vitamin B_6 deficiency, such as acrodynia and convulsions.

The increased requirement for vitamin B_6 by the rat and monkey fed highly processed army combat ration (*13, 19*), and by the rat fed heat-sterilized milk, as herein reported, suggests that this effect of food processing is of a general nature. Fortification of heat-treated food may be deemed advisable for the proper nutrition of infants, combat men, and other humans required to live for extended periods on diets comprised solely of processed food.

Acknowledgment

The authors are indebted to G. T. Durbin for the microbiological assays and to Elizabeth Linden and Glen Kasparek for assistance in the animal room.

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NUTRITIONAL VALUES OF CROPS

Amino Acid Content of West Indies Sugar Cane

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Although the nitrogen content of sugar cane has an important bearing not only on plant nutrition but also on the sugar manufacturing process, little information on this subject is available. These studies are an attempt to remedy this situation. By chromatographic techniques the presence of 11 amino acids has been demonstrated in sugar cane juice. Their distribution in the cane stalk and their quantitative variation with age of the plant and climatic conditions have been examined. Different varieties of cane have essentially the same amino acid picture, although there are quantitative differences. Cane leaves contain the same amino acids as the juice, but have the majority of their nitrogen in protein form. Although under normal conditions the amino acid content of cane juice decreases with age of cane, under drought conditions the amino acid content increases spectacularly. The high amino acid level of drought cane may be related to the difficulties of processing such material in a sugar factory. Cane juices of abnormally high amino acid content are characterized by the formation, on lime-heat clarification, of very small sized flocs which settle extremely slowly and are extremely difficult to deal with in the factory.

IN SPITE of the fundamental importance of amino acids for the growth, behavior, and properties of plants, little information regarding the amino acid status of sugar cane is available. The authors have undertaken to examine the amino acid relationships of typical West Indies sugar cane varieties during growth.

A preliminary report by Pratt and Wiggins (*4*) described the separation of aspartic, glutamic, and γ-aminobutyric acids, glycine, alanine, asparagine, glutamine, lysine, serine, leucine, and valine from the juice of sugar cane variety B.34104 (Barbados Cane Breeding Station selection No. 104 of 1934), using the two-dimensional paper chromatogram technique of Consden, Gordon, and Martin (*2*). Subsequently (*6*) it was found that single-dimensional "strip" chromatograms could be made to give adequate separation of all the amino acids occurring in significant amount, and to show up the presence of the isomeric leucines.

Further studies (*7*) indicated that

qualitatively the amino acid composition of West Indies sugar cane varied but little from one variety to another. The observations recorded in this paper have shown that, on the other hand, large quantitative differences may be encountered, not only between varieties, but with a single variety during growth, and even from one portion to another of a single growing cane.

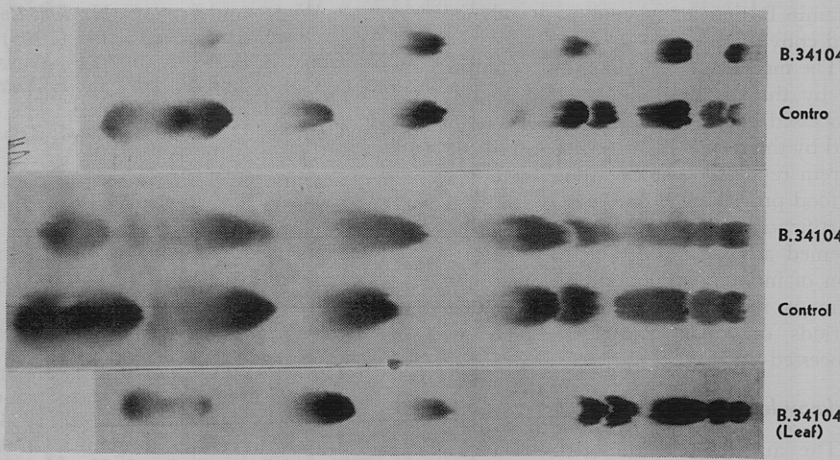


Figure 3. Chromatograms of amino acid concentration

A. Cane variety B.34104, age 5 months
B. Cane variety B.34104, age 7 months
C. Leaves of cane variety B.34104
Amino acids present in control mixture as for Figure 2

