alar</td>
<td>49.58</td>
<td>5.31</td>
<td>10.66</td>
<td>10.54</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>56.95</td>
<td>5.81</td>
<td>10.10</td>
<td>11.67</td>
</tr>
<tr>
<td>Chemical Treatment LSD ≤ .05</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

*Pods of sieve sizes 3 to 5.*
II. TOTAL CHLOROPHYLL, CHLOROPHYLL a, CHLOROPHYLL b,
AND CHLOROPHYLL a/b RATIO IN BEAN LEAFLETS

As shown in Tables IV and V, chemical treatments had no significant effect on chlorophylls.

Since it has been reported in the literature (27) that Alar increased chlorophyll, it was thought that perhaps the addition of succinic acid might increase chlorophyll content of the leaves since it is incorporated into the pyrole structure of the chlorophyll molecule. These data seem to indicate no significant changes occurred in chlorophyll content.

Growth stage had an effect on chlorophylls in the leaflets of snap beans as shown in Tables IV and V.

On a dry weight basis, total chlorophyll and chlorophyll a decreased significantly from the blooming stage to the fruiting stage. These data suggest that considerable stress may have been placed on the chlorophyll system while the fruits were developing.

The chemical-growth stage interaction was not significant, indicating that the bean leaflets responded to chemical treatment much the same way at both growth stages with respect to chlorophyll content.

III. PERCENTAGE ALPHA D-GLUCOSE, BETA D-GLUCOSE, BETA D-FRUCTOSE,
AND D-SUCROSE IN BEAN LEAFLETS, STEMS AND PODS WITH
COMPARISON OF LEAFLETS AND STEMS AT
TWO GROWTH STAGES

Leaflets

As noted from Table VI, chemical treatments had no significant
TABLE IV

EFFECTS OF ALAR AND SUCCINIC ACID ON TOTAL CHLOROPHYLL OF DRIED BEAN LEAVES AT TWO GROWTH STAGES

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>Blooming Stage</th>
<th>Fruiting Stage</th>
<th>Chemical Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg Chlorophyll/g</td>
<td>mg Chlorophyll/Plant</td>
<td>mg Chlorophyll/g</td>
</tr>
<tr>
<td>Check</td>
<td>29.29</td>
<td>142.31</td>
<td>23.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26.53</td>
</tr>
<tr>
<td>Alar</td>
<td>29.84</td>
<td>147.91</td>
<td>25.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27.73</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>29.37</td>
<td>164.86</td>
<td>25.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>27.35</td>
</tr>
<tr>
<td>Growth Stage Mean</td>
<td>29.49</td>
<td>151.69</td>
<td>24.92</td>
</tr>
<tr>
<td>Growth Stage LSD*</td>
<td>1.45</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Chemical Treatment LSD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical x Growth Stage LSD</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

*LSD ≤ .05.
### TABLE V

**EFFECTS OF ALAR AND SUCCINIC ACID ON CHLOROPHYLL a, CHLOROPHYLL b AND a/b RATIO OF DRIED BEAN LEAVES AT TWO GROWTH STAGES**

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>Blooming Stage</th>
<th>Fruiting Stage</th>
<th>Chemical Treatment Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophyll a</td>
<td>Chlorophyll b</td>
<td>a/b</td>
</tr>
<tr>
<td>Check</td>
<td>22.53</td>
<td>6.75</td>
<td>3.35</td>
</tr>
<tr>
<td>Alar</td>
<td>21.46</td>
<td>8.35</td>
<td>2.82</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>22.34</td>
<td>4.77</td>
<td>3.58</td>
</tr>
</tbody>
</table>

**Growth Stage Mean**

<table>
<thead>
<tr>
<th></th>
<th>Chlorophyll a</th>
<th>Chlorophyll b</th>
<th>a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Stage</td>
<td>22.11</td>
<td>6.62</td>
<td>3.25</td>
</tr>
</tbody>
</table>

*Growth Stage LSD* 1.41 N.S. N.S.

