COMPARISON OF PLANT AND GRAIN WAX OF TWO VARIETIES OF SORGHUM

by

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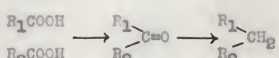
INTRODUCTION

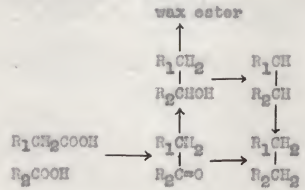
The first work reported concerning a mechanism for the formation of the long chain compounds found in waxes was that of Channon and Chibnall in 1929 (2). They found that the phospholipid fraction of the ether soluble substance of cabbage leaf cytoplasm contained compounds which did not contain phosphorus. This nonphosphorus fraction was found to contain the paraffin, n-nonacosane, and the ketone, 15-nonacosanone. Channon and Chibnall suggested that the paraffin and ketone could be formed by the condensation of two fatty acid molecules to form the ketone which in turn could be reduced to the paraffin.



The formation of the symmetrical 15-nonacosanone by this scheme would necessitate the presence at some time of pentadecanoic acid. The latter, however, has not been isolated from any natural product. In spite of this evidence of the inadequacy of their scheme, Channon and Chibnall felt that the existence of a ketone and paraffin of the same chain length might prove significant in the metabolism of long chain compounds when more information was obtained.

Chibnall et al. (4) used the waxes of forage grasses

since they gave a good yield of wax. However, since the forage grass waxes contained mostly alcohols to the exclusion of paraffins and ketones, they turned to other plant waxes. Apple cuticle wax was found to contain the paraffins, n-nonacosane and n-heptacosane, and the alcohols, d-10-nonacosanol, n-triacontanol, n-octacosanol, and n-hexacosanol. The presence of the secondary alcohol made the picture more complex, but it actually supported the original hypothesis that the long chain compounds are derived from shorter chain fatty acids. An elaboration of the first hypothesis was suggested which gave a scheme with two possible courses of reaction. First, the secondary alcohol is formed as an alternate reduction product of the ketone or, second, the alcohol is formed first with the paraffin being formed from the secondary alcohol via the corresponding olefin.



It was noted that the formation of a double bond with the loss of water such as would occur in this scheme is common in biological reactions. It was also noted that though neither the olefin nor the ketone was found in apple cuticle wax, the scheme

is not necessarily invalid since they may have been present at an earlier stage in the growth of the plant. Chibnall and his co-workers did not have sufficient data to comment on the formation of the primary alcohols. However, they did feel that as close a relationship existed between the primary alcohols and paraffins as seemed to exist between the secondary alcohols and paraffins.

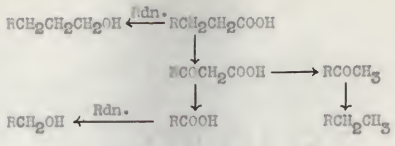
Following the investigation with apple cuticle wax, Sahai and Chibnall (13) studied brussel sprout wax taken from the plants at different stages in plant growth. An attempt was made to show the actual existence of some of the hypothetical intermediate compounds in the formation of the paraffins and other long chain compounds. The brussel sprout wax was found to contain chiefly n-nonacosane, 15-nonacosanone, and 15-nonacosanol. The results of this experiment indicated that the synthesis of the wax began at an early stage in germination and continued throughout the life of at least some of the leaf cells since there was a gradual, but continuous accumulation of the wax. Because of this continuous increase in the wax content, it was believed that the wax is a metabolic by-product, too inert chemically to be further metabolized, rather than an essential component to the physiological activity of the cell. Another fact brought to light was that there is no change in the chemical composition of the wax during the life history of the plant. That is, there is no increase in one component at the expense of another, indicating that if the ketone

is the precursor of the secondary alcohol or paraffin, there is an equilibrium existing between the three substances which is not affected by changes accompanying growth. A third result seen from this experiment was that the slight decrease in the glyceride fatty acids during growth was not sufficient to indicate that these fatty acids could be the starting point in the formation of the longer chain compounds.