Material and Sampling

Except where otherwise stated, all cane was grown from seed pieces on the farm land of the Imperial College of Tropical Agriculture, Trinidad, under supervision of the Agricultural Department, and received artificial fertilizer (standard N, P, K treatment) 3 weeks after planting. Each sample consisted of six canes randomly selected from the appropriate plot (excluding edges) and cut off at ground level and at the emergence of the lowest leaf blade. The canes so cut were immediately milled in the laboratory sugar mill, and the juice was collected, filtered through cotton wool, and clarified either with neutral lead acetate or with a mixture of bentonite, activated charcoal, and kaolin. (This latter mixture was found to be an efficient agent for clarifying diluted cane juice, and allowed the determination of sucrose, glucose, fructose, inorganic phosphate, and amino acids in the same sample. The use of lead, on the other hand, interfered badly with the determination of phosphate and of amino acids by means of ninhydrin.)

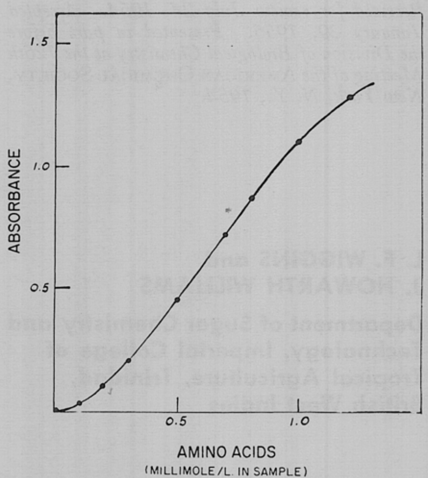


Figure 1. Reference curve for determination of amino acids with ninhydrin in presence of acetate buffer

Each point represents mean value of 3 determinations made with standard solutions of glutamic acid, asparagine, and alanine

Analytical Procedures

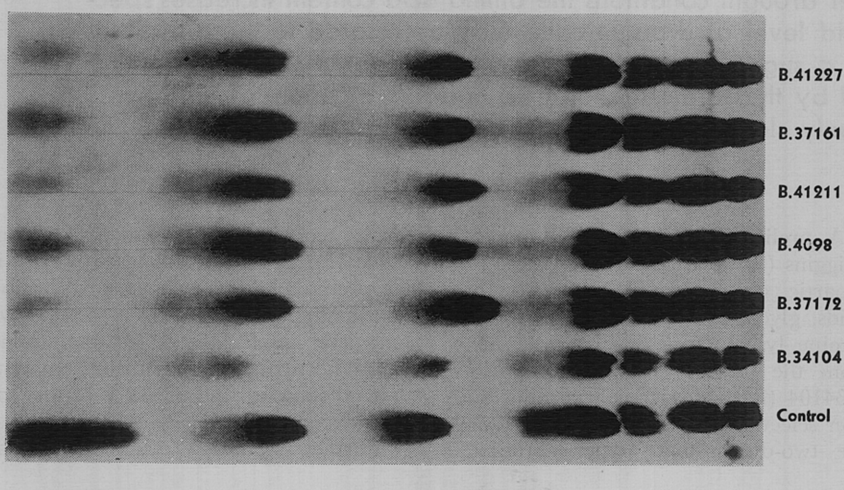
Sucrose was determined polarimetrically in the clarified and diluted juice samples.

Reducing sugars were determined by the modified Luff-Schoorl procedure (*1*).

Amino acids (total) were determined directly in the clarified and diluted juice either by the copper phosphate method of Woiwod (*8*) or by the colorimetric method with ninhydrin as proposed by Moore and Stein (*3*). It was found that, if the reducing agents incorporated in the ninhydrin solution by these authors were omitted, the interference from ammonia or ammonium salts was reduced to negligible proportions; the relationship between amino acid concentration and amount of colored reaction product found, however, was then far from linear, as observed by Moore and Stein (*3*), and a reference curve had to be constructed using standard amino acid solutions. In view of the modifications introduced, the method employed in the determination of amino acids is given here in detail.

Figure 2. Chromatogram of amino acid concentrations from six varieties of West Indies sugar cane at maturity

Amino acids present in control mixture are, in descending order, lysine, asparagine, aspartic acid, glutamic acid, alanine, aminobutyric acid, valine, isoleucine, leucine, and norleucine



Determination of Total Amino Acids in Sugar Cane Juice

Reagents **Ninhydrin.** Ninhydrin, 1.0 gram dissolved in 100 ml. of isopropyl alcohol. The solution, stored in a brown glass bottle, was stable for several months.

