STUDIES ON THE EFFECTS OF TEMPERATURES AND ORGANIC CHEMICAL SUPPLEMENTS ON SEED PRODUCTION OF THE DRY BEAN (*Phaseolus vulgaris* L. and *P. acutifolius latifolius* Freem) and Sugar Beet (*Beta vulgaris* L.)

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A THESIS

Submitted to the College of Agriculture of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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ABSTRACT

Experiments were conducted to determine the effects of high temperature on the seed yields of nine bean varieties and four sugar beet clones. Ten water-soluble vitamins and nucleic acid hydrolysate were applied as foliar sprays during the time of flowering and seed maturation.

Yields of bean and sugar beet were suppressed by the high temperature. Its deleterious effects were enhanced by irregular air movement and competition between plants for light. No effect of the application of organic chemical supplements could be detected due to large experimental error in the data.

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INTRODUCTION

Adaptation of plants to an environment has been considered to depend upon an organism having no inherent deficiency of an essential enzyme or metabolite, or a deficiency induced by the environment (14). The flexibility of the biochemical system of a plant may enable it to adjust to the varying environment. The concept that decreased growth or even death of both higher and lower plants under unfavorable climatic conditions can sometimes be prevented by supplying certain specific metabolites has been supported by the findings of many workers in the past two decades. From the agronomic point of view, the chemical cure of climatic lesions (4) provides a new and prospective avenue of exploration aimed at solving the problem of insufficient food supply for the increasing world population through improving yield in the present cultivated areas or offering a chance to extend crop production to new lands.

The main object of these experiments was to determine whether the poor seed yields of bean and sugar beet strains at high temperature could be improved with organic supplements applied as foliar sprays.

REVIEW OF LITERATURE

The earliest demonstration of the chemical control of a temperature lesion was reported by Bonner (3) in 1943, who grew Cosmos plants at controlled temperatures of 80° and 68°F and applied thiamine in the nutrient solution at a rate of 0.01 mg per liter. Positive growth responses to added thiamine were obtained at a constant temperature of 68° but not at 80°F, nor at a day temperature of 80° combined with a night temperature of 68°F. It should be noted that temperature conditions favoring luxuriant growth appeared unfavorable for demonstration of any apparent growth-promoting effect of thiamine. Earlier experiments conducted by the same author and others (1, 2) also showed that the application of vitamin B_1 was sometimes effective and sometimes not. It was suggested that it was effective only when the plant was low in vitamin B_1 and that leaf vitamin content might be used to diagnose whether or not addition of vitamin B_1 would promote the growth of a given crop or plant species.

Galston and Hand (7) found that adenine stimulated the growth of epicotyl sections, leaf buds and roots of etiolated pea. The thermal inactivation of the growth of excised pea epicotyl-sections was largely prevented by the addition of adenine to the medium.

Highkin (9) used two genetic strains of peas in studies of heat resistance and the inheritance of heat resistance. On the basis of work by Galston and others, he made measurements to determine whether there was a relationship between purine and pyrimidine

composition of these pea strains and their ability to resist heat. The amount of adenine in the heat susceptible variety remained the same at low (14°C) and at high (26°) temperatures, but in the resistant variety, the adenine content at 26°C increased at least 100 per cent as compared to that in plants grown at 14°.

Galston (8) reported the response of pea plants to adenine. All plants were kept at 23°C during the day but were divided into groups which were subjected to different night temperatures from 2° to 30°. Plants made most rapid growth in length at a night temperature of 17°, growth being inhibited about 35 per cent by a 10° deviation in temperature, either upward or downward. Adenine had no effect at the optimum temperature but it promoted stem elongation slightly both at the higher and the lower temperatures. Similar results were obtained on the fresh weight of the pea stem.

Langridge and Griffing (16) grew different homozygous ecotypes of Arabidopsis thaliana aseptically in growth chambers. They found that the genotypic component of the measurable growth variation between races was positively correlated with temperature. Eight out of 43 races showed a disproportionately large decrease in growth at 31.5°C. Five of these 8 races had pronounced morphological symptoms of high temperature damage, and 3 gave significantly increased growth at 31.5° with vitamins, yeast extract, or nucleic acids. The specific stimulating substance for the first two races was found to be biotin, which completely prevented the formation of the temperature lesion; in the third race, a partial alleviation of the temperature lesion resulted from the addition of cytidine.

In their recent work, Ketellaper and Bonner (11) grew plants at different temperatures including the optimal temperature, and found that they produced different amounts of dry matter at different temperatures. Further experiments showed that increases in dry weight up to 40 per cent were obtained by application of vitamin B or ribosides to pea plants grown at 30°C day temperature and 23°C night temperature (30/23°C). Application of the vitamin B mixture or thiamine to Cosmos in cool temperature (17/10 or 20/14°C) produced a 30 per cent increase in dry weight. Vitamin C was effective at 20/14 and 23/17°C.

Much work has been done with lower plants and bacteria because of the extensive background of biochemical studies and the ease of conducting experiments with these microorganisms under controlled conditions. Working with a mutant strain of Neurospora, Mitchell and Houlahan (19) showed that it synthesized riboflavin at a rate approaching that of a wild-type strain at temperatures below 25°C while an external source of this vitamin was necessary for normal growth above 28°C.

In studies on the growth requirements of an Escherichia coli mutant, Maas and Davis (18) found that pantothenate was required by a certain mutant only at temperatures above 30°C. The mutant was derived from an ordinary auxotroph which requires pantothenate at any temperature. For both strains, pantoate and beta-alanine, the intermediates of pentothenate synthesis, were inactive as growth factors, indicating a block in the last stage of this biological reaction. The authors also found that the pantothenate-synthesizing enzyme of the mutant was much more heat-labile than that of the wild types.