*Chemical Treatment LSD* N.S. N.S. N.S.

*Chemical x Growth Stage LSD* N.S. N.S. N.S.

*LSD ≤ .05.*
TABLE VI
EFFECTS OF ALAR AND SUCCINIC ACID ON PER CENT ALPHA D-GLUCOSE, BETA D-GLUCOSE, BETA D-FRUCTOSE, AND D-SUCROSE IN BEAN LEAFLETS

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>αD-Glucose</th>
<th>βD-Glucose</th>
<th>βD-Fructose</th>
<th>D-Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>0.427</td>
<td>1.704</td>
<td>1.737</td>
<td>3.838</td>
<td>7.706</td>
</tr>
<tr>
<td>Alar</td>
<td>0.443</td>
<td>1.511</td>
<td>1.502</td>
<td>3.908</td>
<td>7.364</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>0.471</td>
<td>1.692</td>
<td>1.596</td>
<td>4.809</td>
<td>8.567</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td>N.S.</td>
<td>0.064</td>
<td>0.055</td>
<td>0.151</td>
<td>0.211</td>
</tr>
</tbody>
</table>

LSD = Least Significant Difference
effect on percentage alpha D-glucose in leaflets but did affect beta D-
glucose, beta D-fructose, and D-sucrose.

Leaflets of Alar-treated plants decreased significantly from those of the check in beta D-glucose, beta D-fructose and total sugars, whereas alpha D-glucose and D-sucrose content were not changed.

Leaflets of succinic acid-treated plants were significantly lower in beta D-fructose and significantly higher in D-sucrose and total sugars than in untreated and Alar-treated leaflets.

Comparing D-sucrose to total sugars (Table VI) the relative amounts of sucrose increased with chemical treatments.

These data seem to indicate that both Alar and succinic acid encourage the accumulation of sucrose, while they seem to inhibit the accumulation of reducing sugars.

Those leaflets collected at the blooming stage were found to have higher percentage of alpha D-glucose, beta D-fructose, and total sugars than those collected at the fruiting stage (Table VII). The leaflets from both harvests had essentially equivalent percentages of D-sucrose whereas those leaflets collected at the fruiting stage had a higher percentage of beta D-glucose than those collected at the blooming stage. When all hexoses are added together, the leaflets collected at the blooming stage contained 0.84 per cent more than those leaflets collected at the fruiting stage. Apparently the percentage of sugars in leaflets decreases at fruiting time.

Percentage of sugars in leaflets varied with chemical treatment from one growth stage to another (Table VIII).
### TABLE VII

PERCENT ALPHA D-GLUCOSE, BETA D-GLUCOSE, BETA D-FRUCTOSE, AND D-SUCROSE IN BEAN LEAFLETS AT TWO GROWTH STAGES INCLUDING ALL TREATMENTS