Acetate Buffer. Sodium hydroxide, 40 grams, and glacial acetic acid, 100 ml., dissolved separately in water, were mixed and diluted to 1 liter.

Aqueous Isopropyl Alcohol. Equal volumes of isopropyl alcohol and distilled water.

Clarifying Mixture. Bentonite clay, 15 grams, heavy kaolin, 20 grams, and acid-washed activated charcoal, 50 grams, suspended in 1 liter of distilled water.

Procedure The solution for analysis was prepared by mixing 20.0 ml. of juice, with 5 ml. of clarifying solution, and diluting to 200 ml. in a graduated flask. Filtration through Whatman No. 1 filter paper gave a colorless filtrate representing a tenfold diluted juice.

Table I. Analysis of Juice of West Indies Sugar Cane

(2-foot portion from upper half of stem)

Variety	Sucrose, G./100 Ml.	Amino Acids, Mmoles/L.
B.726	14.0	6.8
B.34104	13.2	10.2
BH.10(12)	13.6	4.0
B.2935	14.5	12.5
B.35187	13.3	7.0
B.3439	13.9	10.2
B.34391	14.0	9.5
B.37172	13.4	0.9
B.37161	13.0	3.8
B.4098	14.3	3.4
B.41227	15.3	3.9
B.41211	12.9	5.0

The filtrate, 10.0 ml., ninhydrin solution, 2.5 ml., and acetate buffer, 2.5 ml., were pipetted into a 50-ml. graduated flask, which was then suspended in a bath of briskly boiling water for 30 minutes (up to eight determinations were made simultaneously). At the end of the heating period the flask was removed and cooled to room temperature and the contents were made up to 50 ml. with aqueous isopropanol, and well mixed by shaking. The absorbance of the solution was measured in a Spekker photoelectric colorimeter, using Ilford yellow filters No. 606 and a 1-cm. cell.

The concentration of amino acids in the solution for analysis was read directly from the curve (Figure 1), constructed by using standard amino acid solutions in the above procedure. The concentration in the original juice was obtained by multiplying this figure by the dilution factor 10. In the case of juices with high amino acid content (> 12 millimolar), a further dilution of filtrate after clarification was made, and the appropriate dilution factor employed.

For chromatography, the amino acids were isolated by absorption on cation exchange columns (Amberlite IR 120 or Zeocarb 225 resins), followed by elution with 2*N* ammonia. After concentration under reduced pressure, the amino acid concentrate was chromatographed on Whatman No. 1 paper using, in the early work, a phenol-water-ammonia solvent, and in later work the butanol-water-formic acid solvent described by the authors (*6*). Glutamine is probably converted into glutamic acid during the ion exchange treatment, since although Pratt and Wiggins (*4*) demonstrated its presence in raw cane juice, it is not detected in juice treated by ion exchange.

Results and Discussion

Qualitative Examination

Following the work of Pratt and Wiggins (*4*), in which the identity of the amino acids found in West Indies sugar cane juice was established by two-dimensional paper chromatography, 12 varieties of cane commonly grown in the West Indies were shipped by air from Barbados to the University of Birmingham, England, where this work was started and the juice analyzed qualitatively by single-dimensional (descending) chromatography. It was at once apparent that the same major constituents were present in all the juices; and although the resolution effected was not very good, it was sufficient to show asparagine as a very minor constituent.

In view of the possibility of deterioration and loss during transit of the cane from Barbados to England, a similar comparison was made in Trinidad with six varieties of mature cane grown there for experimental purposes. The freshly milled juice was analyzed as previously described, using the new solvent mixture reported by the authors (*6*). Again there was a great similarity in the amino acid pictures obtained from all six varieties. A photograph of the chromatogram is reproduced in Figure 2.

Table II. Relative Amounts of Amino Acids in Juice of Cane B.41211

Amino Acids	Concn. in Juice, Millimolar	% of Total Amino Acids
Aspartic acid	1.36	30.2
Glutamic acid	1.20	22.1
Asparagine and glycine	...	...
Alanine	1.65	25.0
Valine, γ-aminobutyric acid, and lysine	0.25	4.6
Leucines	0.98	18.1
Total	5.44	100

An interesting comparison is afforded by Figure 3, which shows the amino acid picture in the juice of one of these varieties (B.34104) when only 5 months old (Figure 3, *A*), and when 7 months old (Figure 3, *B*). Asparagine and valine, both absent at 5 months in this variety, have appeared strongly at 7 months, and leucine has increased, while at maturity (Figure 2) asparagine is still more pronounced, valine is less so, and leucine has almost disappeared. In the leaves of this variety at maturity, however, all these amino acids were present (Figure 3, *C*).

