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THE INFLUENCE OF 2, 4D AND MALEIC
HYDRAZIDE ON THE SUCROSE CONTENT
OF SUGAR CANE

Thesis for the Degree of M. S.

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Rafael Grant

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THESIS

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Hydrazide on the Sucrose Content
of Sugar Cane

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THE INFLUENCE OF 2,4D AND MALEIC HYDRAZIDE ON
THE SUCROSE CONTENT OF SUGAR CANE

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THE INFLUENCE OF 2,4D AND MALEIC HYDRAZIDE ON

THE SUCROSE CONTENT OF SUGAR CANE

INTRODUCTION

The Commonwealth of Puerto Rico is the most eastern soil of the Greater Antilles. On this island, 2,210,700 people are spread over approximately 3,435 square miles, with a density of 645 to the square mile, one of the greatest recorded throughout the world. The main crops of the island are sugar cane, coffee, and tobacco. About \$235,000,000 worth of commodities are exported every year, mainly to the continental United States. Of this figure, 42 percent, approximately, represent sugar exports. The hegemony of sugar is patent and upon it rests the economy of the Commonwealth. The bulk of the sugar cane crop is produced on the coastal plains, mostly close at sea level. Sugar cane (Saccharum officinarum) was introduced by the Spanish Conquistadores as early as 1515. During the last century the crop developed in importance as a local enterprise, mainly to supply the sugar needs of the island. With the incoming of American capital at the beginning of this century and with the opening of the United States market, sugar has steadily grown in importance to become the most decisive factor in the socioeconomic picture of Puerto Rico.

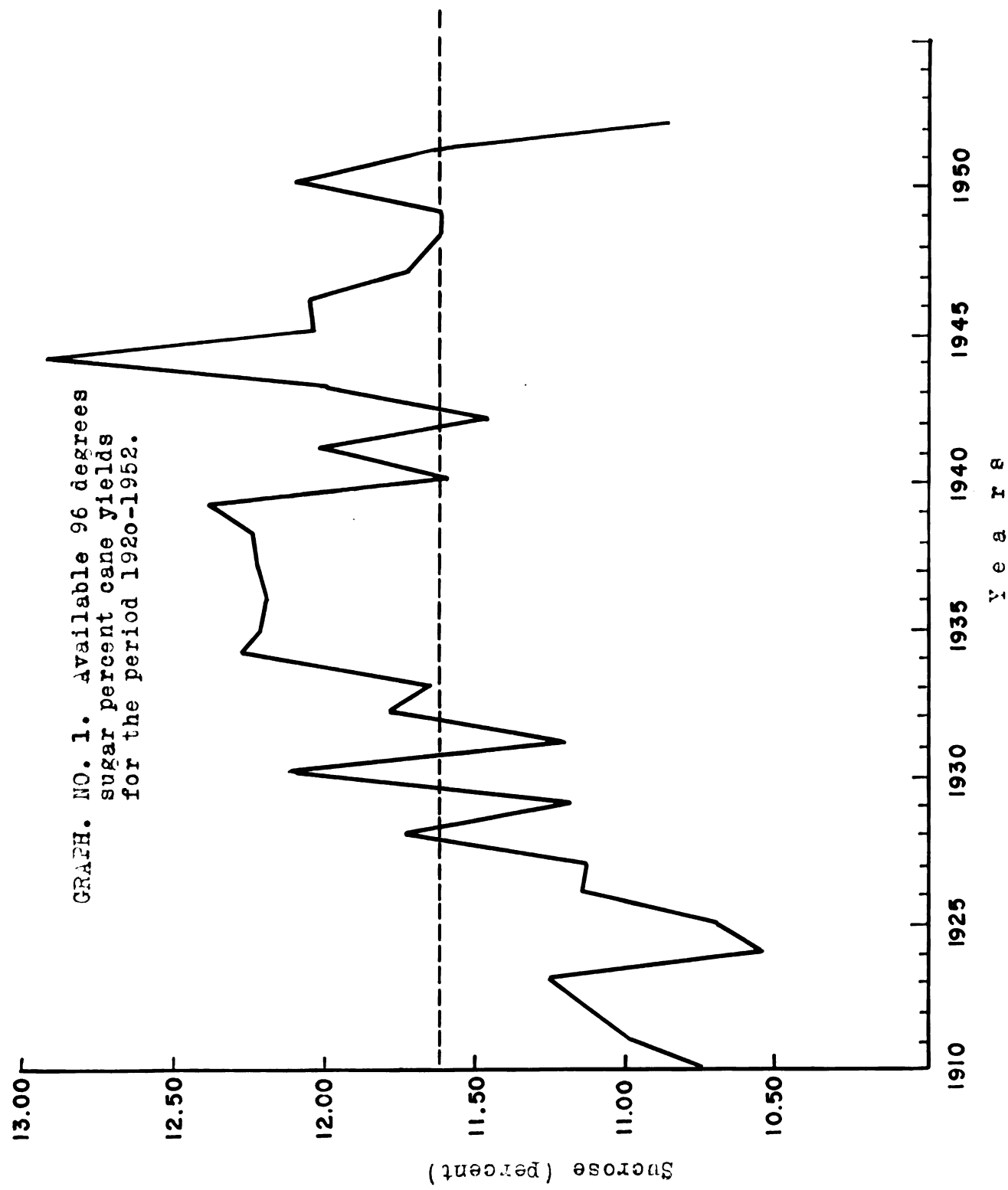
The agricultural phase of this industry has been the