A similar result has been obtained by Horowitz and Fling (10) with Neurospora. The wild strain of Neurospora has a pair of alleles, T^s and T^l, governing the thermostability of the enzyme tyrosinase. Strains carrying T^s produce a tyrosinase characterized by a half-life of at least 30 minutes at 59°C, whereas strains carrying T^l produce a tyrosinase with a half-life of 3 to 4 minutes at the same temperature. The template-modification and rate-modification hypotheses have been mentioned to explain the thermal stability of this enzyme.

Campbell (5) has studied the thermal stability of highly purified alpha-amylase produced at 35° and 55°C by two facultative thermophilic bacteria, Bacillus coagulans and B. stearothermophilus. It was found that the enzymes produced at 55° were more heat stable than those produced at 35°, losing only 6 to 10 per cent of their activity in one hour at 90° whereas the latter preparations lost 90 to 92 per cent of their activity.

From his experience on the nature of biological thermostability, Koffler (12) explained biological individuality on the molecular level. Two possibilities were suggested in regard to the existence of life at extremely high temperature.

1. The essential cell components of thermophiles are relatively more stable than those of mesophiles.

2. Rapid resynthesis is the key to the problem of biological stability to heat.

Koffler and his associates (13), using the flagella from E. coli as a model, found evidence supporting the first suggestion that might explain the remarkable heat stability of thermophile protein.

METHODS AND MATERIALS

Eight commercially grown bean varieties of Phaseolus vulgaris L., one of P. acutifolius latifolius Freem and four sugar beet clones of different thermal sensitivities were used in the experiments.

These experiments were conducted in the Plant Science Greenhouse of Michigan State University, at East Lansing, Michigan. During the time of experimentation, the greenhouse temperatures were controlled by the automatic steam heating system coupled with electrical heaters and manual adjustments of the vents.

The screening procedure for detecting a specific deficiency, suggested by Dr. K. C. Atwood and used successfully by previous workers (17, 15), was adopted in these experiments. Four vitamin solutions having the components shown in Table I and a nucleic acid hydrolysate solution were applied as foliar sprays in all experiments. Stock solutions of ten water-soluble vitamins were prepared at concentrations as shown in Table II. Each vitamin solution was prepared by mixing the four component stock solutions in equal volume before applications. Nucleic acid hydrolysate (NA) was prepared from sodium

Table I. Components of Four Vitamin Solutions

Solutions	Components
V1	riboflavin, nicotinic acid, pantothenic acid, pyridoxine
V2	nicotinic acid, biotin, inositol, folic acid
V3	pantothenic acid, inositol, ascorbic acid, choline
V4	pyridoxine, folic acid, choline, p-aminobenzoic acid

ribonucleate by the method proposed by Langridge (15). Five grams of sodium ribonucleate were hydrolysed and made up to one liter of solution. All of these solutions were made weekly and stored in refrigerators.

Table II. Concentration of the Stock Solutions of Ten Vitamins

Vitamins	Concentrations	Vitamins	Concentrations
riboflavin	10 mg/l	i-inositol	2000 mg/l
nicotinic acid	50	folic acid	10
calcium pantothenate	10	ascorbic acid	200
pyridoxine hydro-chloride	10	choline chloride	10
biotin	0.5	p-aminobenzoic acid	10

Plants were grown in a mixture of sand and peat or vermiculite. Complete nutrient solution containing the following salts was supplied two or three times a week.

$Ca(NO_3)_2 \cdot 4H_2O$	0.4723 gms/l	H_3BO_3	0.0015 gms/l
KH_2PO_4	0.0681	$MnSO_4 \cdot 2H_2O$	0.00086
KNO_3	0.4044	$ZnSO_4 \cdot 7H_2O$	0.00011
KCl	0.2982	$CuSO_4 \cdot 5H_2O$	0.00004
$NH_4H_2PO_4$	0.2302	$(NH_4)_6Mo_7O_{24} \cdot H_2O$	0.00006
$MgSO_4 \cdot 7H_2O$	0.3697	$FeSO_4 \cdot 7H_2O$	0.0143
NH_4NO_3	0.0801	Sequestrene	0.0210

Experiment 1.

On October 16, 1961, seeds of seven bean varieties, namely Columbia Pinto (CP), Michelite #62 (M62), California Dark Red Kidney (CDRK), Idaho Sanilac (IS), Red Mexican #35 (RM35), Great Northern #59 (GN59) and #123 (GN123) of P. vulgaris and Tepary Bean (TB) of P. acutifolius latifolius were sown in ten inch pots filled with the mixture of 1:1 builders sand and peat. Six seeds were sown in each pot.

There were eighteen pots for each variety placed adjacent to each other on the floor of the greenhouse. After the development of foliage leaves, seedlings were thinned to four uniform plants per pot. All plants were kept in a room in the greenhouse at a day temperature varying from 70 to 80°F and a night temperature at approximately 60°F. When a variety started to differentiate flower buds, fourteen very uniform pot cultures were moved into a high temperature room having a day temperature kept above 90° and night temperature around 80°F; the other 4 pots were retained in the lower temperature room and served as controls. The fourteen pots of each variety in the high temperature room were divided into six groups. Five groups, each consisting of two pots, received the five spray treatments respectively (Tables I and II). One of these two pots was sprayed once a week, the other three times a week. The sixth group consisted of four pots and was used as the high temperature control. Pots receiving same treatment were arranged adjacent to each other in plots, but the plots themselves were separated by 18-inch alleyways on two sides, and by 36-inch alleyways on the other two sides.

In the high temperature room, a turbulator (Model T24D, ACME Engineering and Manufacturing Corp.) operated by a 1/6 HP electric motor was used for air circulation during the experiment.

The date of macroscopic flower bud appearance and the starting date of treatment for the eight varieties are given in Table III.

