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The Chemical Composition of Atlas and Dwarf Yellow Milo Plants in Relation to Chinch Bug Resistance

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PREFACE

The importance of sorghum as a grain and forage crop has been known for many years and much study has been devoted to developing new varieties and improving old ones. One of the problems confronting the breeder has been the development of varieties that are, at least relatively, resistant to chinch bug injury. A variety that might otherwise be quite acceptable would prove of little value in many parts of Oklahoma if it were quite susceptible to chinch bug injury. A knowledge of the factor or factors that cause certain varieties to be very resistant to chinch bug injury would prove of value to sorghum breeders and aid in the quicker development of better and more resistant varieties. Research workers, recognizing the need for resistant varieties, have pooled their efforts and are cooperating in a broad program of research seeking the development of better and more resistant varieties.

In Oklahoma, this problem is being studied jointly by the Departments of Entomology and Agricultural Chemistry Research of the Oklahoma Agricultural Experiment Station and by the Dry Land Field Station of the United States Department of Agriculture at Lawton, Oklahoma. Included in the problems for study are: The development of new varieties; the testing of imported varieties under Oklahoma conditions; a study of the best cultural practices to reduce chinch bug injury; and a study of the chemical composition of sorghum, seeking an explanation for the known varietal differences in resistance.

Material in this bulletin deals with the chemical composition of a resistant and a susceptible variety. The differences, of which there are several, can then be studied intensively on a large number of varieties. Thus it is hoped to develop tests that will quickly enable the plant breeder to decide the relative resistance of any variety under examination.

CONTENTS

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INTRODUCTION

The idea that plants show varying susceptibility to insect and fungus attacks is not a new one, but it is only recently that the problem has been subjected to a concentrated study by botanists, chemists, entomologists, and others working together in close cooperation. The data reported in this bulletin represent a part of the chemical study of sorghums being made at the Oklahoma Agricultural Experiment Station, seeking to find an explanation for the known differences in resistance to chinch bug injury shown by various grain sorghums.

Typical chinch bug injury to Dwarf Yellow milo (susceptible) interplanted with Atlas (resistant).

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Resistance in sorghum plants appears to be of two kinds: First, there is a difference in resistance shown by the plants, in that under conditions of equal infestations certain varieties deteriorate much more rapidly than others. Second, certain varieties seem to be more or less unattractive to the insects, so that with equal infestations the net injury to such plants is much less. Work reported in this bulletin is chiefly concerned with changes in composition of sorghum plants that might explain either or both of these observations. No effort has been made to find in these plants unusual compounds that might be responsible for such variations. The aim has been, rather, to find if there are changes in the general composition and growth of sorghums that might be a contributing factor to resistance or susceptibility. A study of special compounds is planned for a later time.

Most serious damage is shown by the younger plants, continuously but in a lessening degree up until heading time, and so this study was made of the younger plants and analyses are not reported after heading time. The analyses of a large number of varieties at different periods of growth over several years is a large task; consequently, only two varieties of sorghums were studied: Atlas, a tall growing type, very resistant; and Dwarf Yellow Milo, a short type showing high susceptibility to injury. Differences in composition of these two varieties will be used as a basis for a more comprehensive study involving a greater number of varieties to see if any such differences as are found are of general significance and can be used to classify resistance in varieties.

REVIEW OF LITERATURE

Insect Resistance

A comprehensive review of the literature on the general subject of insect resistance will not be attempted, due to limited space and the rather complete bibliography in some of the publications mentioned later. Probably the best general discussion of the factors influencing resistance in plants is to be found in the book by Martin (9). Discussed in this volume are such topics (among others) as production of resistant varieties, nature of plant resistance, nature of insect attacks, and influence of nutritional factors.*

The rate of reproduction of the aphis, **Brevicoryne brassicae,** has been plants is to be found in the book by Martin (9). Discussed in this volume of the host plant. The rate of growth, length of larval period, and pupal weights of **Pieris brassicae** were also affected by the chemical composition of the plant.

A discussion of the possible mechanism of chinch bug injury to sorghum is given by Painter (17). The object of insect puncture on the plant is to reach the phloem tubes, and it is suggested that plant injury may come from stoppage of the tubes with a secretion. Tannins are also suggested as a possible factor in resistance. A preliminary report on the resistance of corn and sorghums to chinch bug injury is given in the bulletin by Snelling and Dahms (18). A later bulletin by Snelling et al. (19) is the most comprehensive publication on the subject of chinch bug injury to sorghums. Included in the bulletin is a very complete bibliography on the subject. The authors claim that injury to sorghums may be due to one or more of the following: "(1) The direct withdrawal of plant fluids from cells and especially from the xylem and phloem tubes, (2) the exudation

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* Since this review of literature was prepared, the following article, containing an extensive bibliography, has appeared: Snelling, Ralph O.: "Resistance of Plants to Insect Attack." *Bot. Rev.* 7:543-586 (1941).

of plant fluids from punctures left open after the feeding of the insects with the attendant possible interference with root pressure and translocation, (3) a clogging of the plant conductive tissue with stylet sheath material deposited by the bugs, and (4) openings in the plant tissue are provided through which fungi and bacteria can enter." It is also stated that "Resistance to chinch bug injury is not closely associated with any of the observed morphological or physiological plant characters." Also of interest is their statement that "Resistance may consist of physiological characters involving at least, in part, the ability of a variety to grow or recover in spite of the feeding of the chinch bugs." Dahms and Fenton (4) found that the use of sodium nitrate consistently decreased resistance of certain sorghums to chinch bug attacks and in most cases superphosphate increased resistance.

Chemical Composition

Most of the chemical analyses of the sorghum plant have been made of plants during the later stages of growth, i. e. at heading time or later. The problem of chinch bug injury is, however, chiefly concerned with the younger plants for which there is only a limited amount of data available.

Sorghum is a relatively slow-growing plant during the early stages of development, as is pointed out in a paper by Bartel and Martin (2), and thus is exposed to chinch bug injury for a relatively long period of time.

Martin et al. (10) found little difference in the freezing-point depression and specific conductivity of the juice of older sorghums. Such differences as were found could largely be attributed to differences in juiciness of the stalks. They did, however, find considerable difference in the specific conductivity of Dwarf Yellow Milo and Kansas Orange sorgo (relatively resistant). Such differences would justify an examination of the juice from younger plants.