object of different and varied studies on the part of the producers and the government. In sugar cane, as well as in beet production, the aim is a harvest of heavy tonnage with the highest possible concentration of sucrose. In a large measure, research studies have been dedicated to the improvement of varieties, of fertilizer practices, and of cultivation methods. Considerable progress has been made in obtaining large sugar cane crops; nevertheless, variations in sucrose content still elude practical control. (Graph No. 1.)

Very low percentages of sucrose in this sweet grass have been reported by the Eastern Sugar Associates in the eastern part of the island and by the Land Authority of Puerto Rico in the northern part. In these two areas large sums of money are expended every year in the hope of improving sucrose yields by cultural practices.

At various times consideration has been given to the possibility of modifying the metabolic processes of the sugar cane plant at a time approaching the harvesting season. There are starches and reducing sugars in the stalk as well as in the leaves of the cane. Through this modification in the metabolic processes it was hoped to convert these reserve materials, or at least part of them, into sucrose. As a consequence of these conversions an increase in the final sucrose concentration of the millable cane was contemplated.

GRAPH. NO. 1. Available 96 degrees
sugar percent cane yields
for the period 1920-1952.



REVIEW OF LITERATURE

For the past two decades many plant growth regulators or hormone-like substances have appeared on the market for agricultural use, mainly as weed killers. Among them 2,4D has been used most widely.

Beauchamp (1)* reported that, in Cuban plantations, increases in sucrose of 1.4 to 2.0 percent were obtained from dusting the cane foliage of varieties P.O.J. 2878 and Media Luna 3/18 with four to six ounces to the acre of the sodium salt of 2,4D. The increase in sucrose content was more marked during the first ten days following treatment, than at other dates. Promising results have been reported from some other areas (3).

However, Loustalot, et al., (4) at Mayaguez, obtained no increases in the sucrose content of variety M 336 by spraying the foliage with a 0.2 percent isopropyl ester of 2,4D. In their report the control plots produced about the same sucrose percentage yield at harvest time as the treated plots.

There have been reports that indicate that 2,4D applications may affect the sugar metabolism of other plants. Wort (13), from British Columbia, reported increases in the total sugar content of the stem of buckwheat after treatment with small non-lethal doses of the ammonium salt of 2,4D, (50, 100,

*Numbers in parenthesis denote literature cited.

and 1000 parts per million), within one day after application. Total sugars in the stem and leaves rose considerably within one day after application, but fell again by the second day.