For each spray treatment, 28 ml of vitamin solution or nucleic acid hydrolysate solution were sprayed on the four plants in each pot. All treatments were stopped on January 5, 1962, when the flowering

period was over. Plants in the high temperature room were harvested on January 27, 1962. The low temperature controls were harvested on February 17, 1962. Numbers and weights of pods and seeds produced in each pot were recorded.

Table III. Date of Flower Bud Appearance and the Starting Date of Treatment with Sprays for Eight Bean Varieties

Varieties	Date of flower bud appearance	Starting date of spray treatment
California Dark Red Kidney	Nov. 14, 1961	Nov. 21, 1961
Columbia Pinto	Nov. 15	"
Great Northern #123	"	"
Red Mexican #35	"	"
Great Northern #59	"	"
Idaho Sanilac	Nov. 19	"
Michelite #62	"	"
Tepary Bean	Nov. 26	Nov. 28

Experiment 2.

A further experiment on bean varieties was started on February 18, 1962, based on the differential varietal responses observed in the first experiment. Three varieties, namely California Small White (CSW), Idaho Sanilac (IS) and Tepary Bean (TB) were used. Two rooms in the greenhouse were kept at the same day temperature (80-90°F) and night temperature (70-75°F). Seeds of each variety were sown in 36 pots, initially spaced as in Experiment 1, in each room. Seedlings were thinned in the same manner as in Experiment 1. The earliest variety, Idaho Sanilac, started to initiate flower buds on March 20, 1962. From that date, the temperature in one room was raised to a day temperature above 90°F and night temperature from 80° to 85°F. In the other room,

the day temperature was reduced to 75° to 80°F and the night temperature to approximately 65°F. When spray treatments were started on March 26, 1962, in both high and low temperature rooms, plant cultures were very uniform in size in each variety.

In each room, the experiment consisted of a split plot experiment. Six treatments (five spray treatments and a control sprayed with distilled water) were applied as main plots distributed at random in 2 replications. Each main plot consisted of 3 varieties in a latin square of 3 replications. Individual pots in plots were 5 inches apart in one direction and 8 inches in the other. Main plots were 18 inches apart in one direction and 36 inches apart in the other.

Plants were sprayed twice a week. From March 26 to April 8, 1962, 250 ml of solution were applied to each main plot. After April 11, the volume was increased to 500 ml per plot, and after April 25, the volume was increased to 800 ml per plot, because the plants had grown so large that the original volume could not be sprayed evenly over all leaves. Spray treatments were stopped on May 16, 1962.

In the high temperature room, the Sanilac plants were harvested on May 12; in the low temperature room on May 25, 1962. Plants of the other varieties, bearing mature pods at that time, still continued vegetative growth at both temperatures and started secondary flowering at the lower temperature. Nutrient supply was reduced to once a week after May 16, 1962; watering was stopped on May 29, 1962. Plants were harvested on May 31 in both temperature rooms.

Experiment 3.

Sugar beet cuttings of four clones, namely 59-2, 30-1, 137-11 and 137-8, were made on September 29, 1961. On December 2, 1961, 30 cuttings of each clone were transplanted into ten-inch pots filled with about two inches of sand at the bottom and vermiculite on top. One plant was planted in each pot. These plants were photothermally induced at 48°F under continuous light from December 26, 1961 to March 6, 1962. During this treatment, many plants which were small and weak died. The surviving plants were then grown in a cool greenhouse temperature until bolting.

After bolting, plants were paired according to their size and then separated into two groups. One group was moved into the high temperature room of Experiment 2; another group was left in the cooler room. Five or six treatments of vitamin and nucleic acid hydrolysate solutions were applied on the sugar beet plants in both temperatures with one or two replications according to the number of available plants of each clone. The different treatments and number of plants used are shown in Table IV.

All treatments were started on April 8, 1962, except the second replication of 59-2 which was started on April 22, 1962. A volume of vitamin solutions or nucleic acid hydrolysate sufficient to cover the leaf surface and inflorescences of the beet plants was applied twice a week as a foliar spray. All treatments ended on May 23, 1962. Plants were harvested on June 27, 1962. Fresh weight of tops and seed yields were recorded.

Table IV. Treatments, Replications, and Number of Sugar Beet Plants Used in Experiment 3.

Clones	Temperature	Treatments	Replications	No. of Plants
59-2	high and low	4 vitamin solutions nucleic acid and control	2	24
30-1	high and low	4 vitamin solutions and control	2	20
137-11	high and low	4 vitamin solutions, nucleic acid and control	1	12

RESULTS

Experiment 1.

Temperatures during the time of treatment and seed maturation are shown in Figure 1. In the first half of this period, air temperature of the high temperature room could not be raised using the steam heating system to above 90°F except on sunny days. After December 29, 1961, two electric heaters were used as a reinforcement. The day temperatures could then be raised to above 90°F, however, daily fluctuations were still caused by the external weather. After January 13, 1962, daily maxima were recorded as 100° to 110°F. The exposure of the thermograph to direct sunshine made the recorded temperatures several degrees higher than the true air temperatures on sunny days.

Seed yields of plants in all treatments and at both temperatures are shown in Table V. All of these varieties, except Tepary Bean, performed better at the lower temperature. The differences between yields at these two temperatures were significant at the 1 per cent level. Most of the spray treatments tended to increase the bean yields in California Dark Red Kidney, Great Northern #123, Idaho Sanilac, and Tepary Bean, but for the rest, all spray treatments tended to suppress seed production. No trend can be found from the differences between effects of one and three applications per week.

At the lower temperature, bean plants of all eight varieties grew slower and matured later than those at high temperature. Plants at

Table V. Total Yields of Seeds (in gms) of Eight Bean Varieties for Different Treatments.