Detailed analyses of the various parts of sorghum at later stages of growth are given in the extensive paper by Willaman et al. (21). Following the appearance of the panicles, reducing sugars were found to be much in excess of sucrose, but sucrose rapidly increased and at maturity was greatly in excess of the other forms. Organic acids found in the plants were aconitic, malic, citric, tartaric, and oxalic. Non-sugar solids in the juice consisted chiefly of proteins, cellular material, and true gums. Amide nitrogen was also found in large amounts in the juice. Webster and Mitchell (20) made nitrogen fractionation studies on Atlas and Dwarf Yellow Milo plants and found there were appreciable amounts of amide nitrogen in Atlas plants but not in the other. Most of the observed differences in soluble nitrogen content between the two varieties could be accounted for in the basic and alpha amino nitrogen fractions. Dwarf Yellow Milo, except in the early stages of growth, contained much more of the amino nitrogen fraction than did Atlas. Two papers by Miller (12 and 13) give detailed analyses of the water and carbohydrate content of sorghum and corn leaves, but here again only older plants were used. Miller in his text book on plant physiology (14) concludes from some of his unpublished work that there is little change in the nitrogen content of sorghum leaves during the day, although other varieties of plants may show great changes.

Many papers have appeared dealing with the HCN content of sorghum but only one need be mentioned. In this bulletin by Franzke et al. (7), the factors which control the content of HCN are found to be heritable and the amount of HCN to be very greatly modified by such factors as weather, soil condition, and storage of the plants.

EXPERIMENTAL METHODS

Location of Plantings; Culture

With the exception of one year, all of the plantings were made on the Agronomy farm at Perkins, Oklahoma. Here the sorghums were grown with the regular sorghum plantings in a crop rotation of corn, cotton and grain sorghums.

Typical view of plots from which sorghum plants used in these experiments were taken.

The rows were 3½ feet apart and seed was sown in the rows very thickly. This was neecssary to secure the large number of plants required for the first few samplings when the plants were small. As the plants grew, they were thinned to approximately 10 inches to prevent crowding. The soil was worked as often as necessary to keep it friable and free from weeds. The soil was a light sandy loam classified as Chickasha very fine sandy loam. The varieties were alternated in rows and samplings taken from several rows to eliminate, in so far as possible, soil heterogeneity.

The one year that plants were grown at Lawton, Oklahoma, conditions similar to the above were observed.

Sampling and Preparation of Samples

Material used for these tests was secured by cutting the plant at the surface of the soil, placing in paper sacks and transporting to the laboratory at Stillwater. In all, this consumed from 25 to 35 minutes, since the laboratory is some 11 miles from the field. The number of plants taken varied with the size of the plants, but in no instance was the number sampled less than six, and then only at the latter samplings near heading time. Samples in the field were collected from at least four rows, and, whenever the number permitted, from several locations in the row. Often, as shown by the analyses, plants when small were badly sanded, and thus the ash analyses for the whole plant are without particular significance during the younger stages of growth.

The time of sampling varied with the years. Some were secured at 7:00 a. m., some at noon and others at 3:30 or 4:00 p. m. Sampling times are given in the individual tables.

In the laboratory the samples were measured (height and diameter), and then ground through a power meat chopper. The grinding effectively reduced the sample to a pasty mass, except at the latest samplings, where, due to the fibrous nature of the stalk, most of the samples were reground. Several pounds of this material were then thoroughly mixed and samples immediately weighed out for moisture, nitrogen, and any other determination requiring the whole material, and for preservation in alcohol. Where juice analyses were made, the large cage of a Carver hand press was filled with the ground tissue and the juice immediately expressed to the capacity of the press. This juice was then centrifuged in large cups for 10 minutes and determinations run using the supernatant liquid.

For preserving samples in alcohol, duplicate 50 g. aliquots were weighed out and placed in 500 ml wide mouth erlenmeyers, covered with 95% redistilled alcohol, and boiled for 10 minutes. After cooling, enough alcohol was added to give a final alcohol concentration of 80-85% by volume. Extraction of the preserved samples was completed in large soxhlets, running until the percolate was clear (16-30 hours). After a short extraction, the original alcohol was replaced with fresh alcohol, thus preventing a prolonged heating of concentrated extracts.

Chemical Determinations

Solids.—Solids were secured by drying the samples overnight in an oven at 105° C. Due, probably, to the relatively high non-sugar content of the samples, caramelization was not noticeable and a vacuum oven was not used.

Ash.—The ash is the residue left after ashing the solid samples at a low red heat.

Mineral Analyses (Cl, K, P, Ca).—Mineral determinations were run according to the directions in the Official and Tentative Methods of Analysis (1) found in the section on plant analyses. The only exception to these methods was that nearly all of the chlorine determinations were run on the residue from the ash determinations rather than on alkaline ashings as directed. The data given in Table VII follow the book method in toto.

Specific Gravity.—In determining specific gravity, the Westphal balance was used and the readings made at 20° C.

Titratable Acidity.—Determinations of titratable acidity were run on 5 ml samples, diluted with water to 200 ml. Phenolphthalein was used as an indicator. Results in the tables are expressed in terms of ml of N/10 alkali required to neutralize 100 ml of juice.

Hydrogen Ion Concentration.—At first, determinations of hydrogen ion concentration were made using the Youden quinhydrone, hydrogen ion apparatus. Later, determinations were run using the Coleman glass electrode.

Astringency.—Tannins and related substances were determined essentially as done by Caldwell (3), i. e. by titration with $KMnO_4$ in the presence of indigo carmine. Since this is a method for tannins it is obvious that the expressing of **Total** and **Non-tannin** results as tannins is of comparative value only.

Sugars.—All determinations of sugars were run using the Shaffer-Hartman procedure as outlined in the laboratory manual by Morrow (16).

Neutral lead acetate in excess was added to the centrifuged juices, the excess lead removed with potassium oxalate, and aliquots of the filtrate used for the determinations of **Reducing Sugars.** Such values were calculated as invert sugars. **Total Sugar** values were secured by inverting a sample of the cleared juice with HCl, allowing the sample to stand overnight, neutralizing, and running as reducing sugars. One year, inversion with invertase was used as well, but the differences in results were small and it was discarded in favor of the acid procedure. **Sucrose** results were secured by substracting reducing sugar values from total sugars and multiplying by the factor .95. Where alcoholic extracts were analyzed, the alcohol was first removed from an aliquot by heating on a water bath. The resulting solutions were diluted with water and then analyzed by the same procedure as were the juice samples.

Nitrogen.—Total and **Soluble** nitrogen were run as directed in the Official Methods (1) using the Gunning modification. **Insoluble** values were secured by difference.

Hydrocyanic Acid.—Analyses for hydrocyanic acid were made following essentially the procedure outlined in the paper by Menaul and Dowell (11).

Conductivity.—Conductivity measurements were determined using the conventional Wheatstone bridge apparatus with a microphone hummer —earphone detector system. The constant of the fixed electrode dip cell employed was established by a standard KCl solution.

Enzymes.—Catalase was run on 10 ml of neutralized juice following the procedure outline by Davis (5). **Oxidase** was determined according to the iodimetric method of Guthrie (8). **Peroxidase** determinations were made essentially as directed by Miller (15).