Quantitative Examinations

The first quantitative examination of cane juice for amino acids was made in England on cane flown from Barbados. The upper portions of canes of 12 varieties were received and at once pressed out and the juice was analyzed as described above, using the Woiwod method for amino acid determinations. The results are shown in Table I.

The amino acids were isolated and chromatographed, and showed in all cases a very similar picture [chromatographic separation by means of phenol-water-ammonia as described by Pratt and Wiggins (*4*) was used]. The amino acids from the B.41211 juice, after chromatographic separation, were eluted from the paper and separately estimated. Table II shows the proportions of the main components of the amino acid content of this juice.

A similar analysis performed in Trinidad with a fresh sample of more mature cane (18 months) of variety B.37161 revealed a different distribution of amino acid constituents (Table III). Aspartic and glutamic acids have notably decreased in proportion, leucine has practically disappeared, while asparagine has accumulated to a large extent. It has been observed on more than one occasion, during the examination of fresh cane juice in Trinidad, that with increasing maturity, asparagine tends to accumulate and leucine to disappear from the juice. The appearance of asparagine between the ages of 5 and 7 months in the case of variety B.34104 has been mentioned in connection with Figure 3, *A* and *B*.

The distribution of total amino acids along the stem of a growing cane was studied in variety B.37161. The canes were cut as previously described, and chopped into sections, the uppermost section, between the last emergent leaf blade and the first node of the stem, being designated section 0. The juice was expressed from each section and analyzed by the Woiwod method for amino acids. The results are expressed in Table IV.

In all cases, the amino acids are present in greater concentration in the top and bottom portions of the cane than in the middle; it is evident that there was a steady decrease in over-all amino acid concentration during the period of growth studied.

This latter relationship is, however, by no means always observed, and indeed under certain circumstances, is completely reversed. This was first ob-

Table III. Relative Amounts of Amino Acids in Juice of Ripe Cane B.37161

Amino Acids	Concn. in Juice, Millimolar	% of Total Amino Acids
Aspartic acid	0.16	14.8
Glutamic acid	0.06	5.3
Asparagine (mainly) and glycine	0.47	44.6
Alanine	0.29	27.7
Valine, γ-aminobutyric acid, and lysine	0.08	7.6
Leucines	Trace	...
Total	1.06	100

Table IV. Concentration of Amino Acids in Juice from Successive Portions of Stem of Cane B.37161 during Growth

Age, Months	Section	Internodes	Amino Acids in Juice, Mmoles/L.	Av. Amino Acid Concn., Millimolar
9	0 (top)	0	2.10	2.32
	1	1–4	2.04	
	2	5–8	1.92	
	3	9–12	1.58	
	4	13, 14	2.97	
10	0 (top)	0	...	1.92
	1	1–4	2.08	
	2	5–8	1.96	
	3	9–12	1.46	
	4	13–16	2.20	
11	0 (top)	0	2.60	1.79
	1	1–3	1.52	
	2	4–6	1.40	
	3	7–9	1.40	
	4	10–12	1.65	
	5	13–15	1.60	
	6	16–18	2.40	
13	0 (top)	0	1.50	1.13
	1	1–5	0.98	
	2	6–10	0.94	
	3	11–15	0.86	
	4	16–20	1.38	

Behavior of Cane Grown Under Artificial Drought

In order to test the suggestion that the high amino acid concentration encountered in the previous experiments was due to drought, two varieties of sugar cane having fairly different growth behavior were grown in a large greenhouse covering about 0.25 acre, with controlled irrigation. When the plants were sufficiently advanced, analyses were begun and water was cut off. When the plants showed signs of die-back in the leaves, irrigation was recommenced and the analyses were continued.

The results of these analyses are set out in Table VII. The cane was planted in mid-May 1953, and the water supply cut off on December 15, 1953. Because the water stress was controlled, the initial values for the amino acid content of the juice were higher than those normally encountered in field cane.

