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To the Graduate Council:

I am submitting a thesis written by Umapati Virabhadrayya Sulladmath entitled "Studies on the Influence of Temperature and Soil Moisture on Peroxidase and Hexose Sugars in Snap Beans." I recommend that it be accepted for nine quarter hours credit in partial fulfillment of the requirements for the degree of Master of Science, with a major in Horticulture.

Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:

Dean of the Graduate School

# STUDIES ON THE INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON PEROXIDASE AND HEXOSE SUGARS IN SNAP BEANS

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A Thesis

Presented to

the Graduate Council of

The University of Tennessee

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Umapati Virabhadrayya Sulladmath

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# CHAPTER I

## INTRODUCTION

With the expansion of the growing of vegetables for fresh market, and of the freezing and canning industry since 1920, the production of green beans has been increasing year after year. In 1960, the United States acreage of green beans for processing was 176,160 acres (51) and for fresh market, 139,830 acres (50). In the 1930's as facilities for food freezing and retail distribution of frozen foods developed across the United States, frozen foods began to find their place in commerce. The frozen vegetable pack for 1960 was 1,955 million pounds, being the largest in the history of the industry. Green beans accounted for 86 million pounds valued at 14.2 million dollars (46).

The importance of blanching vegetables before freezing for the inactivation of enzymes causing deterioration in flavor and color has been recognized since the report by Joslyn and Cruess (25). Blanching is now a routine treatment in the processing of frozen pack vegetables.

Inactivation of peroxidase in vegetables is well established as an index of adequacy of blanch. Peroxidase has been found to be more heat resistant than other enzymes such as catalase, ascorbic acid oxidase and polyphenol oxidase. Underblanched beans and other vegetables with some degree of residual peroxidase activity undergo deterioration in quality through loss of ascorbic acid (38), color (13) and the development of off-odors. Overblanching results in the loss of ascorbic acid, poor color and sloughing.

In general, a temperature of 190° F. for a period of 2 to 2½

minutes is used (7). This does not appear to be always adequate or optimum because in recent years defects in texture and flavor have developed in some lots of green beans during prolonged frozen storage (36). It became evident that beans vary in peroxidase content. Maturity, toughness and growing conditions have been considered as some of the factors affecting peroxidase content of green beans. Mundt and McCarty (36) found significant differences among varieties as to peroxidase activity. Climatic factors such as temperature, rainfall and humidity seemed to influence the peroxidase content and blanching time.

The object of the present investigation is to study the influence of temperature and soil moisture on peroxidase and associated quality factors in green beans.

# CHAPTER II

# REVIEW OF LITERATURE

## II. IMPORTANCE OF BLANCHING

That underblanching or overblanching of vegetables is responsible for many undesirable changes has been proven by several workers. Blanching should stop enzyme action. Fisher and Van Duyne (16) found that when snap beans of the Logan variety were blanched for three minutes in hot water they retained more ascorbic acid and scored higher in palatability than beans blanched for 2 minutes. In the study by Paul et al. (38), a high temperature blanch of 200° F. for 2 minutes for green beans and spinach gave better results than the low temperature blanch of 170° F. for 2 minutes. The former resulted in greater peroxidase inactivation in green beans and spinach, and higher ascorbic acid retention in green and lima beans. Incomplete peroxidase inactivation was associated with reduced ascorbic acid content. It was considered that short time low temperature exposures were not sufficient for enzyme inactivation and generally led to increased loss of ascorbic acid by oxidation. Prolonged exposures increased nutrient loss by leaching. In general short-time, high-temperature blanching gave better results. Hartzler and Guerrant (19) working on green beans of Bountiful variety noted that the shortest blanch (200°F. for 1 minute) resulted in greatest loss of ascorbic acid. Maximum losses of ascorbic acid occurred during freezing and frozen storage in the unblanched and underblanched vegetables.

Moyer et al. (35), in extensive studies on the effects of blanching

on the chemical composition of snap beans of Tendergreen variety, sought to correlate losses of ascorbic acid and the residual activity on the three enzymes peroxidase, catalase and ascorbic acid oxidase. Whereas catalase showed varying degrees of heat resistence requiring 4 minutes at 180° F. and 6 minutes at 165° F. for total inactivation, peroxidase required 4 to 5 minutes of heating at 210° F. for complete inactivation. Blanching also appeared to reduce the dry matter content of snap beans. The authors reported losses of 10 to 20 percent sugars, and 69 to 80 percent ascorbic acid. Thus overblanching not only exerted a greater detrimental effect on the ascorbic acid content, but it accentuated the loss of mineral constituents as well.

Unblanched or underblanched vegetables held in frozen storage deteriorate in color and develop 'hay-like' flavors. Not much is known about the reactions that cause such deterioration, though it is definitely known that adequately blanched vegetables retain better color and develop significantly less odor (11, 48). Joslyn and David (26) observed that off-flavor development in frozen vegetables paralleled accumulation of alcohol and acetaldhyde. Lee et al. (29) noted that unblanched Wade beans and Thomas Laxton peas which developed off-flavor, showed positive test for peroxidase.

Inactivation of Peroxidase--Index of Adequacy of Blanch

In 1933, Diehl and Berry (10) stated that catalase activity could serve as an index of adequacy of blanching for peas and indicated that Alderman peas blanched for a time sufficient to destroy catalase activity retained satisfactory quality when stored at -5° F. Later Joslyn (24)

suggested that inactivation of catalase alone was not adequate.

Mergentime (32) and Balls (1) suggested that peroxidase activity might serve as an index of adequacy of heating. That peroxidase was hard to inactivate being more resistent to heat was shown by Hartzler and Guerrant (19). Esselen and Anderson (14) studied the heat resistance of peroxidase in seventeen different vegetables. The resistance tests were made at eight different temperatures through the range of 215° to 300° F. The peroxidase of green beans, broccoli and turnips exhibited marked heat resistance. Masure and Campbell (31) were the first to initiate the study of quantitatively relating the degree of peroxidase inactivation to quality retention in frozen vegetables. Dietrich et al. (12), in an investigation carried out to compare the relative degrees of quality retention as related to the degrees of inactivation of the two enzymes catalase and peroxidase, observed that in green beans at 208° F., catalase was inactivated in 50 seconds, whereas complete inactivation of peroxidase took 150 seconds. Samples blanched to a negative peroxidase test maintained better flavor, color and more ascorbic acid than the samples blanched for shorter or longer time. In an earlier study Dietrich et al. (11) showed that the presence of peroxidase was associated with greater loss of green color and ascorbic acid in peas. Samples containing active catalase showed greater loss of ascorbic acid and degradation of chlorophyll than samples in which both catalase and peroxidase had been inactivated. As regards color retention, Dietrich et al. (13) showed that green beans just adequately blanched to peroxidase inactivation or a little longer, retained a clearer green color when blanched at higher temperature between 200° - 212° F. and shorter time of 1 to 2 minutes than when blanched at

lower temperature between 190° - 195° F. and longer time of 3 to 5 minutes. Gross overblanching at any temperature resulted in poor color due to increased conversion of chlorophyll to pheophytin.

Overblanching is claimed to be one of the most important factors causing sloughing in frozen beans. Van Buren (48) showed that blanching at temperatures above 180° F., and prolonged blanching causes greater sloughing. Sloughing is the separation of the skin resulting from a breakdown in the layer of large thin walled parenchyma cells outside the vascular region of the pod.

The necessity of adequately blanching beans is brought out in the more recent investigations by Mundt and McCarty (36). They observed that at 205° F., a difference of 22 seconds exists in the time of inactivation between the most easily blanched and the most resistant varieties of beans. They further determined that seemingly minute residual quantities of peroxidase, in terms of 10 to 15 units as measured by the Klett-Summerson Colorimeter and hardly detectable as a color change, would result in the effects of underblanching in beans during prolonged frozen storage.

## II. FACTORS INFLUENCING PEROXIDASE ACTIVITY AND BLANCHING TIME

### Influence of Maturity

Bedford and Joslyn (2) considered factors such as maturity, toughness, and freshness as influencing the blanching time and temperature of vegetables. They found that a two minute blanch at 185° F. was adequate for the mature beans and a blanch at 212° F. for those less

mature. Mundt and McCarty (36) however, found no significant difference in blanching time required at 200° F. between beans of small (2-3 sieve) size and beans of medium (4-5 sieve) size. A slightly longer blanch of 2.28 minutes was required for beans of 2-3 grade size as against 2.12 minutes for beans of 4-5 grade size. No differences were noted for medium and large (6 sieve size and above) beans.

Influence of Variety

Mundt and McCarty (36) found significant variations in the blanching requirements for different varieties. In their determinations of blanching times based on peroxidase activity, values of 4.30 to 5.95 minutes at 190° F. and 2.52 to 3.23 minutes at 200° F. were obtained. Bountiful Bunch, Brown Bunch (Bush) and Kentucky Wonder No. 191 required excessive blanching time much in excess of 3 minutes at 210° F. This was attributed to over-maturity since the seed pods of these varieties tend to become overmature quickly.

Isaac and Winch (22) indirectly associated the heat resistance of peroxidase to the amount of lignin in the beans of different varieties. They indicated that possibly in the native state, lignin and peroxidase in combination or lignin specifically could be responsible for enhanced resistance to heat.

Influence of Climatic Factors

Mundt and McCarty (36) observed that climatic factors such as temperature, rainfall and humidity seemed to influence the time of blanching. In the years 1955 and 1956, they studied the blanching requirements of snap beans of Wade, Tenderlong and Top-crop varieties.

During 1955, the growing season was quite hot and dry with low relative humidity, and in 1956 the temperatures were moderate, rainfall was more uniformly distributed and the relative humidity was higher throughout the growing season. Blanching time required for beans obtained in 1956 was slightly less than for those obtained in 1955.

## III. FACTORS INFLUENCING GROWTH AND MATURATION OF BEANS

The problem of maturation is an important one to the food processor. The stage of maturity greatly influences the composition and palatability of any fruit or vegetable (8). Of the environmental factors, temperature markedly influences the rate of every physiological process occurring in plants, including growth (34). In general, increase in temperature results in increased rates of photosynthesis, respiration, and enzymatic reactions, up to a point beyond which at all higher temperatures these rates decline. These are also affected by the time factor (34).