GENERAL INFORMATION

In the tables (pages 14 ff.), the age of plants is recorded as days from planting and is perhaps not as good a guide to the stage of growth as the height of plants. Here, too, ambiguities occur due to the radically different heights of the mature plants from the two varieties. The heights recorded are taken on the cut samples in the laboratory, just before grinding. Dwarf Yellow Milo seedlings are generally the first to appear, and they are large and taller for some time (about 15 inches); then the Atlas plants increase rapidly in height and eventually become much taller.

Considerable difficulty was experienced with infestations of chinch bugs and samplings were cut short in two years due to extreme injury to the Milo plants. At such times care was exercised to secure Milo plants as nearly free from injury as possible and it is not felt that chinch bug injury has appreciably affected the results recorded in the tables. A later paper will deal with the subject of how chinch bug injury influences the chemical composition of the plants.

At the suggestion of Mr. Osborn, Finney Milo was substituted the last two years for Dwarf Yellow Milo. This is a strain of the latter, more disease resistant and just as susceptible to injury as the first strain used.

EXPERIMENTAL RESULTS

Data for the years 1935-40 are given in the accompanying tables. Each year's results are recorded seperately but the discussion of each topic is a summary of the data from the various years, comparing the two varieties to see if there are significant differences. The differences found are to be applied to a study of other varieties, both susceptible and resistant, to find if they can be used as a means of determining the relative resistance of those varieties.

Analyses of the Press Juice

Solids.—Generally speaking, Milo juice was somewhat higher in solids, but not enough so to say that it was consistently higher, and the samples from Lawton (1936) show the Atlas having a higher value during the earlier stages of growth. During 1937 and 1938, Milo values were always higher and the differences became progressively greater as the plants grew. Specifically, then, there did not seem to be enough difference in the solids content of the two varieties to explain differences in susceptibility.

Ash.—Consistent differences were found for ash value, Milo being appreciably the higher in almost every instance. At times, particularly when the plants were small, the differences may be slight, but they became increasingly greater as the crop grew. Atlas uniformily showed decreases as the plants matured, while Milo values increased sometimes quite markedly toward the end of the sampling period. In consequence of this regular variation in the ash content of the juice, further study would seem to be justified seeking to find if other varieties show this difference.

Specific Gravity.—Values and trends in specific much resemble the solids relationship. Although there was a tendency for the Milo values to be higher than Atlas figures, there was not enough difference to say that the variation is significant.

Titratable Acidity.—Titratable acidity varied with the years and with the stage of growth. In two of the four years under consideration, Atlas showed a higher value; and in the other two years Milo was higher. Generally, there was considerable yearly variation; but the net result for a season was that both varieties varied in the same direction and the total values are surprisingly close. Seasonal variations, also, were not great. It is apparent from the foregoing that total acidity cannot be considered as a significant factor in explaining resistance.

Hydrogen Ion Concentration.—Hydrogen ion concentration values were quite similar for the two varieties, with the Atlas plants being slightly more acid but certainly not significantly so. During the season, pH first decreased for a time and then became more acid as heading time was approached. The pH values nearly all fell in the range of 5.0 to 5.3.

Astringency (Tannins and Related Substances).—The presence or absence of tannins has been suggested by Hubert Martin (9) as a possible explanation for varying susceptibility of plants to both insect and disease attacks. Painter (17) also suggests that tannins may influence resistance to chinch bug injury in sorghums. On the basis of results obtained in the present study, however, it is hard to see how there can be any correlation between the amounts of tannins present at any stage of growth and resistance or susceptibility. Young sorghum plants were highest in tannins and total astringency, and older plants showed decreasing amounts. The 1937 results proved a notable exception, however, in that the later stages of growth showed a distinct increase in total values. As in many of the other constituents, all of the values fluctuated some from sampling to sampling

Table I.—Analyses of Press Juice from Whole Sorghum Plants at Different Stages of Growth; Lawton Oklahoma, 1936[1]

Date	Ave. ht. in.	H_2O %[2]	Solids %	Ash %	Sp. Gr.	ACIDITY Titratable	ACIDITY pH	ASTRINGENCY G. PER LITER Total	ASTRINGENCY G. PER LITER Non-Tannin	ASTRINGENCY G. PER LITER Tannin	SUGARS Red. %	SUGARS Total %	SUGARS Sucrose %	Total N.	ENZYMES Catalase	ENZYMES Oxidase	ENZYMES Peroxidase
Atlas																	
June 1	4-6	95.62	4.38	1.11	1.021	61.0	5.23	4.818	2.609	2.084	.96	.96	.00	.208	1.2	22.5	21.1
July 15[3]	14	92.47	7.53	1.74	1.032	79.8	5.01	5.009	2.824	2.750	2.22	2.43	.20	.275	--	--	--
July 15	18	87.79	12.21	1.64	1.056	49.3	4.80	3.596	2.860	.736	4.21	7.07	2.72	.302	--	--	--
Dwarf Yellow Milo																	
June 1	6-8	95.47	4.53	1.33	1.022	55.2	5.22	4.999	2.409	2.590	.85	.87	.02	.208	1.2	21.4	22.0
July 15[3]	6	94.37	5.63	1.41	1.025	59.4	5.07	2.910	2.126	.565	1.26	1.43	.16	.219	--	--	--
July 15	25-30	87.57	12.43	1.60	1.057	47.1	4.95	2.166	1.704	.462	3.12	8.45	5.6	.327	--	--	--

[1] Samples harvested at 7:30 a. m.
[2] Percentages are expressed on a volume basis.
[3] Second planting. Compare with previous line.

Table II.—Analyses of Press Juice from Whole Sorghum Plants at Different Stages of Growth; Perkins, Oklahoma, 1937[1]