Maleic hydrazide, one of the latest hormone-like substances to be described, has been found to have striking effects on plant growth, development, and flowering (7). Naylor and Davis (6) observed that corn plants sprayed with maleic hydrazide exuded droplets of sucrose and accumulated anthocyanin pigments. Quantitative sugar analyses indicated a thirteen-fold increase in sugar concentration. Ririe and Mikkelson (10) obtained evidence that maleic hydrazide influenced sugar beet growth and increased the sugar content. Foliar sprays containing 0.3 and 0.025 percent, made at the rate of 50 gallons per acre, were applied; and sucrose content was determined at three dates of harvest. An exudate appearing on barley after applications of maleic hydrazide was analyzed for sucrose by Currierr, et al. (2). Sucrose and starch were increased in cotton following applications of maleic hydrazide (5). However, Stout (11) and Peto, et al., (9) reported no significant increases in the sucrose content of sugar beets when treated with maleic hydrazide. Wittwer and Hansen (12) reported that sprays with 0.25 percent maleic hydrazide 30 to 40 days prior to harvest, or even as late as 3 or 5 days before harvest, considerably decreased the storage losses of sucrose in sugar beets.

THE RESEARCH PROBLEM

In view of the contradictory reports concerning the role of 2,4D on sugar yields and because of the interest shown by the sugar concerns in its use, work was undertaken to reinvestigate the role of 2,4D on this particular issue in controversy. Furthermore, specific attempts were made to determine the influence of MH30 (maleic hydrazide) as a possible inducer of changes in the sucrose content of the cane.

METHOD OF EXPERIMENTATION

Equipment and Materials. Standing sugar cane which had been harvested a number of years was sprayed with two hormone-like substances: (1) 2,4D, 97 percent sodium salt, (monohydrate dichlorophenoxyacetic acid), equivalent to 82 percent of the acid, commercial type; and (2) MH30, maleic hydrazide, (1,2-dihydropyridazine-3,6-dione), formulated as a water soluble diethalonamine salt, containing 30 percent of the active ingredient by weight as used. "Dreft" at a rate of 0.05 percent was used as a wetting agent.

Layout of the plots. The fields selected in each experiment were divided into six blocks of ten nearly square plots each, thus providing for a randomized block layout, with 10 treatments, replicated six times. Each plot had an area of approximately 0.01 acre. A row of cane was cut around each plot and two rows were left standing between every two plots to serve as buffer strips. Between each two plots there was a 20-foot separation. The row of cane that was cut around each plot provided a convenient pathway to facilitate spraying operations.

Spraying method. Spraying operations with a knapsack sprayer, 8001 nozzle, were started before 6 A.M. in each case, to avoid drafts. The required amount of chemical was dissolved in a gallon of water for each plot, which was enough to wet the

foliage thoroughly. With an extension attached to the hose, and with the nozzle tied to a bamboo pole about 12 feet long, it was possible to raise the nozzle sufficiently to spray the leaves thoroughly from the top.

Sampling and analysis of the juices. At harvest time convenient sugar cane scales were used in the field to obtain the weight data. Sampling was done for every plot and the milling of the cane samples was done at the experimental hydraulic mill of the Station. Juices were sampled and were analyzed by using a saccharimeter for total solids and polarization readings were taken with a polariscope. From that data, purity and available 96 degrees sugar percent cane¹ were calculated, this latter with the use of special conversion formulas and tables².

¹Polarizing 96 degrees, centrifugal sugar assumed that a factory should be able to obtain from canes of a given analysis.

²Capo, B. G., Table of 96° Available Sugar Yields Per Cent Juice. University of Puerto Rico, Agricultural Experiment Station, Rio Piedras, Puerto Rico, October, 1943, 48 numb. leaves.

DESCRIPTION OF THE EXPERIMENTS

The Rio Piedras No. 1 and the Humacao Experiments, 1950.

Two fields were selected, one at the Solis Farm of the Agricultural Experimental Station at Rio Piedras and one at Colonia Mandry of the Eastern Sugar Associates, near Central Pasto Viejo, Humacao. Both fields had been planted to P.O.J. 2878 and about 14 ratoon crops had been harvested. At Rio Piedras the soil had been classified as Vega Alta clay loam, a rather extensive, level sugar cane soil of the north coastal region. At Humacao the soil was originally a muck at a very low level which had been reclaimed from the sea and had undergone considerable mineralization.