Investigations have been conducted on a large number of crops on the development and application of temperature summation to facilitate planning of planting schedules and predicting harvest dates. The cardinal growth temperatures for beans are 50° F. and 80° F., with 60° F. and 70° F. being the optimum monthly minimum and maximum average temperatures (28). Stark and Mahoney (44) suggested a base line of 50° F. for beans for computing temperature summations. This base line was used by Gould (17) who reported the use of temperature summations for 47 varieties of snap beans. He found that most snap bean varieties require 27,000 heat units to reach the optimum stage and suggested planting at every 600 degree hours for smoothness of picking operations. He made it clear that the

number of growing days could not be taken as an accurate index for maturity as these varied with varieties, temperature and other climatic factors. Similar variations in temperature summations were observed by Guyer and Kramer (18). In 1949, they found that the number of degree hours necessary to bring the snap beans to optimum stage of maturity was 29,333 and in 1950 was 24,470. This meant a difference of approximately eight days, which must be explained by factors other than temperature. They observed similar differences for crops maturing in different seasons. Crops planted in spring required a smaller number of heat units than those maturing in midsummer and fall. They, therefore, concluded that other environmental factors such as rainfall could be limiting factors regardless of temperature, and hence indicated that the use of temperature summations for setting planting dates and for predicting harvest dates may not be as practicable for snap beans as for other crops. Similar differences in requirements of heat units have been noted by Magoon (30) for corn, and by Bomalaski (3) for peas. Magoon attributed the maturity of northern grown corn with a smaller temperature unit summation as compared to that of corn grown in south, to various causes such as differences in the rates of respiration at different temperature ranges and differences in the length of day permitting longer periods of photosynthetic activity in the northern regions. Bomalaski explained the variability as due primarily to the temperature factor, and to a lesser extent to soil temperature, soil fertility, soil type and topography.

# IV. FACTORS AFFECTING ORGANIC COMPOSITION AND QUALITY OF BEANS

Factors associated with the climatological environment of a particular location or season exert a great influence on the organic composition of crops.

Boswell (4) reported that no consistent relationship could be observed between mean temperatures and chemical composition, but that there was a good correlation between starch-sugar ratios in peas. Results however, were not uniform for all seasons which he attributed to strain differences, varying nutritional conditions and differences in day length. Janes (23) observed that variety, fertilizer levels and location affected the composition of beans and cabbages. The greatest variation in composition occurred in plants grown at different locations. Dry weight of the Tendergreen variety varied from a minimum of 8.2 percent at Homestead to a maximum of 10.2 percent at Hastings in Florida. Janes, however, did not attribute these variations to any one factor.

Stage of maturity affects the composition and quality of beans. Culpepper (8) showed the best stage of maturity to be when the bean pods had reached their full length, with very little development of seeds. Mature beans had a higher total solids content. Sugars increased from the time of flowering to the 15 to 20-day stage, then decreased gradually to the 40-day stage. Shuey and McCarty (41) reporting the results of an extensive study stated that the varieties for processing are at optimum maturity when the seeds do not exceed 5 to 7 percent by weight of the pod. Hibbard and Flynn (21) noted that green beans that had reached full length

gave maximum production and the best balance of vitamins. The dry matter percentage increased from 10 percent (seed less than 7 mm. diameter) to 30 percent (seed 18 mm. in diameter). The immature seed contributed only 2 percent to the total dry matter, while the most mature seed contributed 57 percent. Similar results were obtained by Guyer and Kramer (18) who correlated percent seed with dry matter. The total solids increased from 9.3 to 17.3 percent as the percent seed increased from 4 to 24 percent, in two varieties Burpees Stringless Green Pod and Pencil Pod Black Wax.

Soil moisture is another factor which is considerably influenced by climatic environment, and which considerably affects composition and quality of beans. That irrigation to maintain available soil moisture within an optimum range, significantly increases the yield of beans has been shown by several workers (5, 37, 40, 45). Byron (6) observed that beans from non-irrigated plots were wrinkled, and had a higher percent dry weight than those receiving irrigation. The beans grown on a non-irrigated plot averaged 10.8 percent dry weight and beans from the heavily irrigated plots averaged 8.6 percent dry weight. There was a significant difference in the total sugars expressed as percent of the dry weight. Beans from the light irrigation plots had 25.4 percent total sugars and 21.4 percent reducing sugars. The beans from heavy irrigation plots had 28.9 percent total sugars and 24.9 percent reducing sugars. Sistrunk et al. (42) found that the beans from the mulched plot contained more reducing sugars than the beans from the non-mulched plots irrespective of irrigation treatment. Mulching would tend to increase the amount of water available to the bean plants. Wadleigh and Ayers (52) in a study of the effects of soil moisture tension on growth and biochemical composition of

bean plants noticed that as long as the soil moisture was maintained well above the permanent wilting point, soil moisture tension had no effect on percentage of reducing sugars in stems.

# CHAPTER III

## METHODS AND MATERIALS

### Variety of Bean

Wade was used since it is one of the popular processing varieties in Tennessee. It is mosaic resistant with dark green, smooth and round pods, and is characterized by a relatively long pod setting period (27).

## I. DESIGN OF THE EXPERIMENT

### FACTORIAL

### Main Treatments

After plants showed flower buds they were handled as follows. Three ranges of temperature as close as possible to the following were maintained in separate greenhouse sections.

1. High temperature range: 68° F. to 80° F.
2. Medium temperature range: 62° F. to 70° F.
3. Low temperature range: 55° F. to 65° F.

The night temperatures approached the lower range and day temperatures the upper range in each house.

### Sub-Treatments

Two soil moisture levels were maintained. These were:

1. High soil moisture level: 150 cc water applied to each No. 5 clay pot to raise the moisture content to approximately the water holding capacity of the soil at every watering. This was usually 1 or 2 times each day.

2. Low soil moisture level: 75 cc water applied to maintain the soil moisture above the permanent wilting point. This was also 1 to 2 times a day, being done at the same time as the plants maintained at high soil moisture level.

Replications:
Four

Soil

Tellico sandstone soil was used for growing the beans in clay pots. The soil has a water holding capacity of 23.99 percent (1/3 atmosphere) and a permanent wilting percentage of 10.20 percent (15 atmospheres). The values were determined according to standard methods (9). Pressure-plate apparatus and pressure-membrane apparatus were used for determining water holding capacity and permanent wilting point respectively.

Fertilizer of a 5-10-5 analysis at a rate of 800 pounds per acre was well mixed with the soil before filling the pots. Each pot contained approximately 1100 gms of soil.

## II. CULTURE OF BEAN PLANTS

From planting to budding, all the plants were given the same treatment as to temperature and watering. They were subjected to different treatments only after they started blooming. Different growing temperatures during the earlier stage would have caused variations in the time required to reach the flowering stage. Earlier investigation by Kattan and Fleming (27) showed that variations in moisture levels had notable effects on quality only during the period of pod development and not during the early period provided soil moisture was adequate.

Planting Seeds

Seeds treated with Captan were planted on January 29, 1961, in 200 5-inch clay pots. Four seeds in each pot spaced about 2 inches apart and 1 inch deep were planted. Watering was done as and when necessary to maintain the optimum levels. Germination was complete on February 6.

Thinning and Staking

Sixteen days after planting and when the plants were 4 to 5 inches high and well established, the number of plants per pot was reduced to two healthy plants. The plants were staked with stiff wire when they were about 12 inches high.

Temperature and Soil Moisture Control

On March 1 (31 days after planting) when more than 50 percent of the plants started showing flower buds, the plants were divided into three lots of 64 plants each and plants in each lot were arranged in three sections of the greenhouse. Two rows containing 4 pots each, with pots spaced 10 inches in the row and 20 inches between rows, formed a sub-treatment which was replicated four times. Recording thermographs, protected from direct sun, were used in each of the three houses.

Approximately 150 cc water for high soil moisture treatment and 75 cc for low soil moisture treatment, were applied at each time to each pot. This raised the soil moisture content from 16 percent to about 29 percent in pots maintained at high soil moisture level, and from 11-12 percent to about 18 percent in pots with low soil moisture level.

Necessity of watering was based on visual observations on condition of plant and soil. Actual soil moisture determinations just prior to

watering showed that the timing and method of watering were quite workable for maintaining soil moisture at desired levels.

Fertilizer Application

The plants were fertilized 8 times. Each pot was given 150 cc of solution prepared by dissolving one teaspoonful each of 7-6-18 soluble fertilizer and ammonium nitrate in one gallon of water.

Plant Protection

Malathion was used 5 times for controlling white flies and aphids, and Tedion twice for control of red spider mites.

## III. HARVESTING BEANS

Flowers were tagged just as the standard petal started opening. Tagging of flowers was done on alternate days. In each of the three sections of the house, the first harvesting was made when the pods were between 2.5 to 3 sieve size ($\frac{16.5}{64}$" - $\frac{21}{64}$" in diameter). Heat units required to mature beans to these sizes at different temperature ranges were calculated, and the subsequent three harvests were made only when the same number of heat units accumulated in each of the three sections, irrespective of the sieve size attained.

## IV. ANALYTICAL METHODS

Measurement of Peroxidase Activity

Immediately after harvesting, the beans were analyzed for peroxidase activity by the staff of the Food Technology Department. The activity was

measured by using guaiacol as substrate in the presence of hydrogen peroxide. The procedure adopted is a modification of the method used by Reddi et al. (39) (Appendix D).

## Determination of Total Solids

Comminuted snap beans in excess of the quantity required for peroxidase measurement, were kept frozen (20° F.) until they were used for other determinations. The total solids were determined by drying the stored comminuted beans at 70° C. for 48 hours (53).

## Determination of Hexose Sugars

Dried beans were ground and hexose sugar determinations were made by adopting a modification of Folin Wu's method (20, 33). The method is based on the ability of glucose (hexose sugars) to reduce copper in hot alkaline solution. The cuprous oxide thus formed is treated with phosmolybdic acid solution, a blue color being obtained. The optical density of the solution is measured in a Spectronic 20 Colorimeter at 430 mu. with the standard adjusted for 100 percent transmission (Appendix C).