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>αD-Glucose</th>
<th>βD-Glucose</th>
<th>βD-Fructose</th>
<th>D-Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blooming</td>
<td>0.504</td>
<td>1.509</td>
<td>2.105</td>
<td>4.197</td>
<td>8.315</td>
</tr>
<tr>
<td>Fruiting</td>
<td>0.390</td>
<td>1.762</td>
<td>1.118</td>
<td>4.174</td>
<td>7.444</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td>0.032</td>
<td>0.079</td>
<td>0.071</td>
<td>N.S.</td>
<td>0.341</td>
</tr>
<tr>
<td>Growth Stage</td>
<td>Chemical Treatment</td>
<td>Per Cent αD-Glucose</td>
<td>βD-Glucose</td>
<td>βD-Fructose</td>
<td>D-Sucrose</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------</td>
<td>---------------------</td>
<td>------------</td>
<td>-------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Blooming</td>
<td>Check</td>
<td>0.556</td>
<td>1.736</td>
<td>2.403</td>
<td>3.968</td>
</tr>
<tr>
<td></td>
<td>Alar</td>
<td>0.430</td>
<td>1.292</td>
<td>1.766</td>
<td>3.766</td>
</tr>
<tr>
<td></td>
<td>Succinic Acid</td>
<td>0.526</td>
<td>1.499</td>
<td>2.144</td>
<td>4.858</td>
</tr>
<tr>
<td>Fruiting</td>
<td>Check</td>
<td>0.297</td>
<td>1.672</td>
<td>1.070</td>
<td>3.709</td>
</tr>
<tr>
<td></td>
<td>Alar</td>
<td>0.457</td>
<td>1.729</td>
<td>1.237</td>
<td>4.051</td>
</tr>
<tr>
<td></td>
<td>Succinic Acid</td>
<td>0.416</td>
<td>1.884</td>
<td>1.047</td>
<td>4.761</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td></td>
<td>0.056</td>
<td>0.134</td>
<td>0.128</td>
<td>0.466</td>
</tr>
</tbody>
</table>
Leaflets from untreated plants contained a higher percentage of the measured hexose sugars but less D-sucrose at blooming time than those treated with succinic acid, which in turn contained more hexoses and D-sucrose than the leaflets of plants treated with Alar. Alar-treated leaflets contained as much D-sucrose as those of untreated plants.

At the fruiting stage the situation was not the same. Untreated leaflets were lowest in percentage alpha D-glucose and total sugars. Alar-treated plant leaflets had a higher percentage beta D-fructose than leaflets of untreated plants or of succinic acid-treated plants. Succinic acid-treated plant leaflets were higher in percentage beta D-glucose than those of either Alar-treated plants or of untreated plants. Leaflets of succinic acid-treated plants were the highest in percentage D-sucrose. Sucrose in leaflets of Alar-treated plants was similar to those of untreated plants.

Comparing percentage hexoses, D-sucrose, and total sugars in Table VIII, untreated plants decreased about 35 per cent in hexoses, 7 per cent in D-sucrose and 22 per cent in total sugars from the blooming stage to the fruiting stage. Leaflets of Alar-treated plants decreased 2 per cent in hexoses, whereas D-sucrose increased 5 per cent and the total sugars increased 3 per cent from the blooming stage to the fruiting stage. With leaflets of succinic acid-treated plants hexoses decreased 20 per cent, sucrose 2 per cent, and total sugars 1 per cent.

**Stems (Containing Petioles)**

Chemical treatment significantly affected the hexose sugars and D-sucrose in bean stems (Table IX).
TABLE IX

EFFECTS OF ALAR AND SUCCINIC ACID ON PER CENT ALPHA D-GLUCOSE, BETA D-GLUCOSE, BETA D-FRUCTOSE, AND D-SUCROSE IN BEAN STEMS

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>αD-Glucose</th>
<th>βD-Glucose</th>
<th>βD-Fructose</th>
<th>D-Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>0.220</td>
<td>1.009</td>
<td>0.480</td>
<td>1.138</td>
<td>2.840</td>
</tr>
<tr>
<td>Alar</td>
<td>0.147</td>
<td>0.822</td>
<td>0.427</td>
<td>1.613</td>
<td>3.009</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>0.164</td>
<td>0.943</td>
<td>0.364</td>
<td>2.283</td>
<td>3.754</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td>0.026</td>
<td>0.064</td>
<td>0.055</td>
<td>0.151</td>
<td>0.211</td>
</tr>
</tbody>
</table>
Stems of Alar-treated plants contained less alpha and beta D-glucose than untreated plant stems whereas they contained higher levels of sucrose. Beta D-fructose and the total sugars content were similar to that of untreated plants.

Stems of succinic acid-treated plants contained lower percentages of alpha D-glucose and beta D-fructose with higher percentages of D-sucrose and total sugars as compared with untreated plants. This was the case in comparing succinic acid treatment results with those of the Alar treatment except that stems from both treatments contained similar percentage alpha D-glucose.