						Acidity		Astringency G. per liter			Sugars				
Age[2] Days	Ave. ht. in.	H_2O %[3]	Solids %	Ash %	Sp. Gr.	Titrat-able	pH	Total	Non-Tannin	Tannin	Red. %	Total %	Sucrose %	Conduc-tivity[4]	Nitrogen %
							Atlas								
24	6	96.41	3.59	1.05	1.014	45.4	5.30	1.844	1.464	.380	.71	.72	.01	.00923	.141
30	8	96.26	3.74	1.19	1.017	39.0	5.30	1.485	1.072	.413	.58	.58	.00	.00913	.184
34	9	96.48	3.52	1.10	1.014	29.2	5.42	1.309	.619	.690	.62	.62	.00	.01348	.203
42	17	96.00	4.00	1.12	1.017	35.7	5.42	1.299	.959	.340	1.28	1.28	.00	.01362	.175
48	22	95.37	4.63	1.15	1.020	42.8	5.05	1.546	1.113	.433	1.68	1.68	.00	.01309	.191
52	26	94.88	5.12	1.17	1.024	42.8	5.20	1.464	1.113	.351	2.36	2.36	.00	-	.177
57	28	93.87	6.13	1.16	1.027	47.6	5.08	1.402	1.196	.206	3.24	3.68	.42	.01363	.155
63	33	93.11	6.89	1.02	1.027	41.2	5.03	1.376			4.25	4.47	.21	.01382	.134
70	37	91.93	8.07	1.02	1.035	41.2	5.10	1.725	1.591	.134	4.93	5.64	.67	- - -	.143
77	39	91.21	8.79	1.13	1.046	49.8	4.87	2.095			5.66	6.42	.72	.01704	.155
87	60	87.22	12.88	1.06	1.054	39.0	5.05	1.766	1.602	.164	6.19	10.04	3.66	- -	.181
							Dwarf Yellow Milo								
24	7	96.36	3.64	1.10	1.014	34.6	5.46	1.215	.777	.438	.56	.56	.00	.00931	.204
30	7	96.13	3.87	1.24	1.017	30.3	5.40	1.113	.701	.412	.56	.58	.02	.00921	.228
34	10	96.06	3.94	1.19	1.016	29.8	5.44	1.102	.639	.463	.72	.72	.00	.00938	.242
42	15	95.82	4.18	1.21	1.017	36.3	5.44	1.206	.876	.330	1.06	1.09	.03	.01372	.224
48	18	94.92	5.08	1.24	1.022	43.2	5.22	1.402	1.200	.202	1.57	1.82	.24	.01357	.231
52	22	94.81	5.19	1.19	1.024	45.4	5.22	1.423	1.072	.351	2.03	2.08	.05		.221
57	22	93.26	6.74	1.30	1.031	51.4	5.10	1.351	1.278	.073	2.35	3.74	1.32	.01382	.250
63	25	92.89	7.11	1.21	1.029	45.0	5.12	1.448			2.39	3.71	1.25	.01382	.225
70	26	91.14	8.86	1.34	1.041	54.0	5.13	1.951			2.51	5.26	2.61		.254
77	28	89.03	10.97	1.66	1.057	60.0	5.01	2.495			2.72	6.48	3.57	.02096	.353
87	33	89.17	10.83	2.02	1.050	47.6	5.26	1.961	1.499	.462	2.41	5.60	3.03	- -	.402

[1] Samples harvested at 7:00-7:30 a. m.
[2] Age, days from planting (first sampling June 4).
[3] Percentages are expressed on a volume basis.
[4] In reciprocal ohms.

Table III.—Analyses of Press Juice from Whole Sorghum Plants at Different Stages of Growth; Perkins, Oklahoma, 1938[1]

						ACIDITY		ASTRINGENCY G. PER LITER			SUGARS				
Age[2] Days	Ave. ht. in.	H_2O %[3]	Solids %	Ash %	Sp. Gr.	Titratable	pH	Total	Non-Tannin	Tannin	Red. %	Total %	Sucrose %	Nitrogen %[3]	Chlorides %
						Atlas									
						First Planting									
55	20	95.85	4.15	.94	1.021	44.8	5.13	1.445	1.176	.269	1.61	1.62	.01	.141	.010
67	33	94.92	5.08	1.13	1.025	42.6	5.20	1.754	.949	.805	2.52	2.61	.09	.145	.013
76	50	92.81	7.19	1.04	1.032	48.0	5.15	1.445	1.176	.269	4.28	4.38	.10	.120	.012
						Second Planting									
26	7	95.25	4.75	1.18	1.022	65.6	5.18	4.312	2.854	1.602	1.10	1.11	.01	.156	.012
45	20	95.68	4.32	1.14	1.023	47.0	5.30	1.630	1.156	.474	1.45	1.50	.05	.159	.018
55	30[4]	94.27	5.73	1.06	1.027	51.2	5.02	1.651	1.424	.227	3.29	3.29	.00	.105	.024
						Dwarf Yellow Milo									
						First Planting									
55	18	95.13	4.87	1.03	1.025	50.4	5.18	1.704	1.211	.493	1.37	1.78	.39	.186	.010
67	23	93.64	6.36	1.35	1.031	51.4	5.22	1.507	1.115	.392	1.60	3.08	1.41	.202	.022
76	39	91.22	8.78	1.41	1.041	59.8	5.20	1.465	1.382	.082	2.14	5.32	3.03	.181	.016
						Second Planting									
26	7	94.81	5.19	1.32	1.024	69.0	5.20	4.456	3.018	1.438	1.06	1.11	.05	.190	.022
45	15	94.17	5.83	1.35	1.031	52.6	5.32	1.960	1.279	.681	1.36	2.04	.65	.228	.027
55	17	92.76	7.24	1.51	1.036	68.6	5.08	2.105	1.630	.475	2.16	3.32	1.10	.220	.046

[1] Samples harvested at 12:30 p. m.
[2] Age, days from planting.
[3] Percentages are expressed on a volume basis.
[4] Heading.

Table IV.—Analyses of Press Juice from Whole Sorghum Plants at Different Stages of Growth; Perkins, Oklahoma, 1939[1]

Age[2] Days	Ave. ht. in.	H_2O %[3]	Solids %	Ash %	Sp. Gr.	ACIDITY		ASTRINGENCY G. PER LITER			SUGARS			Nitrogen %	Chlorides %[3]	Solids whole plant
						Titratable	pH	Total	Non-Tannin	Tannin	Red. %	Total %	Sucrose %			
								Atlas								
32	7	95.01	4.99	1.29	1.024	71.6	5.00	4.103	3.100	1.003	1.57	1.52	.01	.162	.043	14.82
53	28	95.37	4.63	1.26	1.024	42.4	5.00	1.309	1.085	.224	2.06	2.08	.02	.125	.047	13.10
76	60	90.90	9.10	1.17	1.045	71.6	5.20	1.274	1.274	.000	5.20	7.30	2.00	.105	.064	21.39
								Dwarf Yellow Milo (Finney)								
32	8	94.85	5.15	1.44	1.026	66.6	5.00	3.711	2.845	.866	1.28	1.42	.14	.168	.042	14.97
53	20	93.73	6.27	1.39	1.032	55.4	5.05	1.699	1.274	.425	1.77	3.12	1.28	.166	.086	17.49
76	43	86.55	13.45	1.63	1.065	48.4	5.32	1.675	1.580	.095	2.33	9.23	6.37	.334	.100	27.21

1 Samples harvested at 12:30 p. m.
2 Age, days from planting.
3 Percentages are expressed on a volume basis.

and uniform changes were not the rule. Very probably this indicates a correlation of these constituents with the climatic variations and rapidity of growth.

It is true that there may be chemical differences in the tannins and related compounds between the two varieties or there may be differences in the location within the structure of the plant, but certainly on the basis of amounts present in the juice no significant variations can be noted.