## Methods of Statistical Analysis

The experimental data relating to determinations of peroxidase activity, hexose sugars and total solids content were statistically analyzed (43) and F test was applied at 0.01 and 0.05 level of probability. Duncan's new multiple range test as described by Federer (15) was applied to compare more than two means. For comparing two means the Least Significant Mean Difference Test was used.

# CHAPTER IV

## RESULTS AND DISCUSSION

Influence of three ranges of temperature and two soil moisture levels on (1) growth and maturation, (2) peroxidase content, (3) hexose sugars and (4) total solids in snap beans, are discussed separately.

### I. INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON GROWTH AND MATURATION

Heat unit summations were based on hourly temperatures and computed above a base line of 50° F. as was done by Stark and Mahoney (44) and Gould (17). Bean plants in this experiment required 14,393 heat units over a period of 30 growing days from planting to blooming. After the plants started showing flower buds, they were subjected to different temperature ranges. Differences as to the number of heat units required to reach the optimum stage of maturity were noted. The plants required 23,333 and 23,356 heat units at the high and medium temperature ranges respectively. Gould (17) found that most bean varieties require 27,000 heat units. In this experiment, plants at the low temperature range, however, required only 21,662 heat units but four more growing days. This pattern held good on all the four harvest periods (Tables I, II and III).

Similar observations were made by Gould (17). He attributed such variations mainly to temperature and other climatic factors. Guyer and Kramer (18) explained such differences as being due to other environmental factors such as rainfall. In this investigation, since soil moisture was maintained at certain levels, and since other factors such as length of

TABLE I

NUMBER OF DAYS, AND HEAT UNITS ACCUMULATED BETWEEN FLOWERING AND HARVEST OF SNAP BEANS

HIGH TEMPERATURE RANGE

| Harvest Period | Date of Tagging Flower | Date of Harvest | No. of Days | Degree Days | Heat Units | Mean Daily Temperature From Flowering Till Harvesting °F. |
|---|---|---|---|---|---|---|
| 1 | 3/5/1961 | 3/17/1961 | 12 | 279.9 | 6719.0 | 73.2 |
| 2 | 3/11/1961 | 3/24/1961 | 13 | 304.2 | 7302.7 | 73.3 |
| 3 | 3/31/1961 | 4/14/1961 | 13 | 301.1 | 7227.8 | 73.1 |
| 4 | 4/4/1961 | 4/17/1961 | 13 | 298.3 | 7159.2 | 73.1 |

Heat units accumulated from planting till first harvest - 23,333.

TABLE II

NUMBER OF DAYS, AND HEAT UNITS ACCUMULATED BETWEEN FLOWERING AND HARVEST OF SNAP BEANS
MEDIUM TEMPERATURE RANGE

| Harvest Period | Date of Tagging Flower | Date of Harvest | No. of Days | Degree Days | Heat Units | Mean Daily Temperature From Flowering Till Harvesting °F. |
|---|---|---|---|---|---|---|
| 1 | 3/6/1961 | 3/20/1961 | 14 | 274.0 | 6557.6 | 69.6 |
| 2 | 3/11/1961 | 3/25/1961 | 14 | 279.1 | 6698.4 | 69.9 |
| 3 | 4/8/1961 | 4/21/1961 | 13 | 295.0 | 7080.0 | 72.7 |
| 4 | 4/11/1961 | 4/24/1961 | 13 | 288.8 | 6932.8 | 72.2 |

Heat units accumulated from planting till first harvest - 23,356.5.

TABLE III

NUMBER OF DAYS, AND HEAT UNITS ACCUMULATED BETWEEN FLOWERING AND HARVEST OF SNAP BEANS

LOW TEMPERATURE RANGE

| Harvest Period | Date of Tagging Flower | Date of Harvest | No. of Days | Degree Days | Heat Units | Mean Daily Temperature From Flowering Till Harvesting °F. |
|---|---|---|---|---|---|---|
| 1 | 3/6/1961 | 3/21/1961 | 15 | 228.0 | 5472.0 | 65.2 |
| 2 | 3/11/1961 | 3/26/1961 | 15 | 241.6 | 5799.6 | 66.1 |
| 3 | 4/19/1961 | 5/1/1961 | 12 | 250.8 | 5796.9 | 70.9 |
| 4 | 4/21/1961 | 5/3/1961 | 12 | 250.8 | 6020.8 | 70.9 |

Heat units accumulated from planting till first harvest - 21,662.

day, soil temperature and soil fertility were controlled to a degree, the differences are attributable mainly to mean daily temperatures (temperature range). It is quite possible that at the low temperature range, the plants had the optimum conditions for photosynthesis and respired less. At the higher temperature ranges it was observed that plants suffered setbacks due to transient wilting, which obviously resulted in a higher rate of respiration in these plants. Bomalaski (3) attributed such variations to differences in the rates of respiration.

Influence on Number, Size and Shape of Pods

It may be observed from the data presented in Tables IV and V, that the high soil moisture treatment produced a greater number of larger beans than the low soil moisture treatment.

The high temperature combined with low moisture treatment, gave a larger number of small, malformed beans. Beans grown at the low and medium temperature ranges yielded normal well developed straight, uniformly round, attractive pods.

## II. INFLUENCE OF TEMPERATURE ON PEROXIDASE CONTENT

Peroxidase activity, expressed as absorbency units per gram sample, for all four harvests are presented in Table VI. The statistical analysis and multiple range tests are presented in Tables XVII, XVIII, XIX, and XX in Appendix A. From the comparison of the means of peroxidase units in beans from the four harvests as influenced by temperature (Table VII), it is observed that beans from the first harvest that matured at a high temperature range showed the highest peroxidase activity, followed by those at medium

TABLE IV

EFFECT OF SOIL MOISTURE AT MEDIUM TEMPERATURE RANGE ON NUMBER, WEIGHT AND SIEVE SIZE OF SNAP BEANS

| Harvest | Soil Moisture | Number of Pods | Total Weight in Gms. | Average Weight in Gms. | Sieve Size |
|---|---|---|---|---|---|
| 1 | High | 54 | 141.0 | 2.61 | 3 |
| | Low | 44 | 110.9 | 2.52 | 3 |
| 2 | High | 49 | 117.2 | 2.39 | 4 |
| | Low | 47 | 95.2 | 2.03 | 3 & 4 |
| 3 | High | 31 | 113.5 | 4.30 | 3 & 4 * |
| | Low | 30 | 56.3 | 1.87 | 3 |
| 4 | High | 26 | 101.3 | 3.89 | 3,4,5, & 5 |
| | Low | 26 | 101.0 | 3.8 | 3,4 & 5 |

* Predominant sieve size.

TABLE V

EFFECT OF SOIL MOISTURE AT LOW TEMPERATURE RANGE ON NUMBER, WEIGHT AND SIEVE SIZE OF SNAP BEANS

| Harvest | Soil Moisture | Number of Pods | Total Weight in Gms. | Average Weight in Gms. | Sieve Size |
|---|---|---|---|---|---|
| 1 | High | 62 | 164.0 | 2.64 | 3 |
| | Low | 51 | 125.5 | 2.45 | 3 |
| 2 | High | 43 | 117.6 | 2.73 | 2.5 & 3 |
| | Low | 45 | 101.3 | 2.25 | 2.5 & 3 |
| 3 | High | 27 | 83.8 | 3.10 | 3 & 4 |
| | Low | 21 | 61.3 | 2.91 | 3 |
| 4 | High | 19 | 71.7 | 4.48 | 3 & 4 |
| | Low | 16 | 35.2 | 2.20 | 2.5, 3 & 4 |

TABLE VI

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON PEROXIDASE CONTENT OF SNAP BEANS

| Treatment | | Replications | | | | |
|---|---|---|---|---|---|---|
| Temperature Range | Soil Moisture | 1 | 2 | 3 | 4 | Mean |
| | | Absorbency units per gram sample | | | | |
| | | **Harvest I** | | | | |
| High | High | 199 | 144 | 190 | 212 | 186.25 |
| | Low | 259 | 257 | 238 | 252 | 246.50 |
| Medium | High | 153 | 149 | 185 | 156 | 160.75 |
| | Low | 194 | 163 | 133 | 141 | 157.75 |
| Low | High | 107 | 118 | 121 | 121 | 116.75 |
| | Low | 140 | 123 | 116 | 123 | 125.50 |
| | | **Harvest II** | | | | |
| High | High | 142 | 145 | 146 | 117 | 137.50 |
| | Low | 160 | 158 | 160 | 116 | 161.00 |
| Medium | High | 102 | 109 | 128 | 110 | 112.25 |
| | Low | 143 | 112 | 123 | 137 | 128.75 |
| Low | High | 134 | 159 | 144 | 161 | 149.50 |
| | Low | 151 | 182 | 166 | 158 | 164.25 |
| | | **Harvest III** | | | | |
| High | High | 198 | 213 | 189 | 201 | 200.25 |
| | Low | 207 | 207 | 223 | 200 | 209.25 |
| Medium | High | 178 | 185 | 182 | 187 | 183.00 |
| | Low | 174 | 197 | 193 | 206 | 192.50 |
| Low | High | 245 | 217 | 256 | 207 | 231.25 |
| | Low | 220 | 270 | 282 | 277 | 262.25 |
| | | **Harvest IV** | | | | |
| High | High | 186 | 205 | 185 | 181 | 189.25 |
| | Low | 215 | 232 | 200 | 220 | 216.75 |
| Medium | High | 182 | 217 | 203 | 209 | 202.75 |
| | Low | 201 | 201 | 246 | 236 | 221.00 |
| Low | High | 195 | 198 | 230 | 216 | 209.75 |
| | Low | 251 | 209 | 272 | 276 | 252.00 |

TABLE VII

INFLUENCE OF TEMPERATURE ON PEROXIDASE CONTENT OF SNAP BEANS

| Harvest | Treatments | | |
|---|---|---|---|
| | Absorbency units per gram sample | | |
| 1 | 216.37 (High) | 159.25 (Medium) | 121.12 (Low) |
| 2 | 157.87 (Low) | 149.25 (High) | 120.50 (Medium) |
| 3 | 246.75 (Low) | 204.75 (Medium) | 187.75 (High) |
| 4 | 230.87 (Low) | 211.87 (Medium) | 203.00 (High) |

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

Legend:

High: High temperature range
Medium: Medium temperature range
Low: Low temperature range

and low temperature ranges. The differences were highly significant. The peroxidase contents seems to be related to the average mean temperatures during the period from blooming till harvesting, the highest peroxidase activity being obtained with the highest mean temperatures (see Tables I, II and III).