The treatments were as follows: Check, 10, 20, and 30 pounds of 2,4D, acid basis, to the acre. The first set of plots was treated 30 days before harvesting; the second set 20 days prior to harvest; and the third set 10 days prior to harvest. Proper check plots were established at random within every set of plots.

Ten days after the treatment of the last set of plots all the cane from all sets of plots was harvested and weighed in the field. Random samples of 25 canes were obtained from every plot and were taken to the Station mill at Rio Piedras for analysis.

The Rio Piedras Experiment No. 2, 1951.

Another field was selected at the Station Farm. It had been planted to P.O.J. 2878 and several ratoon crops had been harvested. At this location again the soil was classified as a Vega Baja clay, an extensive, level, alluvial sugar cane soil of the north coastal region of Puerto Rico.

The treatments were as follows: 0, 2.5, 5.0, 7.5, 10.0, and 12.5 pounds to the acre, of 2,4D, acid basis. All plots were treated at one time.

Samples of ten canes were taken at random beginning the next day after the application of the 2,4D, and thereafter every day for 15 consecutive days, commencing on April 15, 1951. Later, samples were taken every five to seven days until all the cane was harvested at the end of 46 days.

Analyses of the samples were made the same day they were brought to the Station laboratory.

The Gurabo Experiment, 1952.

The field was selected at Colonia Rio Grande of the Eastern Sugar Associates, near Central Santa Juana between Cuguas and Gurabo. The field had been planted to P.O.J. 2878 and several ratoon crops had been harvested. The soil was classified as a Mabi clay occurring on long gentle slope of nearly level areas throughout the humid sections, in association with the Juncos and Mucara soils derived from tuffaceous materials.

The treatments were as follows: Control, 4 ounces, 8 ounces, one pound, and two pounds of 2,4D, acid basis, to the acre; 4 ounces, 8 ounces, one pound, five pounds, and twenty pounds of maleic hydrazide to the acre. The treatments were administered at one time.

Samples of ten canes were taken at random from every plot for four consecutive days after establishing the treatment differentials on Feb. 26, 1952. Thereafter, samples were taken at intervals of three to eleven days. The sampling intervals were more extended toward the end of the experiment. The last sampling was made on May 9, 1952, some 72 days after the treatments were given.

Samples were analyzed on the same day as they came into the Station laboratory.

ANALYSIS OF THE DATA

Statistical analysis was performed for all the four experiments which included a total of 216 plots. The analysis of variance was applied according to the following breakdown of variables in each experiment:

In the Rio Piedras Experiment No. 1 and in the Humacao Experiment analysis was worked out for such factors as tonnage, polarization, Brix, available 96° sugar percent cane yields, and purity on the 120 plots of approximately 0.01 acre each.

In the Rio Piedras Experiment No. 2 analysis was worked out for daily available 96° sugar percent cane yields for every one of the first 19 days of sampling, as given in Table No. 4, for 36 plots of approximately 0.01 acre each.

In the Gurabo Experiment analysis was applied to the available 96° sugar percent cane yields for every one of the 12 days of sampling, as given in Table No. 5, for 60 plots of approximately 0.01 acre each.

Example of Analysis of Variance.

An example of the analysis of variance of 60 plots of approximately 0.01 acre each is given in Table No. 1 as applied to yields in tonnage, available 96° sugar percent cane yields, Brix, purity, and polarization.

Table 1. Sample analysis of variance. Available 96° sugar percent cane yields in 60 plots of approximately 0.01 acre each. Rio Piedras Exp. No. 1, 1950.

Source	D F	S S	Var.	f
Total	59	19.80		
Treatments	9	1.51	.16	.53
Blocks	5	4.70	.94	3.13
Error	45	13.59	.30	

L.S.D. at 5 % - 2.80 for treatments

L.S.D. at 5 % - 4.44 for blocks

PRESENTATION OF THE DATA

Description of Results.

A resume of the experimental data obtained in the Rio Piedras Experiment No. 1, 1950, and in the Humacao Experiment, 1950, are given in Tables numbers 2 and 3, respectively. Mean values and range of values for Brix, polarization, purity, available 96° sugar percent cane yields, and tonnage for the ten treatments are presented. The minimum difference required for significance at the 5% level are also stated.