Comparing D-sucrose to total sugars (Table IX), D-sucrose content increased with chemical treatment compared with untreated plant stems. The content in stems of succinic acid-treated plants increased about 60 per cent over those of the untreated plants. Furthermore, the relative amounts of D-sucrose in stems of Alar-treated plants increased 29 per cent compared with those of untreated plants. Stems of succinic acid-treated plants showed a relative amount 2.28 times that of the untreated stems and 1.42 times the amount in Alar-treated stems.

These data seem to indicate, as does the leaflet data, that both Alar and succinic acid encourage the accumulation of D-sucrose with succinic acid showing the greater activity.

In comparing stems at the blooming stage with those of the fruiting stage (Table X), those collected at the blooming stage were found to have a higher percentage of alpha D-glucose, beta D-fructose, D-sucrose and total sugars. When all hexoses were added together, the stems collected
TABLE X

PER CENT ALPHA D-GLUCOSE, BETA D-GLUCOSE, BETA D-FRUCTOSE, AND D-SUCROSE IN BEAN STEMS AT TWO GROWTH STAGES INCLUDING ALL TREATMENTS

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Per Cent</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>aD-Glucose</td>
<td>B-GLucose</td>
<td>B-Fructose</td>
<td>D-Sucrose</td>
<td>Total</td>
</tr>
<tr>
<td>Blooming</td>
<td>0.214</td>
<td>1.024</td>
<td>0.451</td>
<td>1.825</td>
<td>3.513</td>
</tr>
<tr>
<td>Fruiting</td>
<td>0.140</td>
<td>0.826</td>
<td>0.396</td>
<td>1.532</td>
<td>2.889</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td>0.019</td>
<td>N.S.</td>
<td>0.043</td>
<td>0.124</td>
<td>0.171</td>
</tr>
</tbody>
</table>
at the blooming stage contained 0.32 per cent more than those stems collected at the fruiting stage. Apparently the percentages of sugars in stems decreased at fruiting time which was the case with the leaflets.

The percentages of sugars in stems varied with chemical treatment from one growth stage to another (Table XI).

Stems from untreated plants contained a higher percentage of hexose sugars at blooming time than those treated with Alar or succinic acid, whereas those stems of plants treated with succinic acid contained a higher percentage of hexoses than those treated with Alar. Stems of succinic acid-treated plants were higher in percentage D-sucrose than those from Alar treatment whereas Alar treatment resulted in greater percentage D-sucrose of stems than untreated plant stems. Stems from succinic acid-treated plants had a higher percentage total sugars than either those from untreated plants or Alar-treated plants which were similar in total sugars.

At the fruiting stage the situation was not the same. Stems of untreated plants were similar in hexoses to those of Alar-treated plants. Stems of succinic acid treatment resulted in the lowest percentage hexoses but had more D-sucrose than those of the Alar treatment. The Alar-treated plants produced more D-sucrose than the untreated plants. With total sugars, Alar and succinic acid-treated plant stems had similar percentages with both higher than the percentage in stems of untreated plants.