There can be no doubt that the effect

Table V. Analysis of Juice of Canes B.34104 and B.37161 Planted September 1951

Age of Canes, Months	Sucrose, G./100 Ml.		Reducing Sugars, G./100 Ml.		Amino Acids, Mmoles/L.	
	B.34104	B.37161	B.34104	B.37161	B.34104	B.37161
3.5	1.90	0.81	2.77	1.09	...	...
5	1.98	2.45	3.24	2.91	2.0	3.0
6	3.50	5.50	3.75	2.80	14.0	8.0
7	3.68	10.15	2.46	1.66	25.4	16.0
8	11.50	10.03	2.39	2.25	20.5	22.0
9	14.45	14.90	2.07	1.79	1.5	1.0
10	14.48	14.36	1.91	1.76	0.75	0.65
11.5	5.70	7.49	2.60	2.51	0.60	0.60
14	11.25	10.67	2.95	1.82	1.29	0.95

served in experiments made during 1951–52 with plots of cane of varieties B.37161 and B.34104 planted in September 1951. Analyses at intervals of approximately one month yielded the highly interesting figures given in Table V.

There is a spectacular rise in amino acid concentration in both varieties, followed by an equally spectacular drop at 8 to 9 months. This drop corresponded closely with the onset of heavy rains, and it was suspected that the preceding rise in amino acid concentration might be due to the effect of a particularly severe drought which was encountered during the early period of growth. The total rainfall in the area was 2.49 inches during the whole period of growth from 3.5 to 8 months, whereas during the period of growth from 8 to 9 months about the same amount of rain fell, and thereafter the rainfall was heavy.

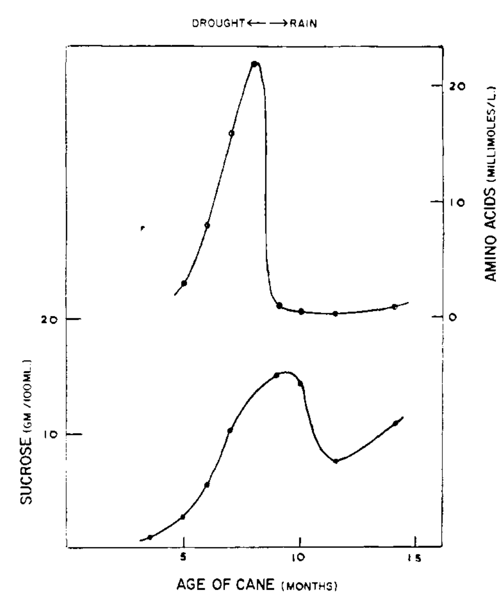
This suspicion has been confirmed by the results of artificial drought experiments described later in this paper. A point of further interest is that the variations in sucrose content of the juice follow those in the amino acid content, but at an interval of about 2 months. In the experiments recorded here the drop in sucrose content was considerable, but it should be remembered that the data refer to immature cane, and that the drought effect began to be observed at an early stage of growth; under more normal conditions of planting the effect would probably be less marked.

The relationship between sucrose and amino acid content in the case of variety B.37161 is shown in Figure 4; the results for variety B.34104 show a similar relationship.

An unexpectedly high amino acid content was found, during the same dry season, for the bulk juice obtained from the six varieties of cane mentioned in connection with Figure 2. The cane (21 months) was milled in the Experimental Sugar Factory of the Imperial College of Tropical Agriculture during March 1952. Amino acid concentrations determined by the ninhydrin method, corrected for the imbibition water added during milling, are shown in Table VI. A comparison of these figures with, for instance, those of Tables II and IV shows that the concentrations of amino acids in all six varieties were remarkably high for mature cane.

Figure 4. Effect of continuing drought followed by rain on amino acid and sucrose content of juice of sugar cane

Variety B.37161
Upper Curve. Sucrose
Lower Curve. Amino acids



of drought is clearly shown in the amino acid levels of the canes of both varieties. As with the field canes affected by natural drought, there is a very sharp rise in amino acid content, up to the remarkable level of more than 30 millimolar, accompanied by a more gradual rise in sucrose content and a fall in reducing sugar content. From the onset of irrigation, this process is reversed, although the response of sucrose to irrigation is once again seen to be slower than that of the amino acids (cf. Table V). It is clear from the figures for reducing sugar content of the juices that the effect is not simply due to loss of water from the plant by transpiration with concomitant increase in the concentration of soluble constituents, and indeed it seems impossible that variations of the magnitude recorded could be due to this factor alone. It seems more probable that, below a particular water stress, the normal path for the conversion into protein of amino acids synthesized by the plant is blocked, and the amino acids accumulate until the protein-building mechanism is once more freed by increasing availability of water.