At both localities the results of the experiments followed about the same pattern.

In continuing the two previous experiments, the Rio Piedras Experiment No. 2, 1951, is described in terms of daily fluctuations in available 96° sugar percent cane yields of the P.O.J. 2878 cane, for six treatments, as is shown in Table No. 4. Deviations from the mean of the treatments are presented.

Table 2. Mean values for Brix, polarization, purity, available 96° sugar percent cane yields and tons of cane per acre of cane treated with three concentrations of 2,4-D at three days prior to harvest. (Rio Piedras experiment No. 1 - 1950)

Number of days prior to harvest	Pounds of 2,4-D per acre	Brix	Polarization	Purity	Available 96° sugar percent cane yields	Tons of cane per acre
10	10	20.2	73.4	85.6	12.3	28.02
10	20	19.7	71.7	85.9	12.1	28.68
10	30	19.9	72.2	85.6	12.2	30.26
20	10	20.0	72.4	84.3	12.3	25.87
20	20	19.7	71.2	87.8	12.0	28.58
20	30	19.8	71.8	85.4	12.1	24.24
30	10	20.1	72.8	85.4	12.3	29.66
30	20	19.9	71.8	85.9	12.0	25.58
30	30	19.7	71.4	85.6	12.0	27.83
	0	20.1	73.3	86.4	12.4	27.70
Range of values		19.7 to 20.2	71.2 - 73.4	84.3 - 87.8	12.0 - 12.4	24.24 - 30.26
Minimum required for significance 5% level		0.7	3.4	2.4	0.7	7.75

Table 3. Mean values for Brix, polarization, purity, available 96° sugar percent cane yields and tons of cane per acre of cane treated with three concentrations of 2,4-D at three dates prior to harvest. (Humacao experiment - 1950.)

Number of days prior to harvest	Pounds of 2,4-D per acre	Brix	Polarization	Purity	Available 96° sugar percent cane yields	Tons of cane per acre
10	10	19.6	74.9	89.6	12.7	38.71
10	20	19.7	74.3	87.1	11.9	43.63
10	30	20.1	74.2	87.7	12.3	45.06
20	10	20.4	75.6	88.0	12.6	43.23
20	20	19.4	70.8	86.7	11.8	39.58
20	30	19.3	72.6	87.1	12.4	41.23
30	10	19.9	72.2	86.4	12.0	44.35
30	20	18.9	73.2	86.4	12.1	44.93
30	30	19.8	72.3	86.6	12.2	41.40
	0	20.6	75.6	86.9	12.5	43.06
Range of values		18.9 - 20.6	70.8 - 75.6	86.4 - 89.6	11.9 - 12.7	38.71 - 45.06
Minimum required for significance 5% level		1.8	6.8	4.3	1.0	10.38

Table 4. Daily fluctuations in available 96° sugar percent cane yields of P.O.J. 2878 cane sprayed with 2,4-D prior to harvesting (Rio Piedras No. 2, 1951.)

		Available 96° sugar										:
		Pounds of 2,4-D per acre										:
		0	2.5	5.0	7.5	10.0	12.5					Mean
		:	:	:	:	:	:	:	:	:	:	:
<u>Number of days</u>		<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>
1	:	14.2	14.9	14.2	13.6	14.9	13.9	:	14.3			
2	:	14.2	14.2	14.8	13.9	14.2	14.2	:	14.3			
3	:	14.2	13.8	14.3	14.2	14.3	13.5	:	14.1			
4	:	13.7	13.8	14.0	13.9	13.9	14.1	:	13.9			
5	:	14.1	14.1	14.1	14.3	14.3	13.4	:	14.1			
6	:	14.2	14.3	13.8	14.1	14.3	14.3	:	14.2			
7	:	14.2	14.7	14.6	13.7	14.1	14.3	:	14.3			
8	:	13.9	14.1	13.7	14.0	13.7	14.0	:	13.9			
9	:	14.2	13.9	13.9	14.4	14.1	14.3	:	14.1			
10	:	13.7	13.9	14.4	14.3	14.0	13.5	:	14.0			
11	:	14.9	13.9	13.9	14.7	14.3	14.5	:	14.4			
12	:	14.4	14.7	14.5	14.0	14.4	14.7	:	14.4			