Treatments	Sprays per week	Varieties							
		CDRK	CP	GN123	RM35	GN59	IS	M62	TB
High temp. control#	0	4.87	16.37	8.50	16.62	6.87	3.62	17.75	12.12
Low temp. control#	0	17.13	23.12	27.62	32.37	21.13	17.13	26.37	1.00
Differences		12.26**	6.75*	19.12**	15.75**	14.26*	13.51**	8.62**	11.12*
High temp. plus									
V1	1	5.5	12.5	9.5	12.5	3.5	6.0	13.0	15.0
	3	3.5	13.0	10.0	13.0	5.0	2.5	12.5	13.0
V2	1	4.5	12.0	11.0	11.5	2.0	4.5	9.5	12.5
	3	4.0	11.5	9.0	18.0	5.5	5.0	11.5	18.5
V3	1	5.5	11.0	13.5	17.0	6.0	5.5	10.0	19.0
	3	6.5	13.5	12.5	18.0	1.5	6.0	14.0	17.0
V4	1	5.5	13.0	6.5	14.5	5.0	6.5	11.0	17.0
	3	5.0	14.0	6.5	19.5	7.0	4.0	12.5	19.5
NA	1	7.5	9.5	12.5	15.5	4.5	2.5	14.5	13.5
	3	3.0	11.0	10.0	8.0	4.0	4.5	4.0	8.0

Average of 4 pots
*, ** Significant at 0.05, 0.01 level, respectively

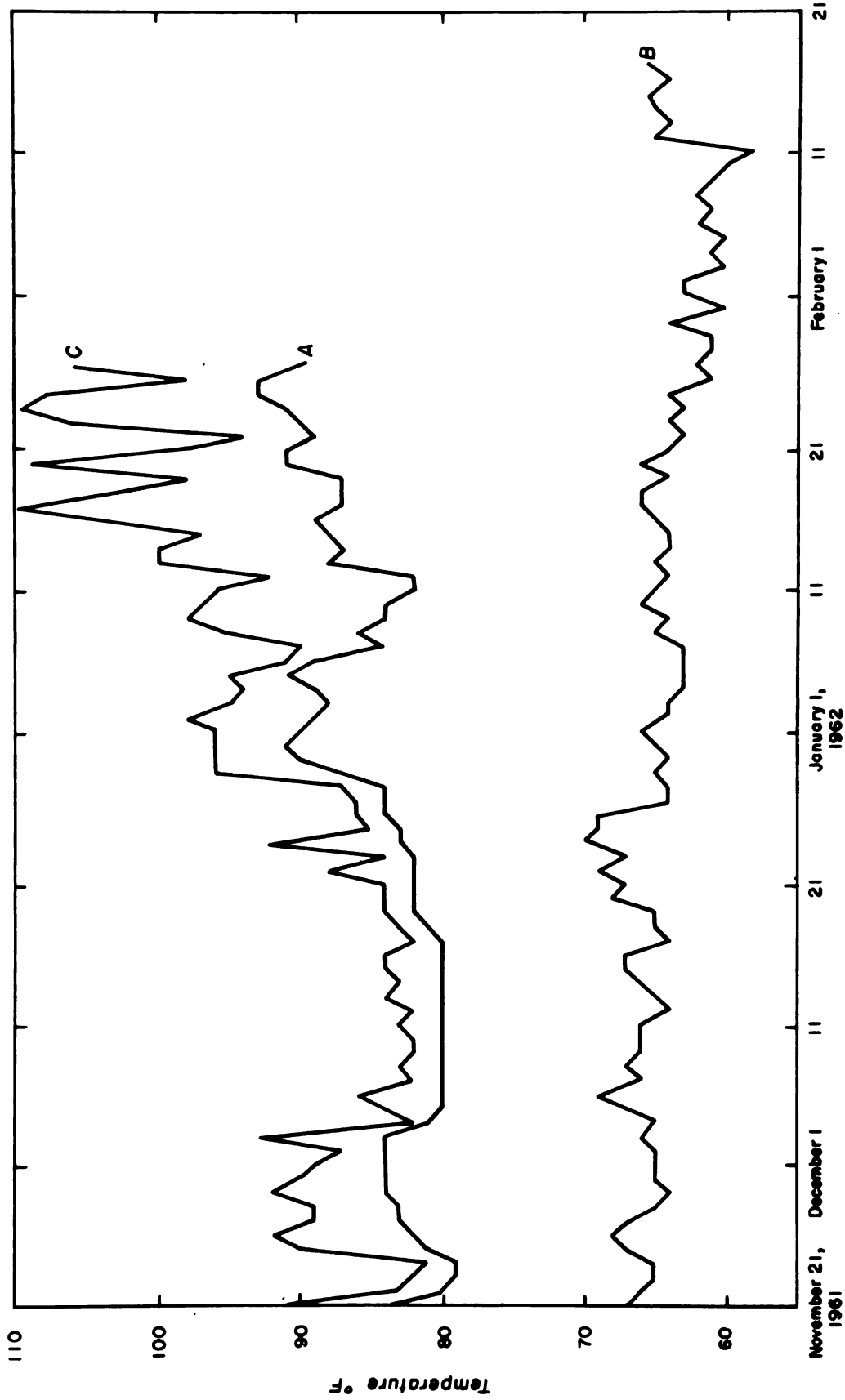

Figure 1. Daily variations of temperature during treatment and seed setting period of Experiment 1. A and B, daily average in high and low temperature room, respectively; C, maximum day temperature in high temperature room.

low temperature had a healthier appearance, and darker green leaves, while those at the high temperature were somewhat elongated, and possessed somewhat yellowish-green leaves. The low yields of the seven bean varieties at high temperature may be attributed to the fact that flower buds of most of these varieties aborted before anthesis. Generally, the bean plants grown at high temperature had fewer pods per pot (four plants), fewer and smaller seeds per pod, and set pods at the higher nodes in comparison with the plants at low temperatures (Table VI). The only exception was the Tepary Bean. It set pods at low temperature but had fewer and poorly developed seeds.