Pods

Chemical treatment had no significant effect on D-sucrose but did effect the percentages of hexoses in bean pods (Table XII).
<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Chemical Treatment</th>
<th>αD-Glucose</th>
<th>βD-Glucose</th>
<th>βD-Fructose</th>
<th>D-Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blooming</td>
<td>Check</td>
<td>0.271</td>
<td>1.141</td>
<td>0.516</td>
<td>1.110</td>
<td>3.038</td>
</tr>
<tr>
<td></td>
<td>Alar</td>
<td>0.141</td>
<td>0.752</td>
<td>0.414</td>
<td>1.702</td>
<td>3.010</td>
</tr>
<tr>
<td></td>
<td>Succinic Acid</td>
<td>0.230</td>
<td>1.178</td>
<td>0.422</td>
<td>2.661</td>
<td>4.491</td>
</tr>
<tr>
<td>Fruiting</td>
<td>Check</td>
<td>0.168</td>
<td>0.877</td>
<td>0.444</td>
<td>1.166</td>
<td>2.641</td>
</tr>
<tr>
<td></td>
<td>Alar</td>
<td>0.153</td>
<td>0.892</td>
<td>0.440</td>
<td>1.524</td>
<td>3.009</td>
</tr>
<tr>
<td></td>
<td>Succinic Acid</td>
<td>0.098</td>
<td>0.707</td>
<td>0.306</td>
<td>1.906</td>
<td>3.017</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td></td>
<td>0.039</td>
<td>0.092</td>
<td>0.078</td>
<td>0.215</td>
<td>0.298</td>
</tr>
</tbody>
</table>
## TABLE XII

-effects of Alar and Succinic Acid on per cent alpha D-glucose, beta D-glucose, beta D-fructose, and D-sucrose in bean pods*

<table>
<thead>
<tr>
<th>Chemical Treatment</th>
<th>αD-Glucose</th>
<th>βD-Glucose</th>
<th>βD-Fructose</th>
<th>D-Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Check</td>
<td>0.253</td>
<td>0.636</td>
<td>1.144</td>
<td>0.327</td>
<td>2.360</td>
</tr>
<tr>
<td>Alar</td>
<td>0.244</td>
<td>0.700</td>
<td>1.162</td>
<td>0.355</td>
<td>2.461</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>0.293</td>
<td>0.917</td>
<td>1.390</td>
<td>0.392</td>
<td>2.993</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td>0.034</td>
<td>0.071</td>
<td>0.076</td>
<td>N.S.</td>
<td>0.274</td>
</tr>
</tbody>
</table>

*Pods of sieve sizes 3 to 5.*
Pods of Alar-treated plants were similar in hexoses measured and D-sucrose content to those of untreated plants whereas pods of succinic acid-treated plants were higher in hexoses.

Plant Parts

Of the three plant parts, the leaflets contained the greatest percentage of sugars (Table XIII). This would be expected since the leaf is the major site of sugar production. Stems were the second highest and the pods the lowest. It appears that the sugars are translocated from the leaf to the stem and then to the pod as they are formed.

Comparing sucrose percentage to the total sugars, D-sucrose content in the leaflets and stems were similar whereas it comprised about 13 percent of the total sugars in the pods. Thus, the reducing sugars were the predominant sugars in the pod.

Beta D-fructose resulted as the predominant sugar in the pod accounting for 47.29 per cent of the total sugars. This agrees with the results of Barker and Solomos as reported by Bonner and Varner (6) since they found beta D-fructose to be the major sugar entering the cytoplasm of the fruit.

It seems apparent that the chemicals had an effect on sugars within the bean plant. The proportion of hexoses to D-sucrose present in leaflets and stems was less with Alar treatment and still less when succinic acid was applied. There appeared to be less change in the proportion of hexoses to D-sucrose in the pods with chemical treatments.

It is the hypothesis of the author that the succinic acid molecule might have been incorporated into the Krebs cycle. With the
TABLE XIII

PER CENT ALPHA D-GLUCOSE, BETA D-GLUCOSE, BETA D-FRUCTOSE, AND D-SUCROSE IN BEAN LEAFLETS, STEMS, AND PODS INCLUDING ALL TREATMENTS