Table 4. Continued

Days of harvesting after treatment	:	Available 96° sugar								:	Mean
		Pounds of 2,4-D per acre									
		0	2.5	5.0	7.5	10.0	12.5	:			
Number of days	:	Percent	Percent	Percent	Percent	Percent	Percent	Percent	:	Percent	
13	:	14.4	14.1	13.8	13.4	14.4	14.0	:	14.0		
14	:	14.4	—	13.7	—	13.9	14.3	:	14.0		
15	:	14.3	14.8	14.4	13.3	13.8	14.4	:	14.2		
20	:	14.7	13.9	13.4	14.1	13.5	13.4	:	13.8		
25	:	14.4	—	—	—	13.5	12.9	:	13.6		
31	:	13.5	12.6	—	—	12.2	13.2	:	12.9		
38	:	13.3	—	—	—	12.1	12.4	:	12.6		
42	:	12.8	—	—	—	12.9	13.2	:	12.9		
46	:	12.8	—	—	—	11.5	12.1	:	12.1		
<hr/>											
Means of treatments	:	14.0	14.1	14.0	14.0	13.7	13.7	:	13.9		
Deviation of treatment	:	-1.2 to +0.9	-1.5 to +0.8	-0.6 to +0.8	-0.7 to +0.7	-2.2 to 0.7	-1.6 to 1.0	:			

The Gurabo experiment constituted a refinement in dosages of those applied in the previous experiments in relation to 2,4D and in addition it included maleic hydrazide treatments. Table No. 5 shows the yields of available 96 degrees sugar percent cane yields at various intervals for P.O.J. 2878 treated with 2,4D and maleic hydrazide. Also the least significant difference needed for comparison at the 5 percent and 1 percent levels are given at the foot of the table and in relative correspondence to the means of treatments for each day of sampling. The mean results of the combined maleic hydrazide and 2,4D treatments, as well as of controls, are shown graphically in Graph No. 2.

GRAPH NO. 2.- The daily variations of the sucrose content of sugarcane receiving preharvest foliage sprays of 2,4D and maleic gydrazide as compared to that of cane from control plots.