Sugars.—Sweetness of juice is another factor that has been mentioned as having a possible relationship to chinch bug injury. That this relationship could be at best only a general one is apparent when we consider that the sugar content of sorghums continued to increase at least beyond any sampling period used here and that the bugs may attack the plant and cause severe damage at any stage here noted. Reducing sugars and total sugar values increased steadily up until heading, when sampling was discontinued. This increase was consistent and regular and occurred for both varieties. Sucrose presented an entirely different picture, for here we find that during the early stages of growth sucrose was almost entirely lacking in the Atlas, while in the Milo there were small but increasingly larger amounts of sucrose. As will be shown later (page 25), this difference was more pronounced the later in the day samples were secured. These data definitely indicate a difference in sugars between the two varieties that merits further study and such work is now in progress. Atlas is often spoken of as a sweet sorghum, but in these data it is clearly shown that during most of the vegetative stages Milo had just as much or more sugar and that it was only after heading that the sugar content varied greatly. The great difference between the two varieties lies in the kind of sugar and amounts of each present, rather than in total sugars. There are indications that this greater content of sucrose in Milo may be correlated with susceptibility, but the data are as yet incomplete.

Nitrogen.—Following the suggestions in the literature (9) that an increase in nitrogen supplied to plants increased the susceptibility, considerable attention was given to the nitrogen content of these two varieties. In confirmation of these statements, data in these tables show that Milo consistently ran higher in nitrogen content and sometimes was markedly higher. This difference led to the work previously reported (20) in which it was shown that nearly all of this difference was due to amide and amino nitrogen. Application of this knowledge to other varieties and to plants grown in cultural solutions is in progress but here again preliminary results have not always been consistent. In general, the percentages in Atlas decrease as growth continues while Milo averages much the same or increases. (Compare with nitrogen in the whole plants.)

Conductivity.—Data are given for only one year, although more determinations have been run with essentially the same results. Values for the two varieties were remarkedly close together at any one sampling period. This is the more surprising in that there were appreciable differences in the ash content between the two varieties. No information is available to explain this fact and it is hoped to make some detailed studies at a later time of conductivity measurements on different parts of the plants.

Chlorides.—Analyses of chlorides run during two years have shown differences consistent enough to justify the belief that there may be some correlation between the higher chloride content of the Milo and its susceptibility. Part of the difference can be explained as due to the greater ash percentages in the Milo; but these greater ash percentages can not entirely account for the differences and some significance may be attached to the observed variations in chlorides.

Enzymes.—Only one set of data are given in the tables, but considerable other data are available on samples not included in the tables that are published here. Summarizing the unpublished data along with the other, there was little difference shown in the oxidase and preoxidase values. Catalase was slightly higher in the Milo, but not enough to be significant.

Analyses of Whole Plants

Total Solids.—More so than in the juice data, we find in the analysis of whole sorghum plants that Milo plants were higher in solids. In most years the differences were small during the earlier stages of growth; but the difference was consistently in favor of the Milo, and as growth continued the differences became progressively greater. With the exception of 1940, however, this difference is probably not significant; and in that year the results may have been modified by the fact that chinch bug injury was greater than usual. Normally, the solids were high while the plants were young and then a marked decrease set in, followed by a later period of ascending values even though the plants were still growing rapidly.

Table V.—Analyses of Whole Sorghum Plants at Intervals During the Season; Perkins, Oklahoma, 1935[1]

Date	Ave. ht. in.	H_2O %[2]	Solids %	Ash %	HCN %	Nitrogen %
			Atlas			
June 1	4-6	95.61	4.39	1.07	.0055	.494
July 15	18-20	80.90	19.10	1.97	.0092	.676
			Dwarf Yellow Milo			
June 1	6-8	95.47	4.53	1.34	----	.432
July 15	25-30	80.67	19.33	2.14	.0084	.616

[1] Samples harvested at 7:30 a. m.
[2] Percentages are expressed on a green weight basis.

Ash.—Unfortunately, as mentioned previously, the values for ash content of the young plants are of only comparative value, due to the large amount of sand held by the sheath. Later in the growth cycle of both varieties, there was a decrease in percentage. In the Milo, this decrease was usually followed by an appreciable increase, and particularly was this true of the data for 1937 (Table VI).

These consistently greater values in Milo under all conditions, in both the juice and whole plants, justifies further work on the ash content in studying other varieties and in making general comparisons.

Sugars.—Data on sugars in the whole plant follow much the same trend as shown by the juice analyses; however, here we find small but definite amounts of sucrose in the Atlas plants. It is hard to account for the appearance of sucrose in these extracts of the whole plants when the press juice for the same time was practically sucrose free or very low. In both the Atlas and Milo, there was an increase in percentage of sucrose for most of the samples, and this was accompanied by a lower total sugar content; i. e. a larger percentage of the total was sucrose. It hardly seems possible that there was a conversion of reducing sugars to sucrose on standing in alcohol, and yet it is hard to conceive of a selective extraction of the sugars in the press juice. Taken as a whole, the sugar relations in these

Table VI.—Analyses of Whole Sorghum Plants at Intervals During the Season; Perkins, Oklahoma, 1937.

Age[1] Days	Ave. ht. in.	H_2O %[2]	Solids %	Ash %	SUGARS[3] Red. %	Total %	Sucrose %	NITROGEN[3] Sol. %	Insol. %	Total %	HCN %
					Atlas						
22	5	85.67	14.33	3.24	.61	.73	.11	.078	.394	.472	--
24	6	76.60	23.70[4]	14.08	.45	.60	.14	.077	.333	.410	.0185
30	8	75.15	24.85[4]	15.11	.42	.53	.10	.088	.311	.399	.0007
34	9	84.25	15.75[4]	6.99	.46	.47	.01	.089	.278	.367	.0053
42	17	88.14	11.86	1.47	1.02	1.21	.18	.081	.270	.351	.0059
48	22	88.62	11.38	1.26	1.30	1.66	.34	.105	.263	.368	.0050
52	26	85.26	14.74	1.24	1.70	2.26	.53	.111	.244	.355	.0038
57	28	84.86	15.14	1.24	2.31	3.04	.69	.103	.232	.335	.0013
63	33	83.97	16.03	.99	3.09	4.00	.86	.081	.186	.267	.0046
70	37	81.72	18.28	1.08	3.53	5.00	1.40	.081	.184	.265	--
77	39	79.15	20.85	1.17	3.86	5.85	1.89	.085	.235	.320	.0008
87	60	76.74	23.26	1.08	4.02	7.03	2.86	.094	.171	.265	.0010
					Dwarf Yellow Milo						
22	7	86.46	13.54	3.05	.53	.73	.19	.115	.435	.550	----
24	7	76.12	23.88[4]	15.92	.38	.65	.16	.089	.333	.422	.0099
30	7	77.24	22.76[4]	14.18	.39	.50	.10	.105	.321	.426	.0002
34	10	82.20	17.80[4]	8.60	.49	.67	.17	.101	.292	.393	.0050
48	18	86.47	13.53	1.47	1.06	1.15	.42	.105	.337	.442	.0067
42	15	87.42	12.58	1.78	.71	1.65	.56	.123	.283	.406	.0060
52	22	85.74	14.26	1.45	1.34	2.01	.64	.111	.286	.397	.0046
57	22	83.57	16.43	1.40	1.68	2.94	1.20	.169	.257	.426	.0027
63	25	82.65	17.35	1.37	1.75	3.23	1.41	.151	.235	.386	.0068
70	26	79.63	20.37	1.40	1.78	4.30	2.39	.149	.214	.363	.0014
77	28	77.63	22.37	1.62	2.30	6.31	3.81	.207	.316	.523	.0011
87	33	77.06	22.94	1.97	1.60	5.19	3.41	.188	.268	.456	.0010