Second harvest beans from the low temperature range showed slightly more peroxidase activity than at other temperature ranges, though there were highly significant differences in peroxidase activity in beans that matured at high and medium temperature ranges. This higher peroxidase activity at low temperature range was more marked in the two subsequent harvests. The increase was highly significant over both medium and high temperature ranges in the third harvest, and over only high temperature range in the fourth harvest. No significant difference was observed between peroxidase content of beans that matured at medium and high temperatures, in the last two harvests. This may be explained as being due to the accumulation of approximately equal number of heat units. Mean temperatures that prevailed during the maturation period seem to influence peroxidase activity. It was 73.1° F. for both third and fourth harvest periods at the high temperature range, and 72.7° F. and 72.2° F. respectively for both these harvest periods at the medium temperature range. (See Figures 1 and 2 and Tables I and II.)

Increased peroxidase activity at the low temperature range in the beans from the second and subsequent harvests may be attributed to three factors. Firstly, this may be due to the increase in the mean temperatures by 1° F. to 5.7° F. during the subsequent maturation periods (Table III and Figures 2 and 3). Secondly, the increased peroxidase activity may be

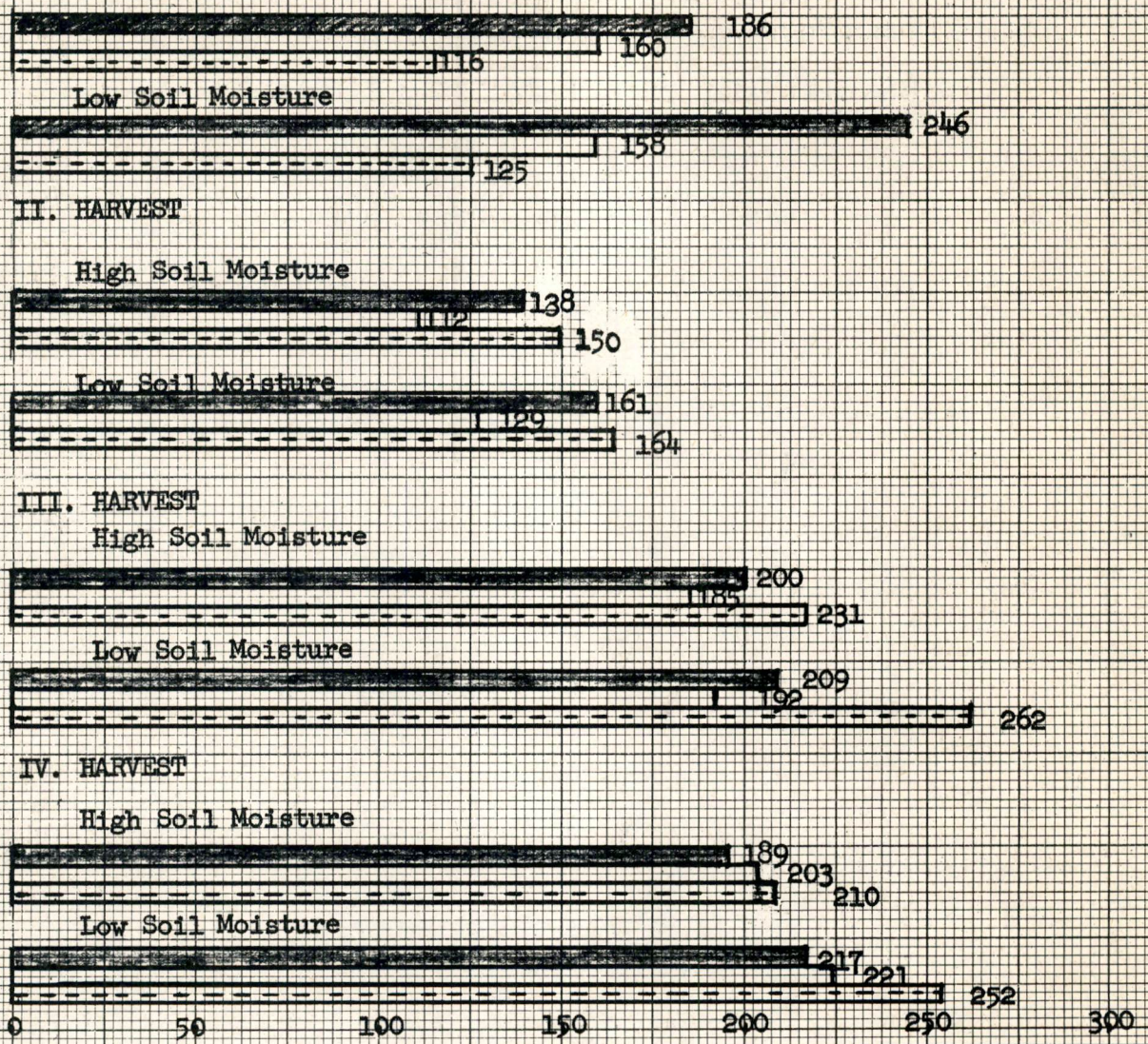



Figure 1. Influence of Temperature and Soil Moisture on Peroxidase Activity in Beans.

High Temperature Range, 68° - 80° F.

Medium Temperature Range, 62° - 70° F.

Low Temperature Range, 55° - 65° F.

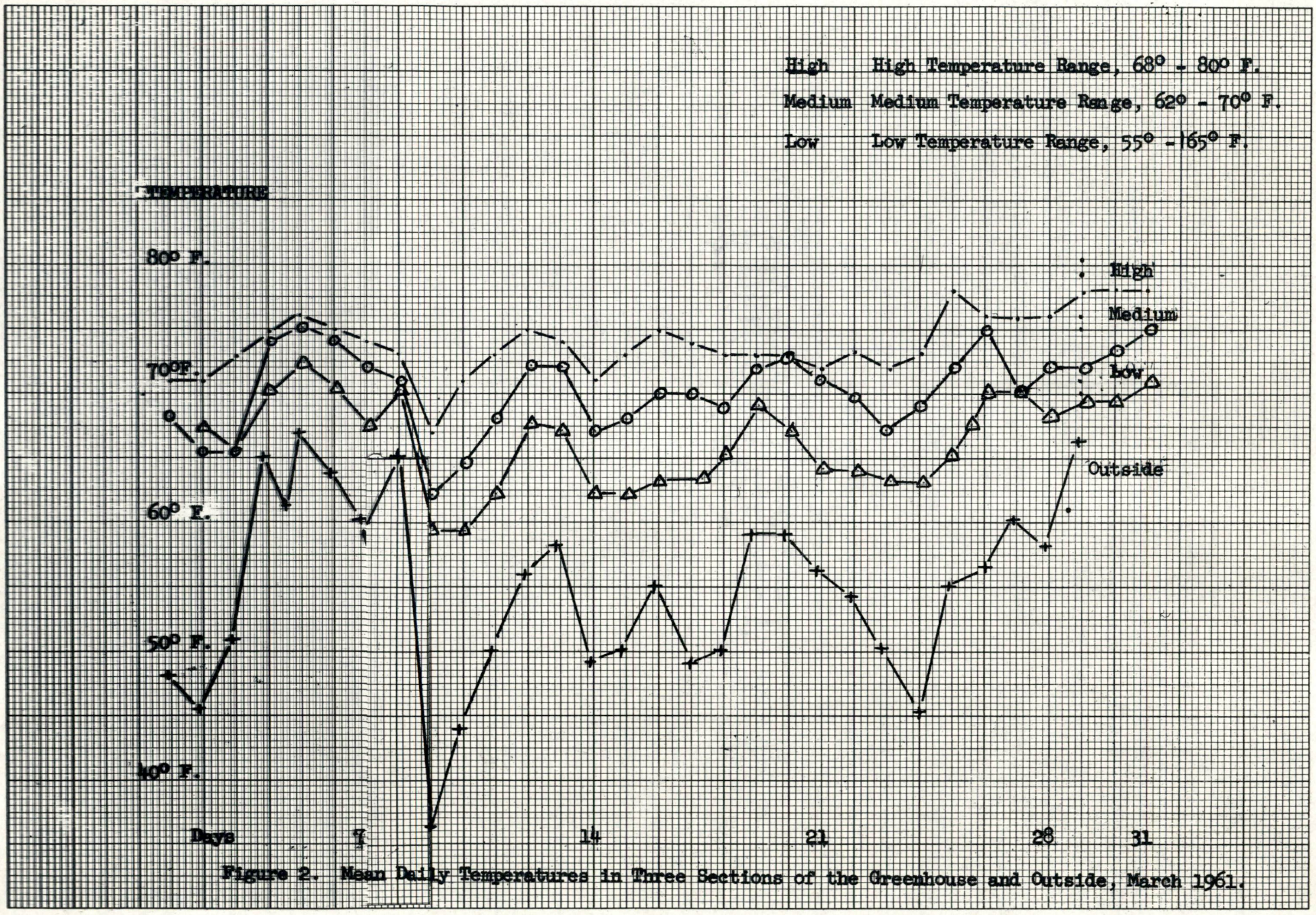



Figure 2. Mean Daily Temperatures in Three Sections of the Greenhouse and Outside, March 1961.