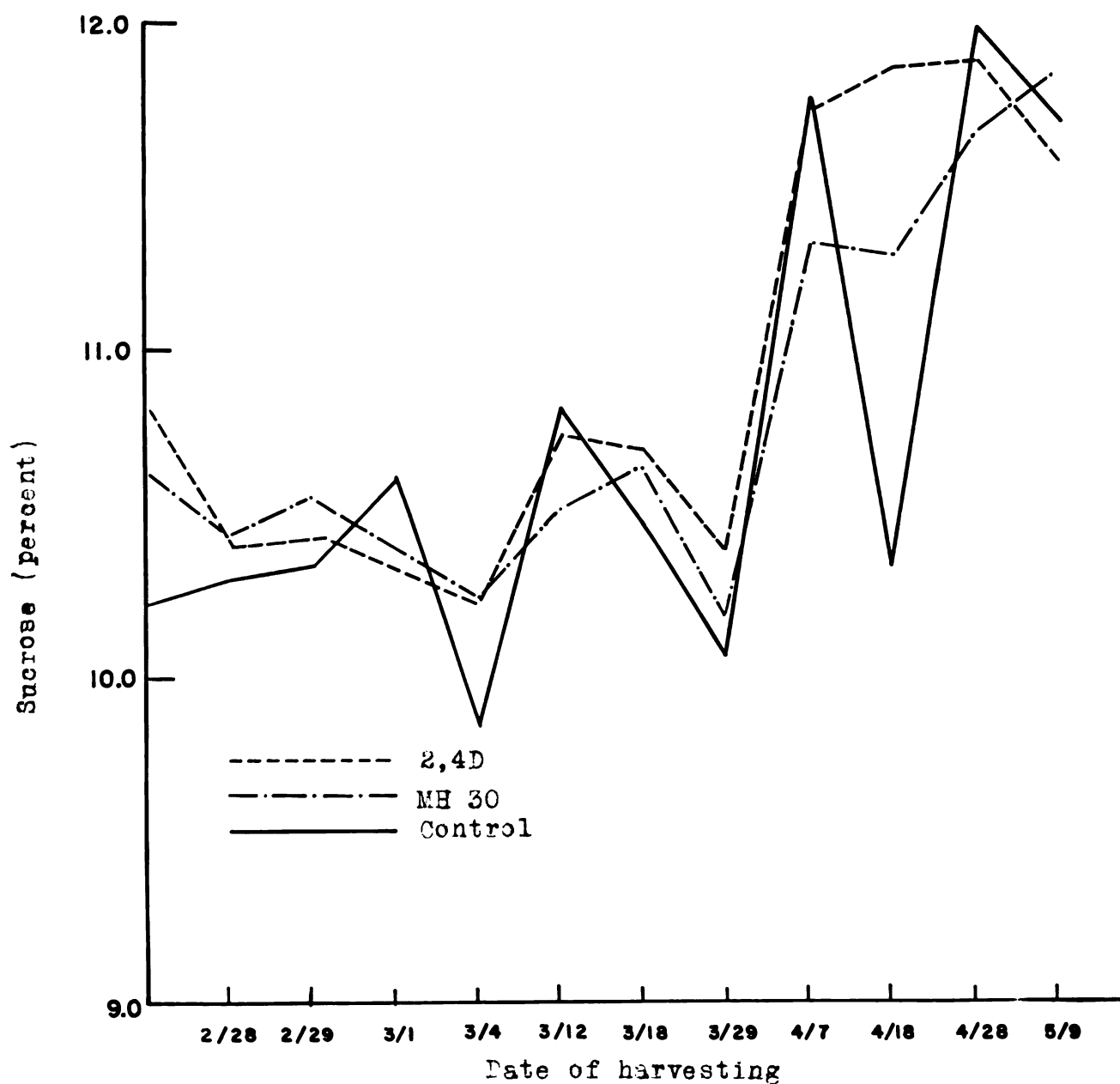


Table 5. The sugar percent cane yields of P.O.J. 2878 at various cutting intervals as influenced by applications of 2,4-D and maleic hydrazide. (Colonia Rio Grande, Gurabo, 1952)

Number	Treatment		Sugar percent cane yields at various dates of cutting				
	Lbs.	Hormone	2/27	2/28	2/29	3/1	3/4
1	Check		10.23	10.30	10.34	10.61	9.85
2	4 oz.	MH ^{1/} 30	11.20	10.38	10.19	9.99	10.19
3	8 oz.	MH 30	10.76	10.39	10.55	10.24	9.79
4	1 lb.	MH 30	10.33	10.50	10.44	10.70	10.55
5	5 lbs.	MH 30	10.61	10.46	10.65	10.46	10.60
6	20 lbs.	MH 30	10.56	10.59	11.13	10.42	10.52
7	4 oz.	2,4-D	10.64	10.53	10.39	10.41	10.17
8	8 oz.	2,4-D	10.73	9.75	10.36	9.88	10.09
9	1 lb.	2,4-D	10.78	10.64	10.61	10.20	10.23
10	2 lbs.	2,4-D	11.10	10.73	10.37	10.81	10.43
Mean of all treatments			10.70	10.43	10.50	10.39	10.24

Least significant difference needed for comparison at the:

5-percent level 0.87 : 0.94 : 0.95 : 0.95 : 0.97 :

1-percent level 1.17 : 1.25 : 1.27 : 1.26 : 1.30 :