[1] From date of planting
[2] Percentages are expressed on a green weight basis.
[3] Samples preserved in alcohol.
[4] Sand, which could not readily be removed, accounts for these large solids and ash values.

sorghums were such that more work can well be done before a decision is reached as to the possible importance of sucrose and possibly other sugars in chinch bug resistance. Certainly it seems there may be a correlation between the higher sucrose content of Milo juice and the greater susceptibility of these plants to injury.

Nitrogen.—Total nitrogen followed much the same pattern shown in the press juice. Milo plants were consistently higher and the percentage increased somewhat as the season progressed, while the Atlas percetages decreased sometimes quite markedly. Soluble nitrogen ranged in much the same manner, with Milo generally increasing as the season progressed.

Hydrocyanic Acid.—Determinations of hydrocyanic acid were run on the samples reported here and on other samples as well. One year corn was included in the plants used for analyses. The amounts are relatively small and fluctuations between the two varieties are uncertain and in no regular direction. Corn was found free of HCN. Considering that corn varieties show varying susceptibilities, despite lack of HCN, and that the differences found in sorghum were negligible, it is considered doubtful if HCN plays any part in sorghum resistance to chinch bug attacks.

Mineral Analyses.—The data given in Table VII for chlorine, phosphorus, potassium and calcium generally show Milo to be higher in each of these elements, as might be expected from the higher ash content. None of these elements on a percentage basis seemed of particular significance, not even the chlorine which in the juice seemed to show variations of significance.

Table VII.—Analyses of Whole Plants at Intervals During the Season; Two Plantings, (Samples correspond to those in Table III) Perkins, Oklahoma, 1938[1]

Age[2] Days	Ave. ht. in.	H_2O %[3]	Solids %	Ash %	Cl %	P %	K %	Ca %	Nitrogen %
				Atlas					
				First Planting					
55	20	86.96	13.04	1.78	.024	.030	.487	.053	.344
67	33	86.66	13.34	1.21	.018	.028	.457	.049	.432
76	50	83.77	16.23	1.14	.023	.033	.422	.068	.398
				Second Planting					
26	7	82.29	17.71	5.22	.024	.038	.536	.074	.462
45	20	85.95	14.05	1.79	.031	.029	.452	.070	.431
55	30[1]	85.38	14.62	1.20	.025	.029	.450	.074	.400
				Dwarf Yellow Milo					
				First Planting					
55	18	15.02	14.98	2.37	.029	.033	.482	.059	.419
67	23	83.73	16.27	1.49	.027	.040	.469	.073	.503
76	39	79.46	20.54	1.56	.022	.043	.527	.087	.610
				Second Planting					
26	7	80.97	19.03	6.04	.022	.028	.582	.094	.507
45	15	83.72	16.28	2.33	.027	.033	.504	.083	.548
55	19	82.64	17.36	1.71	.046	.044	.572	.102	.573

[1] Samples harvested at 12:30 p. m.
[2] Age, days from planting.
[3] Percentages are expressed on a green weight basis.
[4] Heading

Table VIII.—Analyses of Whole Sorghum Plants at Intervals During the Season; Perkins, Oklahoma, 1940[1]

					SUGARS[4]			NITROGEN[4]		
Age[2] Days	Ave. ht. in.	H_2O %[3]	Solids %	Ash %	Red. %	Total %	Sucrose %	Total %	Soluble %	Chlo-rides %
				Atlas						
34	5	79.34	20.66	9.27	.87	1.09	.21	.478	.092	.014
43	11	85.24	14.76	1.69	1.51	1.84	.31	.429	.063	.020
50	19	84.80	15.20	2.31	1.41	2.23	.78	.428	.077	.026
62	29	86.63	13.37	1.56	1.48	1.90	.40	.312	.058	.012
70	49	84.40	15.60	1.29	2.35	2.90	.52	.298	.055	.014
				Dwarf Yellow Milo (Finney)						
34	6	80.79	19.20	7.68	.55	1.09	.51	.468	.109	.016
43	11	81.90	18.10	2.08	.94	2.43	1.42	.521	.085	.021
50	14	75.77	24.23	6.70	.91	3.06	2.15	.748	.243	.023
62	16	79.47	19.53	3.08	1.10	2.75	1.52	.557	.168	.017
70	20	81.13	18.87	1.76	1.23	3.16	1.83	.503	.147	.021

[1] Samples at 3:30 p. m.
[2] Age. days from planting.
[3] Percentages are expressed on a fresh weight basis.
[4] Samples preserved in alcohol.

Daily Changes in Composition of Plant Sap

Tables IX to XI give analyses made at different times during a day or a 24-hour period and the data are intended to show whether any striking differences occurred in composition between juice in the morning and late afternoon. Such changes may also be of value in explaining why certain plants are able to resist injury better than others. Detailed discussions of these tables are not necessary and only certain points will be made that seem of value to the problem.

It is recognized that many of the changes that occur in the juice and whole plant are the result of a simple concentration of the juice resulting from the daily water loss of the plants. Nevertheless, it is this concentrated sap that is available to the chinch bug and our concern is with the juice as the bugs feed on it; consequently, the factor of concentration may be generally disregarded.

Solids and Ash.—Values of solids and ash increased regularly throughout the day, reaching their maximum somewhere around late afternoon, probably between 3:30 and 6:00, although the data are not extensive enough to fix the time exactly. Apparently, the greater part of this increase came before noon, and what data are available show that the changes after 3:00 p. m. were small. Both varieties varied in much the same manner and the differences seem without significance.

Acidity.—Total acidity increased, reaching a maximum around 3:00 p. m. and then starting to fall, the increases being much less than the increases in solids for the same time. Both varieties maintained a relatively constant pH and in much the same range. Certainly there were no acidity changes capable of explaining variety differences.