Experiment 2.

Temperatures for the period of treatment and seed maturation are shown in Figure 2. Air temperatures were recorded under shade in this experiment.

Growth of plants and seed yields were highly variable between replications at high temperature. In each main block, 9 pots of three varieties were arranged as a 3 x 3 latin square. Plants located along the border usually were larger, leafier and had better yields than those located inside the square. Tepary Bean was more sensitive to the border effect than others. In extreme cases, contrast between replications of 5.5 to 96.0 gms (Control) and 2.5 to 80.0 gms (V3) were recorded.

Table VI. Temperature Effects on Yield Constituent of Bean Varieties*.

Character	Temperature	Varieties							
		CDRK	CP	GN123	RM35	GN59	IS	M62	TB
No. of pods	high	11.2	20.0	11.5	24.7	11.0	17.7	23.2	22.7
	low	10.5	21.2	22.3	28.5	17.8	31.3	35.5	18.5
Weight of pods (gms)	high	6.75	20.25	10.50	19.87	8.75	4.87	22.37	14.00
	low	22.75	27.62	32.75	40.75	24.50	23.35	37.75	4.25
No. of seeds	high	13.7	47.0	31.4	58.0	25.2	26.0	90.0	88.0
	low	23.0	48.2	71.3	92.7	55.0	96.3	136.5	38.2
No. of seed per pod	high	1.22	2.35	2.73	2.35	2.29	1.47	3.88	3.88
	low	2.19	2.27	3.20	3.25	3.09	3.08	3.84	2.06
Average weight of seed (gms)	high	0.36	0.35	0.27	0.29	0.27	0.14	0.20	0.14
	low	0.74	0.48	0.39	0.35	0.38	0.18	0.19	0.02
Node where 1st pod was set	high	4.7	8.1	9.1	10.6	8.9	7.0	10.4	8.1
	low	5.4	3.6	5.4	6.9	6.0	6.0	9.4	8.7

* Data are averages of 4 pots.

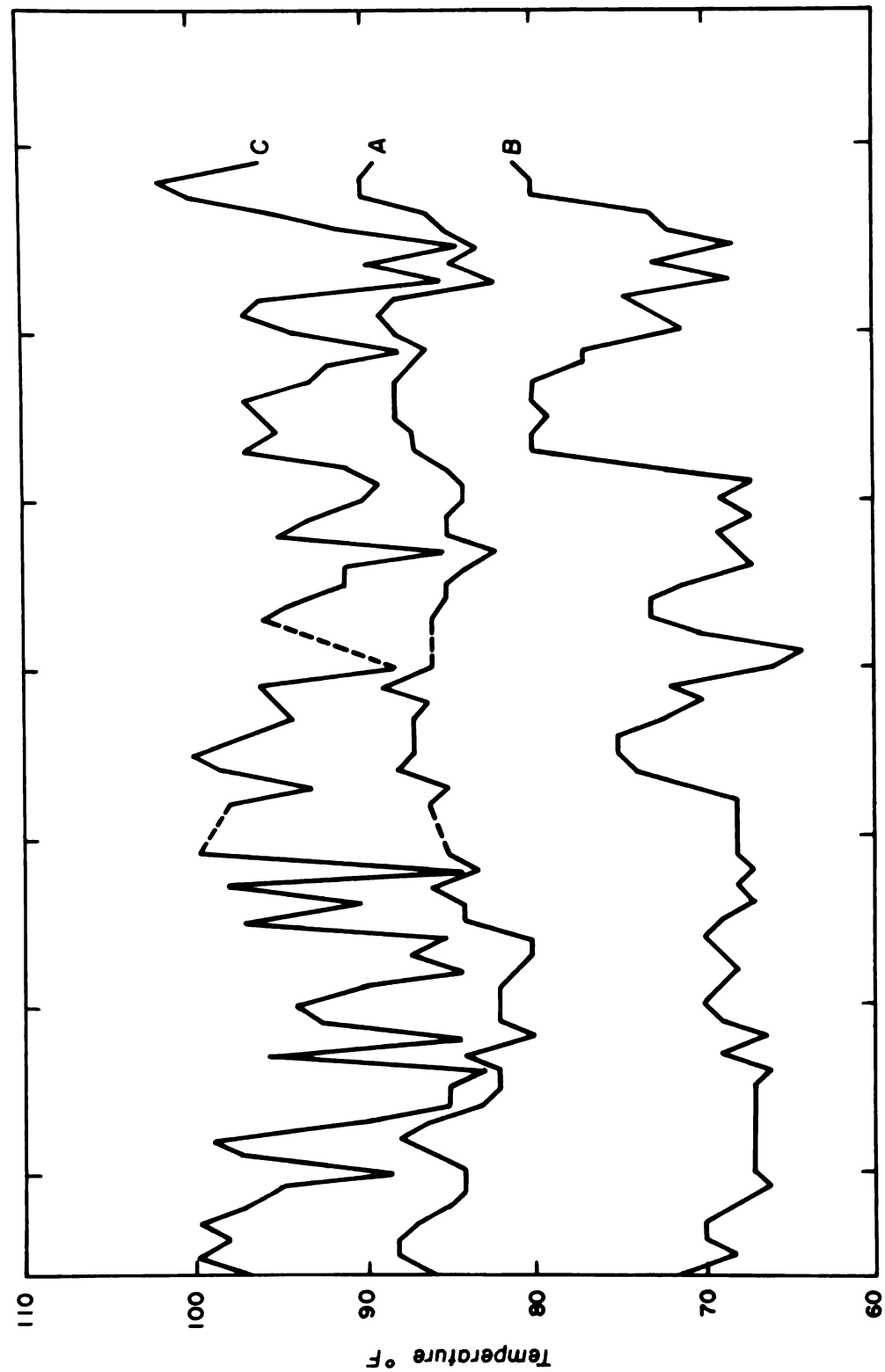

Figure 2. Daily variations of temperature during treatment and seed-setting period of Experiment 2. A and B, daily average in high and low temperature room, respectively; C, maximum day temperature in high temperature room.