Hopkins and Chibnall (8) studied the growth of a mold, Aspergillus versicolor, on higher paraffins to see if they could discover how a living organism attacks a long chain carbon compound having no polar groups when that compound was fed as the sole source of carbon. They found that the mold grew equally well on paraffins containing up to 34 carbon atoms, but would not grow on a paraffin containing 35 carbon atoms. Experimentation on the metabolic end products suggested that the carbon chain had been attacked at several places yielding mostly carbon dioxide and low molecular weight compounds. If the growth of the organism had been restricted by controlling the oxygen supply, the yield of the metabolic products would have been limited so that chemical analysis would have been difficult. Therefore, they used possible metabolic intermediates as the source of carbon for the mold rather than the paraffins. The fact that normal growth occurred on the primary alcohols and ketones whereas no growth occurred on secondary alcohols suggested that in

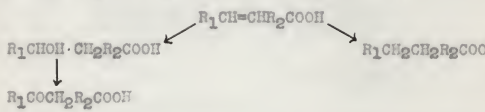
biological oxidation of paraffins, keto groups are introduced at several points in the chain with subsequent disruption of the chain and the formation of shorter fatty acids which are further metabolized through keto acids. It was regarded as highly improbable that the end carbon of a paraffin would be oxidized to form either the corresponding primary alcohol or fatty acid. In a similar study reported in 1941, Bushnell and Haas (1) showed from their experiments that acids and unsaturated hydrocarbons were formed during bacterial decomposition of hydrocarbons.

In 1934 Chibnall et al. (5) published a paper concerning the constituents of many plant and insect waxes. They found that the secondary products such as the ketones and secondary alcohols of the apple cuticle, cabbage leaf, and brussel sprout leaf waxes rarely occurred elsewhere in the plant kingdom. Other plant waxes contained largely primary alcohols with small amounts of fatty acids and paraffins. These findings led them to believe that in their earlier work they had overemphasized the role of the secondary products in the metabolism of the paraffins and that the paraffins were actually more closely related to the long chain fatty acids of the wax phase rather than the shorter glyceride fatty acids. From these later studies Chibnall and Piper (3) presented what was believed to be a more reasonable hypothesis for the metabolism of primary alcohols, paraffins, secondary alcohols and ketones.



This hypothesis was considered to have several strong points. First, it would allow for the synthesis of mixed esters with no restriction on the relative amounts of the acids and alcohols in the wax. This could not be true if the previously upheld Cannizzaro reaction was retained as the only means of formation of the alcoholic and acidic components of the wax esters. Second, it provides for the formation of a series of primary alcohols which, unlike the fatty acids, could not be formed by oxidation of the higher primary alcohols. Third, the hypothesis would uphold the occurrence of primary alcohols and normal fatty acids with an even number of carbon atoms up to 36 carbons and the occurrence of paraffins with an odd number of carbon atoms up to 35 carbons.

The saturated acids, ketonic acids, and hydroxy acids could be formed from the unsaturated acids which in turn were probably synthesized from shorter products.



Dakin (6) showed that such products were biologically interconvertible. Chibnall and Piper (3) refrained from making a

specific suggestion for the formation of the unsaturated acids since the usual positions of the double bonds at carbons 6, 7, 9, 11, 12, 13, and 15 are not entirely reconciled with the idea of oxidation or the idea that the parent acids are acids of six carbon atoms which the followers of Emil Fischer emphasized. Chibnall and Piper did point out, however, that acids with 26 and 28 carbon atoms were found as frequently as those containing 24 and 30 carbon atoms.

Chibnall and Piper (3), in presenting these later schemes for the formation of long chain compounds in plants and insects, noted that variations from one plant to another could be explained by the differences in the functions which the particular compounds performed as suggested by Leathes and Raper (10). The waxes such as cabbage leaf wax which are an integral part of the plant cell are believed to help control the viscosity of the fat phase and might be expected to contain unsaturated acids though as yet this has not been proven. Cuticle waxes such as carnauba and lac wax which probably serve as protection against heat and atmospheric oxidation should necessarily be high melting and saturated. It was suggested also that the long chain compounds are probably not secreted as such by the cell but are metabolized from compounds of lower molecular weight, lower viscosity, and greater solubility which are found in the fat phase of the cytoplasm where these compounds are first made before diffusing to the outside surface. In this same line of thought McNair (11)

showed that the hydrocarbons, acids, and alcohols of waxes from tropical plants had lower melting points, greater molecular weights, and larger empirical formulas than the waxes of the plants in the temperate zone. He also noted that in any one climatic group the wax hydrocarbons have a larger number of carbon and hydrogen atoms per molecule than the alcohols and the alcohols have a larger number of carbon and hydrogen atoms than the acids.