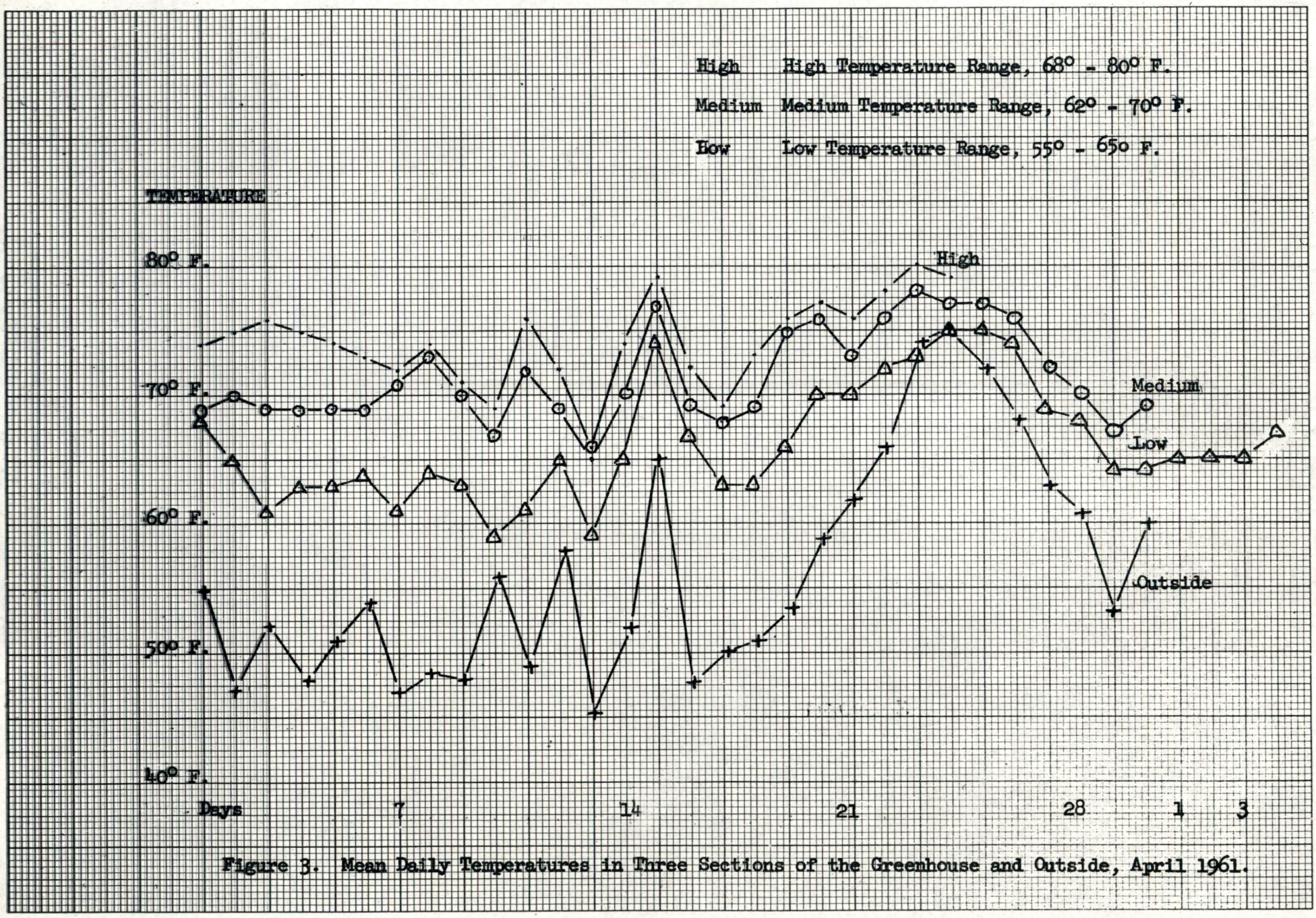



Figure 3. Mean Daily Temperatures in Three Sections of the Greenhouse and Outside, April 1961.

due to the slightly immature condition of the beans, since harvesting was based primarily on the accumulation of heat units. With the increase in mean temperature, a larger number of heat units may be required to bring beans to the same maturity. Actual sieve sizes observed showed that the beans were not so mature as (Table V) those that matured at other temperature ranges. Thirdly, the heterogeneous nature of the sample consisting of beans of smaller sieve sizes in the subsequent harvests might also be responsible for the higher peroxidase activity. In subsequent harvests the beans were not numerous enough to permit proper sampling based on the required sieve sizes.

These observations seem to be in agreement with those of Bedford and Joslyn (2) who noted that less mature beans required a higher blanching temperature, and also of Mundt and McCarty (36) who observed that beans of small size (2-3 sieve size) required a slightly longer blanch than beans of larger size (4-5 sieve size).

## III. INFLUENCE OF SOIL MOISTURE ON PEROXIDASE CONTENT

Results clearly show that peroxidase activity in beans that matured at low soil moisture levels, is significantly higher, for all the four harvests (See Table VIII and Tables XVII, XVIII, XIX and XX in Appendix A).

## IV. INFLUENCE OF TEMPERATURE AND SOIL MOISTURE PEROXIDASE CONTENT

Significant interactions were noticeable only on beans obtained from the first harvest. High temperature combined with low moisture resulted in significantly higher peroxidase content (Table IX). In

TABLE VIII

INFLUENCE OF SOIL MOISTURE ON PEROXIDASE CONTENT OF SNAP BEANS

| Harvest | Treatments | | L. S. D. at probability level | |
|---|---|---|---|---|
| | Low Soil Moisture | High Soil Moisture | 0.05 | 0.01 |
| | Absorbancy units per gram sample | | | |
| 1 | 176.58 | 154.58 | 16.25 | 22.52 |
| 2 | 151.33 | 133.08 | 13.18 | 18.26 |
| 3 | 221.3 | 204.83 | 15.30 | 21.26 |
| 4 | 229.91 | 200.58 | 17.30 | 24.04 |

TABLE IX

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON PEROXIDASE CONTENT OF SNAP BEANS (Inter-action)

| Harvest | | | | | | |
|---|---|---|---|---|---|---|
| 1 | (H X 1) 246.50 | (H X h) 186.25 | (M X h) 169.75 | (M X 1) 157.75 | (L X 1) 125.50 | (L X h) 116.75 |
| 2 | (L X 1) 164.25 | (H X 1) 161.00 | (L X h) 149.50 | (H X h) 137.50 | (M X 1) 128.75 | (M X h) 112.25 |
| 3 | (I X 1) 262.25 | (L X h) 231.25 | (H X 1) 209.25 | (H X h) 200.25 | (M X 1) 192.50 | (M X h) 183.00 |
| 4 | (L X 1) 252.00 | (M X 1) 221.00 | (H X 1) 216.75 | (L X h) 209.75 | (M X h) 202.75 | (H X h) 189.25 |

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

LEGEND:
H, M and L denote high, medium and low temperature ranges respectively.
h and 1 denote high and low soil moisture levels respectively.

subsequent harvests, there were no significant differences attributable to interaction. This is possibly due to the narrowing down of differences between the influences of high and medium temperature ranges as also to increased peroxidase activity at low temperature range, probable reasons for which have been detailed earlier.

In general, the results indicate that temperature and soil moisture influence peroxidase activity. Mundt and McCarty (36) observed that peroxidase activity of beans was influenced by climatic factors. Growing season marked by high temperature and low rainfall accounted for higher peroxidase activity in beans as against the one marked by even temperatures and high rainfall. The trends revealed by this study seem to agree with their observation. Another observation of interest is the gradual increase in peroxidase activity in later harvestings (Figure 1). Exploratory work at the Food Technology Department (47) showed that peroxidase activity in beans obtained from several locations in the valley and plateau areas of the state showed an increase in later harvestings. Higher temperatures as the season advanced appear to be responsible for the increased peroxidase activity.

## V. INFLUENCE OF TEMPERATURE ON HEXOSE SUGARS

The mean percentages of hexose sugar content of beans on dry weight basis as influenced by temperature are given in Table X, and the statistical analyses appear in Tables XXI, XXII and XXIII in Appendix A. Results indicate that growing temperatures affected the sugar content, though not consistently in all harvests (Table XI). In the first harvest, beans that matured at the high temperature range contained 17.94 percent hexose sugars.

TABLE X

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON HEXOSE SUGARS IN SNAP BEANS

| Treatment | | Replications | | | | |
|---|---|---|---|---|---|---|
| Temperature Range | Soil Moisture | 1 | 2 | 3 | 4 | Mean |
| | | Percent dry weight basis | | | | |
| | | Harvest I | | | | |
| High | High | 19.55 | 19.05 | 17.80 | 19.42 | 18.95 |
| | Low | 15.80 | 17.05 | 17.30 | 17.05 | 16.92 |
| Medium | High | 12.80 | 12.70 | 14.80 | 15.17 | 13.99 |
| | Low | 13.40 | 12.25 | 16.65 | 15.70 | 14.51 |
| Low | High | 18.05 | 14.00 | 17.25 | 16.59 | 16.47 |
| | Low | 11.15 | 14.20 | 14.10 | 15.15 | 13.65 |
| | | Harvest II | | | | |
| High | High | 15.55 | 20.62 | 17.20 | 17.30 | 17.92 |
| | Low | 10.70 | 9.82 | 11.85 | 11.27 | 10.91 |
| Medium | High | 15.88 | 16.40 | 12.30 | 16.00 | 15.14 |
| | Low | 17.30 | 17.07 | 16.30 | 16.20 | 16.72 |
| Low | High | 14.27 | 15.20 | 14.35 | 14.60 | 14.60 |
| | Low | 14.11 | 14.81 | 15.70 | 13.17 | 14.45 |
| | | Harvest III | | | | |
| High | High | 12.65 | 16.32 | 14.80 | 15.53 | 14.82 |
| | Low | 11.03 | 11.57 | 12.75 | 15.75 | 12.77 |
| Medium | High | 16.00 | 15.90 | 14.70 | 14.20 | 15.20 |
| | Low | 14.40 | 14.70 | 13.70 | 13.05 | 13.96 |
| Low | High | 9.97 | 10.10 | 10.70 | 12.85 | 10.90 |
| | Low | 12.00 | 11.00 | 11.65 | 10.35 | 11.25 |

TABLE XI

INFLUENCE OF TEMPERATURE ON HEXOSE SUGARS IN SNAP BEANS

| Harvest | Treatments | | |
|---|---|---|---|
| | Percent dry weight basis | | |
| 1 | 17.94 (High) | 15.06 (Low) | 14.25 (Medium) |
| 2 | 15.93 (Medium) | 14.53 (Low) | 14.41 (High) |
| 3 | 14.58 (Medium) | 13.80 (High) | 11.08 (Low) |

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

LEGEND:

| | |
|---|---|
| High: | High temperature range |
| Medium: | Medium temperature range |
| Low: | Low temperature range |

This is highly significant over other treatments. In the second harvest there were no significant differences, though beans at the medium temperature range had a higher percentage of hexose sugars than those at the low temperature range. In the third harvest, the sugar contents of beans at the medium and high temperature ranges though not significantly different from each other, were significantly different from the sugar content of beans at the low temperature range. Not much study has been done on this aspect to date. Boswell (4) reported consistent correlation between mean temperatures and starch-sugar ratios in peas and Culpepper (8) observed that sugars increased till the 15 to 20-day stage.