<table>
<thead>
<tr>
<th>Plant Parts</th>
<th>Per Cent aD-Glucose</th>
<th>Per Cent βD-Glucose</th>
<th>Per Cent βD-Fructose</th>
<th>Per Cent D-Sucrose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaflets</td>
<td>0.398</td>
<td>1.782</td>
<td>1.159</td>
<td>4.239</td>
<td>7.579</td>
</tr>
<tr>
<td>Stem</td>
<td>0.140</td>
<td>0.826</td>
<td>0.396</td>
<td>1.532</td>
<td>2.889</td>
</tr>
<tr>
<td>Pods</td>
<td>0.263</td>
<td>0.751</td>
<td>1.232</td>
<td>0.358</td>
<td>2.605</td>
</tr>
<tr>
<td>LSD ≤ .05</td>
<td>0.034</td>
<td>0.071</td>
<td>0.076</td>
<td>0.191</td>
<td>0.274</td>
</tr>
</tbody>
</table>
compound present the plant might not have synthesized the acid to the same extent as the untreated plant. If this was the case, the part of the Krebs cycle to the point of succinate formation may have been slowed allowing hexoses to accumulate. However, with these sugars not being utilized as rapidly as in untreated plants, a mechanism may have been turned on so that they were synthesized into sucrose. This conversion was apparently more active in the stem than in the leaflet since the proportion of hexoses to sucrose was less.

The results from the use of Alar and succinic acid were similar but the results of the Alar treatment were at a lower level. This as suggested by Martin and Williams (33), tends to indicate the molecule of Alar hydrolyzes slowly. The amount of succinic acid present from Alar hydrolysis would not be as great as that supplied by the succinic acid treatment.

Within pods it appeared the reducing sugars were present in the largest amount. Chemical treatments increased these sugars, with succinic acid resulting in the greater increase. Even though D-sucrose was low in concentration in the pods, a general trend was evident in increasing its content with the chemical treatments.

With the percentages of sugars decreasing by the second harvest (fruiting stage), it might be beneficial to apply another chemical treatment prior to or during blooming. This is another possible area for further research.
V. PERCENTAGE STARCH IN BEAN LEAFLETS AND STEMS

AT TWO GROWTH STAGES

Chemical Treatment

As noted from Table XIV, the percentage starch in leaflets was significantly altered with chemical treatment, whereas in stems this change was not significant.

The percentage starch in leaflets of Alar-treated plants was significantly higher than those of either succinic acid-treated or untreated plants.

Although no significant difference occurred in the percentage starch in stems, there was a general trend for the percentage of starch to increase with chemical treatments. The succinic acid treatment resulted in a higher percentage starch content in stems than those of Alar treated plants.

Growth Stages

There was no significant difference in the percentage starch in leaflets at the blooming stage and fruiting stage (Table XIV). Starch had increased in the stems 26 per cent at the fruiting stage. This seems to indicate translocation of the sugars to the stems where starch conversion and storage apparently took place.

Chemical Treatment–Growth Stage

A significant interaction occurred between chemical treatments and growth stages, indicating the plant did not respond the same to the chemicals at the different growth stages (Table XIV).
TABLE XIV

EFFECTS OF ALAR AND SUCCINIC ACID ON PER CENT STARCH IN BEAN
LEAFLETS AND STEMS AT TWO GROWTH STAGES

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blooming Stage</th>
<th></th>
<th>Fruiting Stage</th>
<th></th>
<th>Chemical Treatments Mean</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaflets</td>
<td>Stems</td>
<td>Leaflets</td>
<td>Stems</td>
<td>Leaflets</td>
<td>Stems</td>
</tr>
<tr>
<td>Check</td>
<td>3.93</td>
<td>27.62</td>
<td>5.62</td>
<td>33.46</td>
<td>4.78</td>
<td>30.54</td>
</tr>
<tr>
<td>Alar</td>
<td>5.73</td>
<td>33.77</td>
<td>6.41</td>
<td>33.76</td>
<td>6.07</td>
<td>33.77</td>
</tr>
<tr>
<td>Succinic Acid</td>
<td>5.62</td>
<td>25.48</td>
<td>4.72</td>
<td>42.97</td>
<td>5.17</td>
<td>34.23</td>
</tr>
<tr>
<td>Growth Stage Mean</td>
<td>5.10</td>
<td>28.96</td>
<td>5.58</td>
<td>36.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Growth Stage LSD* N.S. 5.36

Chemical Treatment LSD  .69 N.S.