In determining the effects of temperatures, spray treatments and varietal responses, analysis of variance of the data (Table VII) showed highly significant differences between varieties and their interaction with temperatures (Table VIII). The varietal means in Table IX show that, only at low temperatures are the differences in seed yields significant. The yields of these three varieties were very low at high temperature. No significant difference was obtained.

Table VII. Seed Yield (in gms) for Different Bean Varieties and Treatments at High and Low Temperatures.

Treatments	High temperature				Low temperature			
	CSW	IS	TB	Total	CSW	IS	TB	Total
V1	34.5	24.5	6.5	65.5	407.5	86.5	413.5	907.5
V2	31.5	36.5	12.5	80.5	437.0	61.5	364.5	863.0
V3	34.0	37.0	82.5	153.5	408.5	110.5	371.0	890.0
V4	46.0	35.0	13.0	94.0	427.0	87.5	368.0	882.5
NA	55.5	34.0	49.5	139.0	450.0	66.0	415.0	931.0
Control	38.5	46.0	101.5	186.0	500.5	95.5	403.0	999.0
Total	240.0	213.0	265.5	718.5	2630.5	507.5	2335.0	5473.0

The yield of Tepary Bean at the lower temperature was much better than in Experiment 1 (see Tables V and IX, 1.0 gm per pot in Expt. 1 vs. 194.58 gms per plot or 64.86 per pot in Expt. 2). It is believed that the high day temperature after May 14, 1962 (Figure 2) favored the development of seeds of this variety.

Table VIII. Analysis of Variance of the Total Seed Yields of the Experiments at Both High and Low Temperatures.

Sources	DF	SS	MS	F
Temperatures	1	313962.09	313962.09	415.420*
Replications	2	3748.75	1874.38	2.480
Treatments	5	3281.56	656.31	0.868
Temp. X Treat.	5	510.23	102.05	0.135
Error (a)	10	7557.73	755.77	
Varieties	2	114302.78	57151.39	190.963**
Var. X Treat.	10	2185.97	218.60	0.730
Var. X Temp	2	106206.69	53103.34	177.437**
Var. X Treat. X Temp.	10	3683.30	368.33	1.231
Error (b)	24	7182.65	299.28	
Total	71	562621.75		

* Significant at 0.05 level
** Significant at 0.01 level

Table IX. Average Yields (in gms) of Seeds of Three Bean Varieties at High and Low Temperatures.

Varieties	Temperatures	
	High	Low
CSW	20.00	219.21
IS	17.75	42.29
TB	22.12	194.58
LSD at 0.05	16.13	14.63
LSD at 0.01	22.64	20.53

The effects of vitamin and nucleic acid treatments were not significant (Table VIII). Further effort was directed toward detecting the effect of the vitamin solutions and nucleic acid hydrolysate. When

the yields of CSW in the low temperature room were analysed separately, the effects of treatments were significant at the 5 per cent level (Table X). Statistically significant differences were obtained between mean yields (Table XI), however, the degrees of freedom are too small, in this case, for reliable conclusion.

Table X. Analysis of Variance of the Yield of CSW Bean at Low Temperature.

Source	DF	SS	MS	F
Replications	1	6.02	6.02	
Treatments	5	331.91	66.38	5.96*
Error	5	55.70	11.14	
Total	11	393.63		

* Significant at 0.05 level

In comparing the data presented in Tables VII or IX, the differences between yields in the high and low temperature rooms are very striking, however, the effects cannot be analysed statistically because no replication was possible for the temperature factor.

Table XI. Average Yields (in gms) of CSW Bean Seed from Plants receiving Different Spray Treatments at Low Temperature.

Treatments	Yields
V1	67.92
V2	72.84
V3	68.08
V4	71.16
NA	75.00
Control	83.42
LSD at 0.05	8.74
LSD at 0.01	13.70

Experiment 3.

The seedball yields from the beets are expressed in the form of yield in grams per gram of fresh top, as shown in Table XII, because the different plant sizes at harvesting time made the data variable and interpretation difficult.

Table XII. Seedball Yields (gms/gm of fresh top) for Three Sugar Beet Clones and Different Treatments at High and Low Temperatures.

Temperature	Treatments	Clones		
		59-2*	137-11**	30-1*
High	V1	0.000	0.000	0.023
	V2	0.000	0.000	0.029
	V3	0.000	0.000	0.019
	V4	0.001	0.000	0.011
	NA	0.001	0.000	---
	Control	0.001	0.000	0.008
Low	V1	0.018	0.024	0.147
	V2	0.011	0.052	0.158
	V3	0.034	0.018	0.161
	V4	0.021	0.035	0.123
	NA	0.014	0.037	---
	Control	0.015	0.033	0.139

* Average of two replications
** Data of one replication

The most apparent fact is that all three clones produced more seeds per gram of fresh top at the lower temperature. Clones 59-2 and 137-11, which have been categorized as poor seed producers at high temperature (22), produced only a few seeds or none at high temperature. Vitamin solutions and nucleic acid hydrolysate treatments could not overcome the pronounced effects of environmental factors,

but it seems that the yields of clone 30-1 at high temperature have been improved by the first three vitamin solutions.

DISCUSSION

The hypothesis, first presented by Bonner (4) that the temperature-induced deficiencies may be overcome by the application of appropriate chemicals offers new opportunities for agronomic researchers. However, these experiments failed to show whether or not it is applicable in the cases of bean and sugar beet seed production. The yields of most bean varieties and sugar beet clones used in these experiments were suppressed by the high temperature. In the first experiment, the spray treatments of vitamin solutions and nucleic acid hydrolysate appeared to improve seed setting in some varieties at high temperature, but it could not be verified in the more detailed studies of Experiment 2.