It is interesting to note that the hexose sugars decreased gradually as harvesting advanced.

## VI. INFLUENCE OF SOIL MOISTURE ON HEXOSE SUGARS

Results obtained show that soil moisture significantly affects the sugar content expressed as percent dry weight in beans (Table XII). In the first two harvestings, beans that developed at the high soil moisture content had significantly higher percentage of sugars. It may be noted here, that reduced peroxidase activity was associated with high soil moisture content. In the third harvest a similar trend was evident, though there were no significant differences apparent. The results are in agreement with those of Byron (6).

## VII. INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON HEXOSE SUGARS

The interactions are not significant except in the second harvest

TABLE XII

INFLUENCE OF SOIL MOISTURE ON HEXOSE SUGARS IN SNAP BEANS

| Harvest | Treatments | | L. S. D. at probability level | |
|---|---|---|---|---|
| | High Soil Moisture | Low Soil Moisture | 0.05 | 0.01 |
| | Percent dry weight basis | | | |
| 1 | 16.43 | 15.02 | 1.14 | 1.58 |
| 2 | 15.30 | 14.02 | 1.17 | 1.62 |
| 3 | 13.64 | 12.66 | N.S. | -- |

(Table XIII). But the results indicate that beans have a higher sugar content (dry weight basis) under the influence of higher temperatures within the optimum range, and with soil moisture maintained close to the field capacity.

## VIII. INFLUENCE OF TEMPERATURE ON TOTAL SOLIDS

Janes (23) found differences in total solids in snap beans grown in different locations and attributed them to climatic factors. The results show that temperature significantly affects the total solids in beans (Table XIV and Tables XXIV, XXV, and XXVI in Appendix A). In all the three harvests, beans that matured at high temperature range, have total solids ranging from 11.63 percent to 12.07 percent, which are highly significant over those of beans that matured at lower temperature ranges (Table XV). Significant differences, due to medium and low temperature ranges in the second and third harvests are not observed, which again may be attributed to the narrowing down of temperature differences in later stages.

## IX. INFLUENCE OF SOIL MOISTURE ON TOTAL SOLIDS

That the total solids in beans are significantly higher at low soil moisture levels, is evident only in the second harvest (Table XVI). The beans average 10.01 to 11.21 percent dry weight in the third harvest at low soil moisture level, and beans at the high soil moisture levels average 9.45 to 10.83 in the third harvest. Those results are in conformity with those of Byron (6). Results also indicate that, while total solids in beans tend to decrease under the influence of higher soil moisture, the

TABLE XIII

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON HEXOSE SUGARS IN SNAP BEANS
(Inter-action)

| Harvest | Percent dry weight basis | | | | | |
|---|---|---|---|---|---|---|
| 1 | (H X h) 18.95 | (H X 1) 16.92 | (L X h) 16.47 | (M X 1) 14.51 | (M X h) 13.99 | (L X 1) 13.65 |
| 2 | (H X h) 17.66 | (M X 1) 16.72 | (M X h) 15.14 | (L X h) 14.60 | (L X 1) 14.45 | (H X 1) 10.91 |
| 3 | (M X h) 15.20 | (H X h) 14.83 | (M X 1) 13.96 | (H X 1) 12.77 | (L X 1) 11.22 | (L X h) 10.90 |

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

LEGEND:
H, M and L denote high, medium and low temperature ranges respectively.
h and 1 denote high and low moisture levels respectively.

TABLE XIV

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON TOTAL SOLIDS IN SNAP BEANS

| Treatment | | Replications | | | | |
|---|---|---|---|---|---|---|
| Temperature Range | Soil Moisture Level | 1 | 2 | 3 | 4 | Mean |
| | | Percent dry weight basis | | | | |
| | | Harvest I | | | | |
| High | High | 12.25 | 11.34 | 11.32 | 10.34 | 11.31 |
| | Low | 12.50 | 11.96 | 11.85 | 11.54 | 11.96 |
| Medium | High | 9.58 | 9.09 | 10.06 | 10.42 | 9.78 |
| | Low | 10.08 | 9.64 | 9.92 | 10.43 | 10.02 |
| Low | High | 8.38 | 8.25 | 7.82 | 8.31 | 8.19 |
| | Low | 8.00 | 7.76 | 8.11 | 8.36 | 8.06 |
| | | Harvest II | | | | |
| High | High | 12.01 | 11.51 | 12.19 | 7.86 | 10.89 |
| | Low | 12.34 | 13.48 | 12.66 | 12.50 | 12.74 |
| Medium | High | 8.29 | 8.40 | 7.86 | 8.45 | 8.25 |
| | Low | 8.80 | 8.75 | 8.62 | 8.42 | 8.65 |
| Low | High | 9.45 | 10.26 | 7.57 | 9.64 | 9.23 |
| | Low | 10.09 | 10.60 | 10.84 | 10.86 | 10.59 |
| | | Harvest III | | | | |
| High | High | 13.47 | 12.01 | 12.65 | 11.42 | 12.38 |
| | Low | 12.56 | 11.22 | 13.04 | 10.25 | 11.76 |
| Medium | High | 10.75 | 10.61 | 10.31 | 9.09 | 10.19 |
| | Low | 9.87 | 10.34 | 11.72 | 11.11 | 10.76 |
| Low | High | 9.80 | 9.88 | 10.00 | 10.00 | 9.92 |
| | Low | 10.52 | 10.67 | 11.18 | 12.04 | 11.10 |

TABLE XV

INFLUENCE OF TEMPERATURE ON TOTAL SOLIDS IN SNAP BEANS

| Harvest | Treatments | | |
|---|---|---|---|
| | Percent dry weight | | |
| 1 | 11.6375 (High) | 9.9025 (Medium) | 8.1237 (Low) |
| 2 | 11.8187 (High) | 9.9137 (Low) | 8.4487 (Medium) |
| 3 | 12.0775 (High) | 10.5112 (Low) | 10.4750 (Medium) |

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

LEGEND:
High: High temperature range
Medium: Medium temperature range
Low: Low temperature range

TABLE XVI

INFLUENCE OF SOIL MOISTURE ON TOTAL SOLIDS IN SNAP BEANS

| Harvest | Treatment | | L. S. D. at probability level | |
|---|---|---|---|---|
| | Low Soil Moisture | High Soil Moisture | 0.05 | 0.01 |
| | Percent dry weight | | | |
| 1 | 10.0125 | 9.7633 | N.S. | |
| 2 | 10.6600 | 9.4500 | 0.0873 | 0.1209 |
| 3 | 11.2100 | 10.8300 | N.S. | |

total solids tend to increase with increase in mean temperatures in later harvestings.

# CHAPTER V

## SUMMARY AND CONCLUSIONS

A study of the influence of three ranges of temperature and two levels of soil moisture on peroxidase, hexose sugars and total solids in snap beans of Wade variety was made. Heat unit summations as indices of maturity were used. All the analytical data were statistically analyzed and interpreted.

Results indicated that both growing temperature and soil moisture level influence peroxidase activity, hexose sugars and total solids in snap beans.

The following are the chief findings in this investigation:

1. At the low temperature range, beans required a smaller number of heat units and more growing days to reach the desired stage of maturity. At the high and medium temperature ranges they matured to the same extent with a greater accumulation of heat units, but in comparatively fewer growing days.
2. Peroxidase activity in snap beans was influenced by growing temperatures. Increased peroxidase activity was associated with higher growing temperature ranges. A correlation seems to exist between the enzyme activity and mean temperatures during the entire maturation period. With an increase in mean daily temperature as the harvest season advanced, peroxidase activity increased.
3. Increased peroxidase activity was associated with low soil moisture due possibly to the immature state of beans

developed under high soil moisture tension. Maximum peroxidase activity appeared to be associated with high growing temperatures combined with low soil moisture levels.

4. Increase in hexose sugars in beans on dry weight basis was associated with higher growing temperature in each of the three harvest periods. Higher sugar content was significantly related to high soil moisture content.

At the three temperature ranges used, there was a gradual decrease in sugar content as the harvest season advanced, which may account for lower palatability scores for beans obtained in later harvests.

5. Snap beans had a higher percentage of total solids when grown at a high temperature range and at a low soil moisture level. Beans obtained from later harvests had increasingly higher total solids due possibly to the rising daily mean temperatures.