Astringency Values.—Significant varietal differences were not found in the tannins or other fractions, and there is no reason on the basis of amounts present for assuming that tannins exert any selective action on chinch bugs.

Table IX.—Daily Changes in Composition of Press Juice from Sorghum Plants; Perkins, Oklahoma, 1937

Date and Time		Age[1] Days	Ave. ht. in.	H_2O %	Solids %	Ash %	Sp Gr.	ACIDITY Titratable	ACIDITY pH	ASTRINGENCY G. PER LITER Total	ASTRINGENCY G. PER LITER Non-Tannin	ASTRINGENCY G. PER LITER Tannin	SUGARS Red. %	SUGARS Total %	SUGARS Sucrose %	Conductivity	Nitrogen %
Atlas																	
June 9	3:00 p. m.	29	8	95.70	4.30	1.25	1.019	42.2	5.40	1.753	1.113	.640	1.15	1.15	.00	.00911	.195
June 10	7:00 a. m.	30	8	96.26	3.74	1.19	1.017	39.0	5.30	1.485	1.072	.413	.58	.58	.00	.00913	.184
July 28	7:00 a. m.	48	22	95.37	4.63	1.15	1.020	42.8	5.05	1.546	1.113	.433	1.68	1.69	.01	.01309	.191
July 28	2:30 p. m.	48	22	93.99	6.01	1.30	1.026	45.0	5.38	1.485	1.278	.207	2.88	2.88	.00	.01348	.204
Dwarf Yellow Milo																	
June 9	3:00 p. m.	29	7	95.51	4.49	1.38	1.020	36.8	5.20	1.268	.619	.649	.96	.96	.00	.00918	.226
June 10	7:00 a. m.	30	7	96.13	3.87	1.24	1.017	30.3	5.40	1.113	.701	.412	.56	.57	.01	.00921	.228
July 28	7:00 a. m.	48	18	94.92	5.08	1.24	1.022	43.2	5.22	1.402	1.200	.202	1.57	1.82	.24	.01357	.231
July 28	2:30 p. m.	48	18	94.25	5.75	1.27	1.025	46.2	5.35	1.361	1.258	.103	1.62	2.43	.77	.0169	.219

[1] Age, days from planting.

Table X.—Changes in Composition of Press Juice from Sorghum Plants During a 24-hour Period and at Two Stages of Growth; Perkins, Oklahoma, 1939

Time of sampling	Age[1] Days	Ave. ht. in.	H_2O %	Solids %	Ash %	Sp. Gr.	ACIDITY Titratable	ACIDITY pH	ASTRINGENCY G. PER LITER Total	ASTRINGENCY G. PER LITER Non-Tannin	ASTRINGENCY G. PER LITER Tannin	SUGARS Red. %	SUGARS Total %	SUGARS Sucrose %	Nitrogen %	Chlorides %
Atlas																
5:00 a. m.	41	10	96.71	3.29	1.07	1.016	43.4	5.02	1.777	1.240	.537	.64	.71	.07	.150	.046
10:00 a. m.	41	10	96.10	3.90	1.18	1.019	46.4	5.10	1.613	1.193	.420	1.18	1.20	.02	.156	.052
3:00 p. m.	41	10	95.09	4.91	1.47	1.024	53.4	5.00	1.941	1.824	.117	2.17	2.17	.00	.163	.060
6:00 p. m.	41	10	95.00	5.00	1.44	1.025	47.4	5.00	1.941	1.717	.234	2.02	2.07	.05	.196	.056
5:00 a. m.	61	42	96.17	3.83	1.07	1.019	43.4	5.05	1.250	.944	.306	1.91	1.93	.02	.111	.046
10:00 a. m.	61	42	95.33	4.67	1.14	1.022	47.4	5.00	1.250	1.062	.188	2.27	2.27	.00	.108	.053
3:00 p. m.	61	42	94.86	5.14	1.20	1.028	47.4	5.05	1.486	1.132	.354	2.95	3.19	.23	.127	.055
6:00 p. m.	61	42	94.98	5.02	1.17	1.028	47.4	5.05	1.227	.991	.236	2.78	3.00	.21	.102	.051
Dwarf Yellow Milo (Finney)																
5:00 a. m.	41	13	96.29	3.71	1.21	1.018	45.4	5.10	1.613	1.193	.420	.71	.84	.13	.178	.051
10:00 a. m.	41	13	95.67	4.33	1.44	1.022	46.4	5.05	1.426	1.146	.280	1.25	1.40	.15	.186	.063
3:00 p. m.	41	13	94.30	5.70	1.58	1.030	55.0	5.00	1.777	1.286	.491	1.54	2.56	.97	.198	.075
6:00 p. m.	41	13	94.19	5.81	1.64	1.028	42.4	5.10	1.917	1.146	.771	1.50	2.45	.90	.214	.070
5:00 a. m.	61	29	94.11	5.89	1.22	1.030	55.4	5.05	1.510	1.227	.589	1.77	3.13	1.29	.172	.088
10:00 a. m.	61	29	93.37	6.63	1.31	1.033	57.4	5.05	1.344	1.227	.117	2.12	3.57	1.38	.190	.090
3:00 p. m.	61	29	92.08	7.92	1.47	1.037	58.4	5.12	1.604	1.250	.390	1.99	4.58	2.46	.216	.092
6:00 p. m.	61	29	91.84	8.16	1.44	1.039	58.0	5.15	1.699	1.321	.378	2.78	4.62	2.68	.220	.098

[1] Age, days from planting.
[2] Percentages are expressed on a volume basis.

Table XI.—Composition of Press Juice From the Leaves and Stalks of Sorghum Plants at Three Times During the Day; Perkins, Oklahoma, 1940

Time of Sampling	H_2O %[1]	Solids %	Ash %	SUGARS Red. %	Sucrose %	Total %	Nitrogen %	Chlorides %
			Atlas[2]					
			Whole Plants					
5:15 a. m.	95.22	4.78	1.25	1.42	1.46	.04	.211	.015
11:00 a. m.	94.36	5.74	1.49	2.02	2.08	.06	.240	.026
3:45 p. m.	93.63	6.37	1.65	2.28	2.41	.12	.252	.024
			Leaves					
5:15 a. m.	94.79	5.21	1.41	1.17	1.18	.01	.242	.012
11:00 a. m.	93.63	6.37	1.64	1.72	1.80	.08	.271	.024
3:45 p. m.	92.62	7.38	1.71	2.01	2.01	.00	.306	.015
			Stalks					
5:15 a. m.	95.88	4.12	1.26	1.56	1.64	.08	.150	.022
11:00 a. m.	95.43	4.57	1.27	1.90	1.95	.05	.165	.026
3:45 p. m.	94.74	5.26	1.30	2.13	2.29	.15	.198	.014
			Dwarf Yellow Milo (Finney)[3]					
			Whole Plants					
5:15 a. m.	92.66	7.34	1.55	1.20	2.42	1.16	.352	.017
11:00 a. m.	92.18	7.82	1.69	2.06	3.21	1.09	.346	.035
3:45 p. m.	90.29	9.71	1.98	1.55	3.64	1.99	.450	.042
			Leaves					
5:15 a. m.	92.38	7.62	1.60	1.07	1.97	.86	.340	.010
11:00 a. m.	89.85	10.15	2.09	1.92	2.99	1.02	.494	.032
3:45 p. m.	89.27	10.73	2.09	1.45	3.68	2.12	.516	.024
			Stalks					
5:15 a. m.	93.71	6.29	1.48	1.20	2.67	1.40	.260	.030
11:00 a. m.	91.18	8.82	1.59	1.74	3.26	1.44	.312	.033
3:45 p. m.	89.27	10.73	2.09	1.45	3.46	1.91	.318	.028