^{1/} Maleic hydrazide

Table 5. Continued

3/12	3/18	3/27	4/7	4/17	4/28	5/9	Mean
10.82	10.47	10.05	11.76	10.33	11.98	11.7.	10.70
10.58	10.80	10.34	11.52	11.70	11.86	12.20	10.91
9.99	11.03	9.97	11.51	11.25	10.84	11.87	10.68
10.55	11.31	10.29	11.15	11.55	11.56	12.05	10.92
10.43	9.79	10.32	11.08	11.38	11.66	11.66	10.76
10.74	10.51	10.19	10.97	11.61	12.15	11.73	10.92
10.32	10.87	10.44	11.59	11.63	11.45	11.45	10.82
10.96	10.70	10.34	11.83	11.73	11.91	11.08	10.78
10.88	10.39	10.28	11.58	12.04	11.90	11.62	10.93
10.81	10.85	10.48	11.90	12.09	12.31	12.16	11.17
10.60	10.67	10.27	11.49	11.53	11.76	11.75	10.86

0.82 : 0.92 : 1.13 : 0.88 : 1.15 : 1.25 : 0.49

1.09 : 1.23 : 1.51 : 1.18 : 1.53 : 1.67 : 0.66

DISCUSSION

In discussing the results of these experiments it should be noted that findings of the experiments were mainly based on chemical analysis of the juices sampled.

For the first two experiments, Rio Piedras and Humacao, in general, the difference between the means for Brix, polarization, purity, available 96 degrees sugar percent cane yields, and tons of cane to the acre are not significant. This preliminary attempt to alter the metabolism of the sugar cane plant at harvest time was not successful as evidenced by the data presented in Tables No. 1 and No. 2. It was felt that perhaps applications in large dosages of 2,4D did not affect the metabolic activity of the cane plant as did smaller doses used by Beauchamp (1). However, other factors may be of importance, such as the physical properties of the chemical used. In the literature available there is no mention to anything concerning the pH, wettability, rate of absorption by the plant, and other properties of the chemical used, which may have a decisive importance in the final results obtained.

Additional work was undertaken to determine daily fluctuations in available 96 degrees percent cane yields after applications of 2,4D with smaller dosages. After tracing the daily fluctuations for sucrose for 15 consecutive days, and thereafter every five to seven days for more than one and a half months, no significant differences were observed between the

means of the various treatments.

Further refinements in the dosages with 2,4D were tried in the Gurabo experiment. There were no significant differences between the means of the treatments. The same holds true for the means of the treatments using maleic hydrazide. The yields from the plots receiving a 0.20 percent solution of 2,4D were quite consistently higher throughout the harvesting period than those from the check plots, but the differences were not significant even at the 5 percent level. There were some definite high and low sucrose levels during the harvesting season, but through the season as a whole the canes from the treated plots were more uniform in the sucrose content than those of the check plots. This trend is shown in Graph No. 2.

A general inspection of the data indicates that the daily variations in the sucrose content throughout the harvesting season were attributable rather to weather, or other conditions, than to treatments. In conclusion, the evidence of the experimental data, in Puerto Rico, to this date, is rather discouraging for the use of plant growth regulators, such as 2,4D and maleic hydrazide, in an effort to affect the composition of the green leaf-millable stalks of the cane at harvesting.

SUMMARY

Data are presented here on the effect of applications of 10, 20, and 30 pounds to the acre of the sodium salt of 2,4D, acid basis, at three intervals, 10, 20, and 30 days, prior to harvest. Field experiments were conducted at Rio Piedras and Humacao following a randomized block layout. Each treatment was replicated six times. The mean available 96 degrees sugar percent cane yields was 12.2 at both locations with very small deviations above or below it. No significant differences were observed between the mean Brix, polarization, purity, available 96 degrees sugar percent cane yields, and tons of cane to the acre at either location.

Data are presented from further experiments on the effect of the application of smaller dosages of 2,4D and maleic hydrazide to the sugar cane plant at varying intervals prior to harvest time. Field experiments were conducted again in Rio Piedras in northern Puerto Rico and at Colonia Rio Grande, between Caguas and Gurabo, in east-central Puerto Rico. Daily fluctuations in Brix, polarization, purity, and available 96 degrees sugar percent cane yields were followed for 46 and 72 day periods, respectively. No significant differences were observed between the mean available 96 degrees sugar percent cane yields that could be ascribed to treatments. Seasonal variations attributable to weather conditions may have been rather important factors at both locations.

LITERATURE CITED

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