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# APPENDICES

# APPENDIX A

TABLE XVII

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON PEROXIDASE CONTENT OF SNAP BEANS
First Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 400.28 | |
| Temperature | 2 | 18385.67 | 52.41 ** |
| Moisture | 1 | 2904.00 | 8.28 * |
| Temperature x Moisture | 2 | 2263.65 | 6.45 ** |
| Error | 15 | 350.82 | |

MULTIPLE RANGE AND L. S. D.[2] TESTS

| No. of Means | | | | 2 | | 3 | | |
|---|---|---|---|---|---|---|---|---|
| L. S. Range[1] for temperature at | P | 0.05 | | 19.92 | | 20.98 | | |
| | | 0.01 | | 27.60 | | 28.92 | | |
| No. of Means | | | 2 | 3 | 4 | 5 | 6 | |
| L. S. range for temperature x moisture at | P | 0.05 | 28.20 | 29.60 | 30.45 | 31.01 | 31.48 | |
| | | 0.01 | 39.07 | 40.94 | 42.16 | 42.91 | 43.47 | |
| L. S. D. for moisture at | P | 0.05 | | 16.25 | | | | |
| | | 0.01 | | 22.52 | | | | |

Comparison of Means
Absorbency units per gram sample

| | | | | |
|---|---|---|---|---|
| Temperature | 216.37 (high) | 159.25 (medium) | 121.12 (low) | |
| Moisture | 176.58 (low) | 154.58 (high) | | |
| Temperature x moisture | 246.50 (Hxl) | 186.25 (Hxh) | 160.75 (Mxh) | 157.75(Mxl) |
| | 125.50 (Lxl) | 116.75 (Lxh) | | |

1. Least Significant Range
2. Least Significant Difference
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

Legend:
H, M and L denote high, medium and low temperature ranges respectively.
h and l denote high and low soil moisture respectively.

TABLE XVIII

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON PEROXIDASE CONTENT OF SNAP BEANS
Second Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 44.09 | |
| Temperature | 2 | 2943.81 | 12.79 ** |
| Moisture | 1 | 1998.41 | 8.68 ** |
| Temperature x Moisture | 2 | 42.84 | 0.34 |
| Error | 15 | 230.00 | |

MULTIPLE RANGE AND L. S. D.[2] TESTS

| | | | |
|---|---|---|---|
| No. of Means | | 2 | 3 |
| L.S. Range[1] for temperature at P | 0.05 | 16.16 | 16.93 |
| | 0.01 | 22.35 | 23.42 |
| L.S.D. for moisture at P | 0.05 | 13.18 | |
| | 0.01 | 18.26 | |

Comparison of Means

Absorbency units per gram sample

| | | | |
|---|---|---|---|
| Temperature | 157.87 (low) | 149.25 (high) | 120.50 (medium) |
| Moisture | 151.33 (low) | 133.08 (high) | |

1. Least Significant Range
2. Least Significant Difference
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

TABLE XIX

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON PEROXIDASE CONTENT OF SNAP BEANS
Third Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 303 | |
| Temperature | 2 | 7378 | 24.03 ** |
| Moisture | 1 | 1633 | 5.31 * |
| Temperature x Moisture | 2 | 316 | 1.02 |
| Error | 15 | 307 | |

MULTIPLE RANGE AND L. S. D.[2] TESTS

| | | | |
|---|---|---|---|
| No. of Means | | 2 | 3 |
| L. S. Range[1] for temperature at P | 0.05 | 18.75 | 19.68 |
| | 0.01 | 26.06 | 27.36 |
| L. S. D. for moisture at P | 0.05 | 15.30 | |
| | 0.01 | 21.26 | |

Comparison of Means

Absorbency units per gram sample

| | | | |
|---|---|---|---|
| Temperature | 246.75 (low) | 204.75 (medium) | 187.75 (high) |
| Moisture | 221.3 (low) | 204.83 (high) | |

1. Least significant range
2. Least significant difference
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

TABLE XX

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON PEROXIDASE CONTENT OF SNAP BEANS
Fourth Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 488 | |
| Temperature | 2 | 1622 | 4.15 * |
| Moisture | 1 | 5163 | 13.20 ** |
| Temperature x Moisture | 2 | 293 | 0.74 |
| Error | 14 | 391 | |

MULTIPLE RANGE AND L. S. D.[2] TESTS

| No. of Means | | 2 | 3 |
|---|---|---|---|
| L. S. Range[1] for temperature at | P 0.05 | 21.17 | 22.23 |
| | 0.01 | 29.43 | 30.89 |
| L. S. D. for moisture at | P 0.05 | 17.30 | |
| | 0.01 | 24.04 | |

Comparison of Means

Absorbency units per gram sample

| | | | |
|---|---|---|---|
| Temperature | 230.87 (low) | 211.87 (medium) | 203.00 (high) |
| Moisture | 229.91 (low) | 200.58 (high | |

1. Least significant range
2. Least significant difference
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

TABLE XXI

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON HEXOSE SUGARS IN SNAP BEANS
First Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 4.478 | |
| Temperature | 2 | 32.574 | 18.93 ** |
| Moisture | 1 | 12.136 | 7.05 * |
| Temperature x Moisture | 2 | 4.827 | 2.80 |
| Error | 15 | 1.72 | |

MULTIPLE RANGE AND L. S. D.[2] TESTS

| | | | |
|---|---|---|---|
| No. of Means | | 2 | 3 |
| L. S. Range[1] for temperature at | P 0.05 | 1.38 | 1.45 |
| | 0.01 | 1.92 | 2.01 |
| L. S. D. for moisture at | P 0.05 | 1.14 | |
| | 0.01 | 1.58 | |

Comparison of Means

Percent dry weight basis

| | | | |
|---|---|---|---|
| Temperature | 17.94 (high) | 15.06 (low) | 14.25 (medium) |
| Moisture | 16.43 (high) | 15.02 (low) | |

1. Least significant range
2. Least significant difference
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

Any two means underscored by the dotted line are significantly different from each other at 0.05 level of probability.

TABLE XXII

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON HEXOSE SUGARS IN SNAP BEANS
Second Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 1.480 | |
| Temperature | 2 | 6.304 | 3.44 |
| Moisture | 1 | 9.514 | 5.20 * |
| Temperature x Moisture | 2 | 38.647 | 21.14 ** |
| Error | 15 | 1.829 | |

MULTIPLE RANGE AND L. S. D.[2] TESTS

| No. of Means | | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| L. S. Range[1] for temperature x moisture at | P 0.05 | 0.63 | 0.66 | 0.68 | 0.69 | 0.70 |
| | 0.01 | 0.87 | 0.92 | 0.94 | 0.96 | 0.97 |
| L. S. D. for moisture at | P 0.05 | | | 1.17 | | |
| | 0.01 | | | 1.62 | | |

Comparison of Means
Percent dry weight basis

| | | |
|---|---|---|
| Moisture | 15.30 (high) | 14.02 (low) |

Temperature x Moisture

17.66 (Hxh) : 16.72 (Mxl) : 15.14 (Mxh) : 14.60 (Lxh) : 14.45 (Lxl) : 10.91 (Hxl)

1. Least significant range
2. Least significant difference
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

Legend:
H, M and L denote high, medium and low temperature ranges respetively.
h and l denote high and low soil moisture levels respectively.

TABLE XXIII

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON HEXOSE SUGARS IN SNAP BEANS
Third Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 3.766 | |
| Temperature | 2 | 27.067 | 14.04 ** |
| Moisture | 1 | 5.777 | 2.99 |
| Temperature x Moisture | 2 | 2.963 | 1.53 |
| Error | 15 | 2.060 | |

MULTIPLE RANGE TESTS

| | | | |
|---|---|---|---|
| No. of Means | | 2 | 3 |
| L. S. Range[1] for temperature at | P 0.05 | 1.53 | 1.65 |
| | 0.01 | 2.12 | 2.23 |

Comparison of Means
Percent dry weight basis

| | | | |
|---|---|---|---|
| Temperature | 14.58 (medium) | 13.80 (high) | 11.08 (low) |

1. Least significant range
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

TABLE XXIV

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON TOTAL SOLIDS IN SNAP BEANS
First Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 0.2146 | |
| Temperature | 2 | 24.6316 | 109.32 ** |
| Moisture | 1 | 0.3725 | 1.65 |
| Temperature x Moisture | 2 | 0.3641 | 1.61 |
| Error | 15 | 0.2253 | |

MULTIPLE RANGE TESTS

| | | | |
|---|---|---|---|
| No. of Means | | 2 | 3 |
| L. S. Range[1] for temperature at P | 0.05 | 0.511 | 0.537 |
| | 0.01 | 0.708 | 0.743 |

Comparison of Means
Percent dry weight basis

| | | | |
|---|---|---|---|
| Temperature | 11.6375 (high) | 9.9025 (medium) | 8.1237 (low) |

1. Least significant range
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

TABLE XXV

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON
TOTAL SOLIDS IN SNAP BEANS
Second Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Square | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 0.8143 | |
| Temperature | 2 | 22.8431 | 22.05 ** |
| Moisture | 1 | 8.7246 | 8.42 ** |
| Temperature x Moisture | 2 | 1.0975 | 1.05 |
| Error | 15 | 1.0360 | |

MULTIPLE RANGE AND L. S. D.[2] TESTS

| | | | | |
|---|---|---|---|---|
| No. of Means | | | 2 | 3 |
| L. S. Range[1] for temperature at | P | 0.05 | 1.087 | 1.137 |
| | | 0.01 | 1.501 | 1.573 |
| L. S. D. for moisture at | P | 0.05 | 0.0873 | |
| | | 0.01 | 1.2093 | |

Comparison of Means
Percent dry weight basis

| | | | |
|---|---|---|---|
| Temperature | 11.8187 (high) | 9.9137 (low) | 8.4489 (medium) |
| Moisture | 10.66 (low) | 9.45 (high) | |
| Temperature x Moisture | ____ | ____ | |

1. Least significant range
2. Least significant difference
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.01 level of probability.

TABLE XXVI

INFLUENCE OF TEMPERATURE AND SOIL MOISTURE ON TOTAL SOLIDS IN SNAP BEANS
Third Harvest

ANALYSIS OF VARIANCE

| Source of Variation | Degrees of Freedom | Mean Squares | Observed F |
|---|---|---|---|
| Total | 23 | | |
| Replication | 3 | 0.8481 | |
| Temperature | 2 | 6.6966 | 10.30 ** |
| Moisture | 1 | 0.8550 | 1.31 |
| Temperature x Moisture | 2 | 1.6801 | 2.57 |
| Error | 15 | 0.6527 | |

MULTIPLE RANGE TESTS

| | | | |
|---|---|---|---|
| No. of Means | | 2 | 3 |
| L. S. Range[1] for temperature at P | 0.05 | 0.8428 | 0.8848 |
| | 0.01 | 1.1676 | 1.2236 |

Comparison of Means

| | | | |
|---|---|---|---|
| Temperature | 12.0775 (high) | 10.5112 (low) | 10.4759 (medium) |
| Moisture | ——— | | |
| Temperature x Moisture | ——— | | |

1. Least significant range
* Significant at 0.05 level of probability
** Significant at 0.01 level of probability

Any two means underscored by the same line are not significantly different from each other at 0.05 level of probability.