Warth (14), in his recent book, concludes that the metabolism in plants probably involves some of the following reactions. The alcohols assimilate carbon dioxide to form hydroxy acids which can unite with hydrocarbons to form esters. Esters also may be formed by the union of acids and alcohols with the loss of water. The esters in turn may be decarboxylated to form hydrocarbons. This is a reaction often found in plants growing in arid areas. The acids may be formed by the oxidation of the corresponding aldehydes and alcohols.

The volume of literature on waxes has continued to grow since the work of the men mentioned above, but little more has been written about the metabolism of the compounds found in waxes. One possible exception is the speculation of Hall (7) in which he considered the possibility of forming the long chain wax compounds as well as other plant products from the hexose sugars formed during photosynthesis. Since work on the characterization of sorghum grain wax has been carried on in this laboratory for some time, it was decided that a study of

the sorghum plant wax might give an interesting comparison. It was thought that if the wax was taken from plants at different stages in plant growth some information leading to a better understanding of the metabolism and of the period of maximum deposition of the wax might be gained.

EXPERIMENTAL PROCEDURES

The lipids were extracted from samples of Atlas sorghum and Western Blackhull sorghum cut at intervals during plant growth starting before the development of the grain heads. The lipids were extracted with hot solvent and separated into the "wax" and the "remaining lipid fraction" on the basis of solubility. The melting range, acid number, saponification number, and acetyl number were determined on the samples in order to detect any changes during growth in the amount of free acid, free alcohol, hydroxy acid, and/or ester in the wax laid down by the plant.

Preparation of the Samples

Atlas, a forage type sorghum, and Western Blackhull, a grain type sorghum, were planted by the Agronomy Department on the Agronomy Farm at Kansas State College. Nine samples of the Atlas were cut at ground level at approximately one-week intervals from July 22, 1948 to September 20, 1948. Ten sam-

ples of Western Blackhull were cut, also at ground level, at similar intervals from August 9, 1948 to October 25, 1948. The grain heads were apparent within the boot at the August 6 cutting, the third cutting, of Atlas and at the August 16, the second cutting, of Western Blackhull. The samples were dried at room temperature for one week and were then stored at 6° C. until the time of extraction.

Some of the same seed used by the Agronomy Department was planted without fertilizer in vermiculite in the greenhouse. The seedlings were cut at ground level after 15 days' growth when they were three to four inches tall. The cuttings were dried at 65° C. for 17 hours before extraction.

The waxes from the grain heads and the rest of the plant were extracted separately with hot Skellysolve B in percolators and a large Soxhlet extractor after being chopped in a Wiley mill without a screen. Since there appeared to be a greater amount of wax on the stalks under the leaf sheath than on the leaves, the leaves including the leaf sheath were separated from the stalks for separate extraction for the final samples, the harvest cutting, of each type of sorghum. After extraction the volume of the lipid solution was reduced to about one-eighth of the volume of the solvent used in extraction. The extracts were chilled to -20° C. and filtered cold. The material which crystallized from cold Skellysolve B was taken as "wax" and the residue left after removal of the sol-

vent from the filtrate was taken as the "remaining lipid fraction". The wax and the remaining lipid samples thus obtained were dried in a vacuum oven at 60° C. for 14 hours.

Melting Range

The melting range of a wax sample was determined by packing the pulverized wax into the sealed end of a capillary tube. The latter was attached to a thermometer and placed in a Thiele tube. The temperature of the oil bath was allowed to rise at a rate of not more than two degrees per minute. The temperature at which the wax first began to soften to the temperature at which the wax was completely melted was taken as the melting range.

Acid Number

The acid number is the number of milligrams of potassium hydroxide necessary to neutralize the free acids in one gram of sample.

In the determination of the acid numbers 0.2 to 0.5 gram samples were used depending on the color intensity of the particular sample. The weighed sample was placed in a 250 ml Erlenmeyer flask which was equipped with a 50 ml buret and a side arm with a reflux condenser attached so that the determination could be carried on without vaporization of the hot

solvent into the room. Fifty ml of 95 percent ethanol and 10 ml toluene were used as the solvents. The mixture was heated to boiling and titrated hot to a phenolphthalein end point of 10 seconds with 0.05 N aqueous sodium hydroxide using a magnetic stirring device. A blank determination was carried out on the solvents used. The acid number was calculated in the following manner.

$$\text{acid number} = \frac{(V_s - V_b)(N)(56)}{w}$$

where V_s is the volume of the sodium hydroxide used of the sample, V_b is the volume of base used for the blank, N is the normality of the sodium hydroxide, and w is the weight of the sample. Two determinations were made on each sample and the average was taken as the acid number.