Chemical x Growth Stage LSD  .69 6.56

*LSD ≤ .05.
With the leaflets, the succinic acid treatment appeared to be losing its effect on influencing starch accumulation by the time of the fruiting stage. In the stem the percentage of starch present at fruiting time was 42 per cent as compared with 25 per cent at blooming time.
CHAPTER V

SUMMARY

A study of the effects of Alar and succinic acid on fresh weight, dry weight, percentage dry matter, chlorophyll content, alpha D-glucose, beta D-glucose, beta D-fructose, D-sucrose and starch of *Phaseolus vulgaris* plants collected at two growth stages was carried out from June 1968 to July 1970 on Morgan Farm, of the University of Tennessee, Knoxville.

Plants treated with chemicals resulted in no significant change in fresh and dry weight, or in percentage dry matter of the leaflets. Considering growth stage, dry weight and percentage dry matter of the leaflets increased from the blooming stage to the fruiting stage.

With stems, chemical treatments significantly altered fresh and dry weights but did not change percentage dry matter. The Alar-treated plants resulted in the lowest values with the succinic acid-treated plants the highest values of these constituents.

No significant difference occurred between growth stages in terms of fresh and dry weights but percentage dry matter increased from the blooming stage to the fruiting stage.

No significant chemical-growth interaction occurred for leaflets or stems indicating these plant organs responded to chemical treatment much the same during both growth phases with respect to fresh weight, dry weight and percentage dry matter.

Pod number, fresh and dry weight, and percentage dry matter of pods were not significantly influenced by chemical treatments. However,
a general trend occurred for pod number to increase with chemical treatments resulting in a 22 per cent increase with succinic acid treatment.

Chlorophyll content of the leaflets was not significantly altered with chemical treatments. However, chlorophyll content decreased from the blooming stage to the fruiting stage.

The chemical-growth stage interaction also was not significant indicating bean leaflets responded to chemical treatment similarly during both growth stages in terms of chlorophyll.

In leaflets the reducing sugars, alpha, beta, D-glucose and beta D-fructose generally decreased, based on percentage of total sugar, with chemical treatment while D-sucrose and starch increased.

In stems the reducing sugars generally decreased with chemical treatment whereas D-sucrose increased. There was a general trend for starch percentage to increase with chemical treatments.

In pods the reducing sugars generally increased with chemical treatment with no significant difference in D-sucrose.

The reducing sugars were highest in percentages in the leaflets, the site of production, and the lowest in the stems. Whereas D-sucrose was highest in the leaflets and lowest in the pods.

Some significant interactions occurred indicating that plants did not respond the same to chemical treatment in all cases.

In summary, these data indicate Alar and succinic acid both changed sugar content of bean plant with succinic acid producing the greater effect of the two chemicals. This seems to indicate the Alar molecule hydrolyzed into succinic acid and the hydrazine moiety.
These data suggest that succinic acid might be utilized as a growth regulator to increase sugar content of plants, and might warrant further study to determine its effect on fruit set and fruit quality.
LITERATURE CITED


VITA

The author, James Glendon Staley, was born in DeKalb County, Tennessee, on June 18, 1940. He attended Smithville Elementary School of that county and was graduated from Smithville High School in 1958. He entered The University of Tennessee in September, 1958, and received the Bachelor of Science degree in 1963 with a double major in Agriculture Education and Horticulture.

In 1963, he entered graduate school at The University of Tennessee and received the Master of Science degree with a major in Ornamental Horticulture in 1966. In 1970, he was granted the Doctor of Philosophy degree from The University of Tennessee with a major in Agricultural Plant and Soil Science. While working toward both advanced degrees, he was employed by The University of Tennessee Agriculture Experiment Station as a Research Assistant in Horticulture.

He is married to the former Bobbie Nell Allen of Alexandria, Tennessee and they have two children.