# APPENDIX B

TABLE XXVII

MEAN DAILY TEMPERATURES IN DIFFERENT SECTIONS OF THE GREENHOUSE AND OUTSIDE, MARCH, 1961
(In degrees Fahrenheit)

| Date | Greenhouse * | | | Outside |
|---|---|---|---|---|
| | I | II | III | |
| 1 | 71.8 | 67.9 | ... | 48.0 |
| 2 | 71.4 | 66.2 | 67.0 | 46.0 |
| 3 | 73.7 | 66.1 | 64.9 | 51.0 |
| 4 | 75.6 | 74.2 | 70.5 | 65.0 |
| 5 | 76.3 | 75.0 | 72.4 | 67.0 |
| 6 | 75.6 | 74.5 | 70.5 | 64.5 |
| 7 | 74.8 | 72.2 | 67.0 | 60.5 |
| 8 | 73.9 | 71.2 | 70.0 | 65.0 |
| 9 | 67.9 | 62.3 | 59.0 | 36.0 |
| 10 | 70.7 | 64.2 | 59.3 | 44.0 |
| 11 | 73.0 | 68.1 | 61.8 | 50.0 |
| 12 | 74.6 | 72.0 | 68.5 | 56.0 |
| 13 | 73.9 | 71.7 | 66.9 | 58.0 |
| 14 | 71.0 | 67.2 | 62.4 | 49.0 |
| 15 | 73.1 | 68.5 | 62.1 | 50.0 |
| 16 | 74.6 | 70.4 | 63.3 | 55.0 |
| 17 | 73.8 | 70.0 | 62.7 | 49.0 |
| 18 | 72.7 | 69.2 | 65.2 | 50.0 |
| 19 | 72.6 | 72.2 | 69.2 | 59.0 |
| 20 | 73.3 | 73.4 | 67.5 | 59.5 |
| 21 | 72.0 | 70.9 | 64.5 | 56.0 |
| 22 | 73.0 | 70.1 | 64.5 | 54.0 |
| 23 | 72.2 | 67.1 | 62.8 | 50.0 |
| 24 | 73.2 | 67.8 | 63.4 | 45.0 |
| 25 | 78.3 | 71.9 | 66.5 | 55.0 |
| 26 | 77.8 | 74.7 | 70.0 | 56.0 |
| 27 | 76.5 | 70.4 | 70.5 | 60.0 |
| 28 | 75.8 | 72.5 | 68.0 | 58.0 |
| 29 | 78.1 | 72.4 | 68.6 | 66.0 |
| 30 | 77.6 | 72.9 | 68.8 | - |
| 31 | 78.0 | 74.6 | 70.9 | - |

Greenhouse section 1: High temperature range
Greenhouse section 2: Medium temperature range
Greenhouse section 3: Low temperature range

Note:
Mean daily temperatures in greenhouse derived by averaging hourly temperatures.
Mean daily temperatures outside derived by averaging maximum and minimum temperatures.

TABLE XXVIII

MEAN DAILY TEMPERATURES IN DIFFERENT SECTIONS OF THE GREENHOUSE AND OUTSIDE, APRIL AND MAY 1961
(In degrees Fahrenheit)

| Date | Greenhouse | | | Outside |
|---|---|---|---|---|
| | I | II | III | |
| 1 | 74.3 | 69.2 | 67.6 | 50.0 |
| 2 | 74.8 | 70.0 | 65.2 | 47.0 |
| 3 | 76.5 | 69.4 | 61.5 | 52.0 |
| 4 | 75.4 | 69.4 | 62.8 | 48.0 |
| 5 | 73.9 | 69.1 | 63.1 | 51.0 |
| 6 | 73.0 | 69.5 | 64.4 | 54.0 |
| 7 | 72.2 | 71.2 | 60.6 | 47.0 |
| 8 | 74.2 | 73.5 | 63.9 | 48.5 |
| 9 | 71.5 | 70.5 | 63.2 | 48.0 |
| 10 | 69.5 | 66.9 | 59.0 | 56.0 |
| 11 | 76.4 | 72.0 | 61.3 | 49.0 |
| 12 | 72.4 | 69.4 | 65.0 | 58.0 |
| 13 | 66.6 | 65.4 | 59.1 | 45.0 |
| 14 | 74.2 | 70.4 | 65.3 | 52.0 |
| 15 | 79.3 | 77.0 | 74.5 | 65.0 |
| 16 | 72.4 | 68.7 | 67.1 | 48.0 |
| 17 | 69.3 | 67.7 | 63.0 | 50.0 |
| 18 | 73.0 | 69.0 | 63.0 | 51.0 |
| 19 | 75.7 | 75.0 | 66.0 | 53.5 |
| 20 | 77.0 | 76.2 | 70.1 | 59.0 |
| 21 | 75.6 | 73.1 | 69.7 | 62.0 |
| 22 | 78.4 | 76.5 | 72.5 | 66.0 |
| 23 | 80.3 | 78.1 | 73.4 | 74.0 |
| 24 | 79.4 | 77.5 | 75.5 | 75.0 |
| 25 | - | 77.1 | 74.7 | 72.0 |
| 26 | - | 76.1 | 74.1 | 68.0 |
| 27 | - | 72.2 | 68.9 | 63.0 |
| 28 | - | 70.0 | 67.8 | 61.0 |
| 29 | - | 67.4 | 64.0 | 53.0 |
| 30 | - | 69.5 | 64.5 | 60.0 |
| | | MAY 1961 | | |
| 1 | - | - | 65.0 | 63.0 |
| 2 | - | - | 65.3 | 58.0 |
| 3 | - | - | 65.1 | 56.5 |
| 4 | - | - | 67.0 | 64.0 |

Greenhouse section 1: High temperature range
Greenhouse section 2: Medium temperature range
Greenhouse section 3: Low temperature range

Note:

Mean daily temperatures in greenhouse derived by averaging hourly temperatures. Mean daily temperatures outside derived by averaging maximum and minimum temperatures.

# APPENDIX C

DETERMINATION OF HEXOSE SUGARS IN SNAP BEANS

(Adapted from the Method of Folin Wu[1])

1. One gram of dry ground material is placed in a 50 ml. volumetric flask. 80 percent ethyl alcohol is added up to the mark, shaken and allowed to stand for 24 hours.
2. More alcohol is added to bring up to volume if necessary and mixed well. One ml. each of aliquot is pipetted into two 100 ml. beakers, allowed to stand and evaporate completely so that no traces of alcohol remain.
3. Two ml. of distilled water are added and with a policeman the dried material is thoroughly scraped. 16 m. of 1/12 N sulphuric acid are run into it from a burette, and the mixture is allowed to stand for 2 minutes. Then 2 ml. of 10 percent of sodium tungstate is added, mixed well and allowed to stand for 5 minutes.
4. About 1/4 teaspoonful of activated charcoal is added, mixed well and allowed to stand for at least 10 minutes. It is then filtered through good quality filter paper.
5. Two ml. of filtrate is pipetted into 25 ml. test tubes. 2 ml of Folin's Copper Reagent are added and the test tube is put in boiling water bath for exactly 8 minutes. It is then cooled under running cold water for exactly 2 minutes.
6. Two ml. of phosmolybdic acid are added and mixed well. The test tube

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[1]Methods and Calibrations, Bausch and Lomb Spectronic 20 Colorimeter Catalogue No. 33-29-40.

is again placed in boiling water bath for exactly 5 minutes. Cooling is done as before for 2 minutes.

7. Distilled water is added to make up the volume to 25 ml.mark. The optical density is measured in a Spectronic 20 Colorimeter at 420 mu. with the blank[2] adjusted for 100 percent transmission.

The reading is correlated with calibrations[3] to obtain percent hexose sugars on dry weight basis.

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[2]The Folin Wu blank is prepared by adding 16 ml. of N/12 sulphuric acid and 2 ml. of 10 percent sodium tungstate to 2 ml. of water.

[3]Methods and Calibrations, Bausch and Lomb Spectronic 20 Colorimeter Catalogue No. 33-29-40.

# APPENDIX D

## MEASUREMENT OF PEROXIDASE ACTIVITY IN SNAP BEANS

(Adapted from the method of Reddi et al.[1])

1. 50 grams of snap beans are passed through a food chopper. 5 grams of comminuted material are put in a pint jar and 245 ml. phosphate citrate buffer are added. The buffer is prepared by combining 14.2 parts of 0.2M disodium hydrogen phosphate and 5.8 parts of 0.1M citric acid. pH is adjusted to 6.5 by addition of sodium hydroxide.
2. The mixture is blended on an osterizer for 5 minutes. 10 ml. of aliquot are transferred to a second pint jar and 240 ml. buffer solution are added. This mixture is blended on an osterizer for 3 minutes.
3. 5 ml. of aliquot are transferred into macro type Evelyn Photoelectric Colorimeter tube, to which are added 5 ml. buffer solution, 13 ml. distilled water, and 1 ml. of 0.3 percent hydrogen peroxide solution.
4. The Evelyn tube is placed in a water bath at 25° C. and after allowing the reaction mixture to attain the temperature of water bath, 1 ml. of 1 percent alcoholic solution of guaiacol is added, and the reaction is allowed to proceed for 45 minutes.
5. The tube is then taken out of the water bath and the outside wiped clean. The optical density of the solution is measured in an Evelyn Photoelectric Colorimeter at 420 mu. with the blank adjusted for 100 percent transmission. The blank is prepared under the same conditions except that the enzyme extract is boiled for 5 minutes, cooled and made up to original volume. The activity is expressed as absorbency units per gram sample.

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[1] R. R. Reddy, W. B. Esselen, and C. R. Fellers. "Peroxidase Activity of Apple Tissue." Food Technology IV (2):63,1950.