Table XIII. Average Yields (in gms) of Seeds of Different Treatments at High and Low Temperatures.

Treatments	Temperatures	
	High	Low
V1	10.92	151.25
V2	13.58	143.83
V3	25.58	148.33
V4	15.67	147.08
NA	23.17	155.17
Control	31.00	166.50
LSD at 0.05	46.18	34.57
LSD at 0.01	72.42	54.20

In comparing the mean yields between different chemical treatments the least significant difference at 1 per cent level was as high as 234 per cent of the mean yield of the control at high temperature (Table XIII). (It should be pointed out that the LSD in Table XIII was calculated from a generalized error over all treatments and varieties;

but since the treatment variation in TB far exceeds the treatment variation of the other strains, there is a marked lack of homogeneity of errors, and the LSD as given is not critically valid for any comparisons involving treatments.) The increases of fresh or dry weight in other higher plants induced by similar chemical sprays have been reported to fall at levels below 50 per cent of the weight of control plants (Table XIV). Therefore, it would have been impossible to detect any effect of the supplements from data of this large variability except in the presence of an unusual increase in yield. It is believed that inadequate environmental control and the resulting high error components masked all the effects that these supplements might have had.

Table XIV. Increase of Fresh or Dry Weight in Higher Plants Induced by Organic Supplements.

Plants	Supplements	Effect in increasing fresh or dry weight of begetative organs	References
Cosmos	Vitamin B_1	20-48%	Bonner (1943)
	Vitamin B mixture	30%	Ketellapper and Bonner (1961)
	thiamine	30%	"
Pea	adenine	1-13%	Galston (1957)
	Vitamin B	40%	Ketellapper and Bonner (1961)
	ribosides	40%	"
Arabidopsis thaliana	vitamins	44%	Langridge and Griffing (1959

Experiments were conducted in the greenhouse. The first difficulty encountered in these experiments was the control of temperature.

At the beginning of Experiment 1, the temperature in the high temperature room could not be raised effectively to a level around 90°F with the steam-heating system. During the time of Experiment 2, the high temperature could me maintained somewhat constant with a new thermostat, but in the latter part of this experiment, the hot spring weather after May 14, 1962 made the temperature in the low temperature room impossible to maintain at the low level initially established. During the second half of May, the day temperatures usually were above 90°F. Tepary Bean grew well at high temperature and it was the only bean variety which yielded poorly at the lower temperature of Experiment 1. This variety set pods satisfactorily at low temperature (day temperature 70°-80°, night temperature 60°F) but the seeds did not develop well (see Table VI). In Experiment 2, this variety set only some empty pods at low temperature as was expected, but after the middle of May, all seeds developed rapidly as the day temperature increased. Finally, it had a seed yield only somewhat lower than that of California Small White at the same temperature. If there were some effects of the applied supplements on seed setting at low temperature, they may have been masked by the subsequent rapid growth of seeds at the increased day temperature. It is evident that a well regulated greenhouse or growth chamber is necessary for conducting critical experiments of this nature.

The second difficulty which was encountered was the limitation of space in the greenhouse in Experiment 2. At the beginning, a split latin square design with six treatments as main plots and 3 varieties as subplots was adopted for balancing the effects of light variation but

it was found impracticable to spray the chemical solutions on a plot without contaminating others. The change of the design reduced the degrees of freedom for treatments; and the intra-plot competition was found to be very serious. Idaho Sanilac was the only bush type variety used in Experiment 2. When it was located between plants of the two vining varieties according to the latin square arrangement in plots, it grew well during the early stage but was finally completely shaded by its neighboring plants which became vegetatively dominant in the latter half of the experimental period. It has been pointed out that, in Experiment 2, the location of plants greatly affected the yields of bean plants grown at high temperature. Although the plants were very uniform in size when spraying with organic supplements was initiated, crowding in subsequent growth led to excessive variation in yield between replicates. Significant differences occurred between yields of plants along the borders and in the inner part of plots in all three varieties (Table XV). The border effects were not appreciable at low temperature.

In Experiment 2, bean plants showed luxuriant vegetative growth at high temperature. Plants of the two vining varieties had spindly growth, long internodes and small yellowish leaves like those of Experiment 1, but they grew more rapidly. The mean temperature at the time of treatment and seed maturation in the high temperature room were 84.53° and 85.30°F for Experiments 1 and 2, respectively. This difference did not seem to have much effect on the growth habit and yields of bean plants. However, during Experiment 2 the high temperature room was more crowded with bean plants than in Experiment 1. In order to raise the temperature of that room, door and vents were usually closed. The turbulator used for improving ventilation in the high temperature room

of Experiment 1 was not available for Experiment 2. Therefore, the possible effects of high relative humidity of stagnant air between plants on transpiration and on preventing the loss of heat of leaves by radiation in the infra-red (6, 21) are worth noting. The prevention of heat transfer between plants and their environment and the resulting high leaf temperature might have enhanced the temperature effect in the high temperature room.

Table XV. Border Effect on the Seed Yield (in gms) of Three Bean Varieties.

Varieties	Temperature	Location of plants	Mean yields per pot	Difference between mean yields
CSW	high	border	8.86	3.59*
		inside	5.27	
	low	border	73.64	0.94
		inside	72.70	
IS	high	border	8.33	3.98**
		inside	3.71	
	low	border	14.61	0.32
		inside	13.77	
TB	high	border	16.07	14.90*
		inside	1.17	
	low	border	67.73	4.92
		inside	62.81	

* significant at 0.05 level

** significant at 0.01 level

The effects of light can be considered separately according to its effects on different physiological activities. As pointed out by Neale (20), in the glasshouse, there was a correlation between the transpiration rate of tomato, lettuce and carnation plants and the total light radiation. It is possible that the greater amount of light which was available for bean plants along the border would also help in aeration and water relations.