The magnitude of this drought effect is clearly seen by reference to Figure 5. Here the amino acid content of the juice from one of the greenhouse canes is compared with that from cane grown concurrently in the open; the figures for the field cane are averages from seven plots of cane variety B.37172 which had different levels of nitrogen, phosphorus, and potassium fertilizer.

Considerable importance is attached to the observation of this drought effect, as sugar cane that has been subject to prolonged natural drought yields juice which is very difficult to clarify in the factory; simple lime-heat treatment of such juices leads to the formation of very small flocs with poor settling characteristics (*5*). The juice from the canes grown under artificial drought did in fact show these symptoms increasingly as the drought effect continued, and it is believed that the accumulation of amino acids may be directly concerned with the physicochemical changes responsible for such behavior. An account of investigations into this aspect of the matter will be published elsewhere.

Table VI. Amino Acid Content of Bulk Samples of Cane Juice Milled from Mature Cane in March 1952

Variety	*Amino Acids, Mmoles/L.*
B.41.227	11.4
B.37.161	9.2
B.41.211	10.6
B.4098	12.6
B.37.172	8.8
B.34.104	11.3

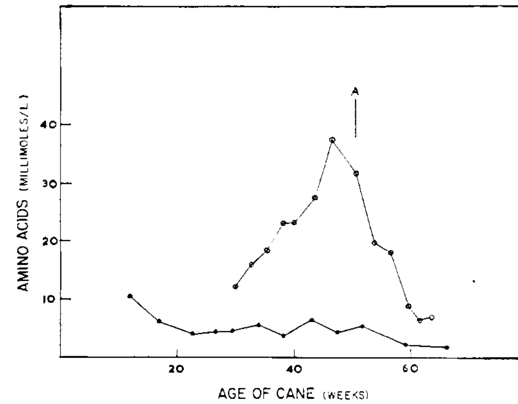


Figure 5. Variation in amino acid content of cane

○ Subject to artificially induced drought
● Grown under natural conditions
A indicates commencement of irrigation of drought cane. Each point on lower curve represents mean of seven analyses, each relating to a different plot of same cane, variety B.37172

Conclusions

From the data presented, it is concluded that different varieties of sugar cane grown in the British West Indies exhibit great similarity qualitatively in the amino acid composition of their juice.

It is not possible to say that the quantitative differences found from one variety to another are true varietal differences, as the amino acid content of the juice is profoundly affected by environmental factors such as water availability.

Low water availability leads to a marked rise in amino acid content of the juice of growing cane; increased water availability results in rapid decrease in amino acid content.

The response of sucrose content to changes in water availability is similar to that of amino acid content, but less pronounced and less rapid.

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Table VII. Effect of Applied Drought and Irrigation on Amino Acid Content of Sugar Cane Juice

Variety B.37161				*Variety POJ.2878*		
Sucrose, %	*R. S. %*	*Amino acids, mmoles/l.*	*Date of Analysis*	*Sucrose, %*	*R. S., %*	*Amino acids, mmoles/l.*
			Drought			
10.1	1.84	13.6	Dec. 3, 1953	6.4	2.99	12.0
10.9	1.55	26.0	Dec. 21, 1953	9.4	2.81	15.9
12.8	1.38	17.8	Jan. 7, 1954	6.7	2.96	18.3
16.4	1.05	18.0	Jan. 26, 1954	9.5	2.61	23.0
15.1	1.18	32.5	Feb. 10, 1954	11.5	2.30	23.0
...	0.43	...	Mar. 4, 1954	15.2	1.88	27.5
19.0	0.76	32.5	Mar. 24, 1954	15.7	2.50	37.5
19.3	0.50	23.5	Apr. 23, 1954	16.5	4.02	31.5
			Irrigation			
...	...	...	May 12, 1954	17.0	17.2	19.5
18.3	0.85	22.0	May 20, 1954	...	...	...
...	...	...	May 31, 1954	17.3	1.69	17.8
15.3	1.11	14.0	June 21, 1954	15.5	0.56	8.5
14.9	0.41	8.1	July 5, 1954	14.2	0.39	6.3
10.9	0.64	7.0	July 19, 1954	8.9	0.88	6.9