[1] Percentages are expressed on a volume basis.
[2] Atlas plants were 15″ tall, 48 days old.
[3] Milo plants were 13″ tall, 48 days old.

Sugars.—Total sugar percentages increased markedly until the 3:00 to 3:30 p. m. sampling is reached, and then fell slightly before the 6:00 p. m. sampling. Greatest variations were found in the sucrose. Not only was Milo consistently higher, but as the day progressed the differences became more strikingly apparent. Only small amounts of sucrose were present in Atlas even at the peak, while there was a great piling up of sucrose in the Milo plants. This comparatively large amount is shown in detail on the graph, Figure 1. Apparently sucrose is an active metabolic form of sugar in Milo but not in Atlas. This daily fluctuation was so pronounced that further study is in progress and hopes are held that sucrose may finally be found to show a varietal correlation with susceptibility.

Nitrogen Changes.—Changes in nitrogen were not so pronounced as in other constituents. Percentages usually increased, but somewhat slower than solids. Milo was always higher and the difference became more pronounced in the older plants.

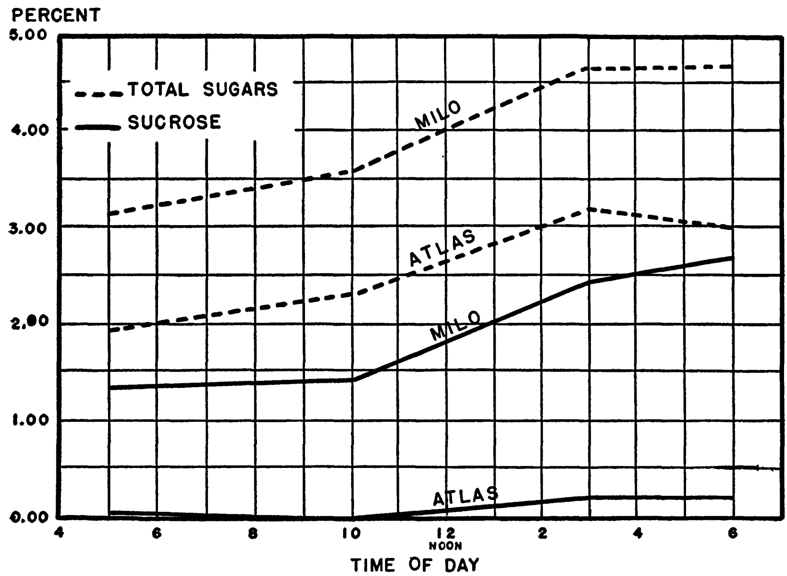

Figure 1. Changes in sugar content of Atlas and Milo press juice over a 24-hour period. Plants 61 days old. (See Table X.)

Comparison of Juice from Leaves and Stalks

As might be expected, the juice of the leaves was somewhat higher in solids over the period of a day than was that from the stalks, although Milo stalks reach nearly as high a percentage late in the afternoon. The ash content of both varieties seems to follow directly the solids content of the juice. As in the previous tests, the ash of Milo was higher in both the leaves and stalks; but this does not seem significant because of its direct relation to the change in solids content. As found in the previous tests, Milo consistently contained more sucrose, both in the leaves and stalks, while only traces occurred in any part of the Atlas. Nitrogen percentages also were higher in Milo and much higher in the leaves than in the stalks. At this stage of growth of the plants (15 inches), Milo was markedly higher in nitrogen; and this probably is a significant difference. Excepting only the greater percentage of chlorides in the Milo, the values do not show any unusual trends.

SUMMARY

1. Plantings of Atlas and Dwarf Yellow Milo sorghums were sampled at various stages of growth over a four-year period. Analyses were not continued beyond the heading stage.
2. The chemical analyses were studied with the purpose of finding if any of the components vary in a manner that can be correlated with resistance or susceptibility to chinch bug injury.
3. Solids content of the juice, generally higher in the Milo, did not seem to show a significant difference.
4. The Milo plants were consistently higher in ash content under all conditions, and this value would seem to merit further consideration.
5. Neither of the acidity measurements (total titratable acidity and pH) showed significant differences. The pH values fluctuated in the range of 5.0 to 5.5, with samplings at the same time for both varieties running close together.
6. Astringency values, including tannins, on the basis of the amounts present, do not seem capable of explaining any of the differences in resistance that are known to occur. Tannin values fluctuated greatly from year to year. The younger plants were highest and the amounts decreased as growth continued.
7. Sugars in both varieties increased progressively at least up until heading time. Although Atlas is known as a sweet sorghum, during the periods under examination Milo plants invariably contained more sugars. The sucrose content of the varieties was markedly different in that Atlas rarely contained any appreciable amounts while Milo always had some and generally contained large amounts. The percentage of sucrose in Milo at late afternoon was always much greater than in the morning and the difference between the two varieties at this time of day seems fundamental. At present, this difference seems more noteworthy than any other found in this study.
8. Marked differences were found in the total nitrogen content of the plants and juices. Atlas normally showed a decreasing content with growth, while Milo percentages increased. Some differences in nitrogen content have been reported elsewhere and work is being continued on these differences.
9. Conductivity studies indicated little variation between the two varieties. More work is in progress to find why such data do not show better correlation with the ash values.
10. Analyses for chlorides were made on many samples, with some indication that the higher values usually found for Milo may be significant.
11. Only a few enzyme analyses are recorded in this paper, but these, together with unpublished data, fail to show any significant correlation between resistance and the catalase, oxidase, or peroxidase activity.
12. Determinations were run for phosphorus, potassium, and calcium on one set of samples and Milo plants were generally found to be higher in all. Such results normally would follow the higher ash content of Milo plants.
13. The hydrocyanic acid content of both varieties was found to be generally alike; and this, together with other observations, seems to exclude this compound from consideration in explaining resistance.
14. Separate analyses of leaves and stalks failed to yield any significant data.

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