It has been reported (23) that under high temperature, bean plants needed a relatively large amount of light energy for photosynthesis. For normal growth, flowering and fruit development, light of 1200-2000 f. c. from above should be supplemented by light of equal intensity from the sides of the plants. Experiment 2 was conducted in winter and early spring under the natural short day and low light intensity. Six 500 W tungsten lamps, at a level about two feet above the top of bean plants, were used in each temperature room as supplementary light from 7 a. m. to 7 p. m., but these do little to improve the photosynthesis of bean plants. During the course of this experiment, most plants in the high temperature room lost their leaves on the lower portion of stems, except for the plants located along the borders. In the latter case, there is no doubt that those plants had larger photosynthetic area for synthesizing carbohydrates to meet the necessities of rapid vegetative growth and the consumption of a large amount of organic food in the greater respiration caused by high temperature.

In Experiment 3, no significant effect of treatments was obtained. The experimental plants were propagated asexually. The limited number of plants, their different initial size and deteriorated growth vigor made the data insufficient for a concrete conclusion.

SUMMARY

Bean varieties and sugar beet clones were grown under high temperature (day temperature 90°-100°, night temperature 80°-85°F) and lower temperature (day temperature 70°-80°, night temperature 60°-65°F) in the greenhouse during anthesis and seed maturation. Ten water-soluble vitamins and nucleic acid hydrolysate were applied as foliar sprays during anthesis and seed development.

1. Seed yields of bean varieties and sugar beet clones were greater at the lower temperature except for the Tepary Bean which needs a moderately high day temperature (around 90°F) for good seed development.

2. Variations between replications were so great as to obviate the possibility of reliability of observation of effects of chemical supplements.

3. High temperature effects were enhanced by poor ventilation and competition between plants for light.

LITERATURE CITED

1. Bonner, J., and J. Greene. Experiments on the relation of vitamin B_1 to the growth of green plants. Bot. Gaz. 100: 226-237. 1938.

2. Bonner, J., and J. Greene. Further experiment on the relation of vitamin B_1 to the growth of green plants. Bot. Gaz. 101: 491-500. 1939.

3. Bonner, J. Effects of application of thiamine to Cosmos. Bot. Gaz. 104: 475-479. 1943.

4. Bonner, J. The chemical cure of climatic lesions. Engng. Sci. Mag. 20: 28-37. 1957.

5. Campbell, L. L. Crystallization of alpha-amylase from a thermophilic bacterium. J. Amer. Chem. Soc. 76: 5256. 1954.

6. Curtis, O. F. Leaf temperature and the cooling of leaves by radiation. Plant Physiol. 11: 343-364. 1936.

7. Galston, A. W., and M. E. Hand. Adenine as a growth factor for etiolated peas and its relation to the thermal inactivation of growth. Arch. Biochem. 22: 434-443. 1949.

8. Galston, A. W. In "The experimental control of plant growth" (Ed. F. W. Went) pp. 313-317. (Chronica Botanica Co.:Waltham, Mass.) 1957.

9. Highkin, H. R. The relationship between temperature resistance and purine and pyrimidine composition in peas. Plant Physiol. Suppl. 32: L. 1957.

10. Horowitz, N. H., and M. Fling. Genetic determination of tyrosinase thermostability in Neurospora. Genetics 38: 360-374. 1953.

11. Ketellapper, H. J., and J. Bonner. The chemical basis of temperature responses in plants. Plant Physiol. Suppl. 36: 21. 1961.

12. Koffler, H. Protoplasmic differences between mesophiles and thermophiles. Bact. Revs. 21: 227-240. 1957.

13. Koffler, H. G. E. Mallett, and J. Adye. Molecular basis of biological stability to high temperatures. Proc. Natl. Acad. Sci. 43: 464-477. 1957.

14. Kurtz, C. B. Biochemistry of Adaptation in Plants to Environment. Amer. Nat. 94: 237-242. 1960.

15. Langridge, J. A hypothesis of developmental selection exemplified by lethal and semi-lethal mutants of Arabidopsis. Aust. J. Biol. Sci. 11: 58-68. 1958.

16. Langridge, J., and B. Griffing. A study of high temperature lesions in Arabidopsis thaliana. Aust. J. Biol. Sci. 12: 117-135. 1959.

17. Lindegren, C. C., and G. Lindegren. Linkage relations in Saccharomyces of genes controlling the formation of carbohydrates and the synthesis of vitamins, amino-acids and nucleic acid components. Indian Phytopath. 4: 11-20. 1951.

18. Maas, W. K., and B. D. Davis. Production of an altered pantothenate-synthesizing enzyme by a temperature-sensitive mutant of Escherichia coli. Proc. Natl. Acad. Sci. 38: 785-797. 1952.

19. Mitchell, H. K., and M. B. Houlahan. Neurospora. IV. A temperature sensitive riboflavinless mutant. Amer. J. Bot. 33: 31-35. 1946.

20. Neale, F. E. Transpiration of glasshouse tomatoes, lettuce and carnations. Neth. J. Agr. Sci. 4: 48:56. 1956.

21. Raschke, K. Heat transfer between the plant and the environment. Ann. Rev. Plant Physiol. 11: 111-126. 1960.

22. Snyder, F. W. and G. J. Hogaboam. Effect of temperature during anthesis and seed maturation on yield and germinability of sugar beet seed. Journal article No. 2188, Michigan Agricultural Experiment Station. (in press).

23. Viglierchio. D. R., and F. W. Went. Plant growth under controlled conditions. IX. Growth and fruiting of the Kentucky Wonder Bean (Phaseolus vulgaris). Amer. Jour. Bot. 44: 449-453. 1957.