Saponification Number

The saponification number is the number of milligrams of potassium hydroxide necessary to saponify one gram of sample.

The method of Knight (9) was used with a few modifications. In the determination of the saponification numbers 0.29 to 0.5 gram samples were placed in 250 ml Erlenmeyer flasks and refluxed for one hour with 20 ml 0.5 N alcoholic potassium hydroxide and 20 ml diethylene monoethyl ether. The excess potassium hydroxide was back titrated while hot with 0.5 N HCl to the disappearance of the phenolphthalein end point. After

the apparent end point had been reached the mixture was heated to boiling again to redissolve the precipitated wax and then retitrated. A blank determination was carried out on the reagents. The saponification number was calculated in the following manner.

$\frac{(V_b - V_s)(N)(56)}{w}$ = saponification number, where V_b is the volume hydrochloric acid needed to neutralize the blank, V_s is the volume HCl needed to neutralize the excess NaOH in the sample, N is the normality of the HCl, and w is the weight of the sample. Two determinations were made on each sample and the average of the two determinations was taken as the saponification number.

Acetyl Number

The acetyl number is the number of milligrams of potassium hydroxide needed to neutralize the acetic acid obtained by saponifying one gram of acetylated sample.

A modification of the Roberts and Schuette (12) method was used. An approximately 0.5 gram sample was weighed carefully and placed in a reaction tube which consisted of a piece of 12 mm pyrex tubing about 12 cm long which had been sealed at one end. The wax was weighed as pellets and the remaining lipid fraction was melted and pipetted into the tube in an attempt to obtain a uniform sample. About 0.15 ml freshly distilled acetic anhydride was added to the sample. The tube was

then drawn out so that it could be sealed without prolonged heating, to give a tube about 10 cm long. After reweighing the tube with the acetic anhydride, the tube was sealed and placed in hot water until the wax melted. The mixture was then shaken and allowed to acetylate in an oven at 130 C. for one hour. When the tube was cooled, it was broken in the middle and placed in a 500 ml Erlenmeyer flask with 50 ml water and heated under reflux until the wax had melted. Twenty-five ml standard 0.05 N carbonate free NaOH was added through the condenser and rinsed down with water. The mixture was again heated to boiling to hydrolyze the excess acetic anhydride. The mixture was then cooled and titrated with more NaOH to a phenolphthalein end point. It was necessary to reheat, cool, and retitrate the mixture in order to reach neutrality. A blank determination on the sample without acetic anhydride was made. The acetic anhydride was standardized by treating it in the same manner as indicated above except for the absence of the sample. The acetyl number was calculated in the following manner.

$$\text{acetyl number} = \frac{[(W \cdot l) - V_s](m)}{w_1} - \frac{(V_b)(m)}{w_2}$$

where W is the weight of the acetic anhydride used, l is the ml NaOH per gram of acetic anhydride, V_s is the volume of NaOH needed to neutralize the excess acetic anhydride, m is the mg KOH equivalent to one ml NaOH, V_b is the volume of NaOH needed to neutralize the blank sample, w_1 is the weight of the sample,

and w_2 is the weight of the blank. Two determinations were made on each sample and the average of the two values obtained was taken as the acetyl number.

RESULTS

The experimental results obtained from the waxes extracted from plants taken at different stages in plant growth are given in Table 1. Graphical expressions of the chemical constants of the same samples are given in Figs. 1, 2, 3, and 4. There are some significant facts which can be pointed out from these data. There appeared to be a considerable amount of wax on the plants at an early stage in germination. Apparently the wax was laid down throughout the growth of the plant and reached a somewhat constant percentage about the time that the grain heads became apparent.

In addition to the increase in the amount of the wax, there were some definite changes in the chemical constants of the waxes. The acetyl number of the Western Blackhull grain wax, the Western Blackhull plant wax, and the Atlas plant wax decreased during the period of growth studied. The acetyl number of the Atlas grain wax on the other hand increased during the same period to a value almost equal to that at which the acetyl number of the Atlas plant wax started to decrease. The amount of free acids, as indicated by the acid number, remained almost constant for both types of grain

wax. There was a slight increase in the fatty acid content of the Atlas plant wax. The Blackhull plant wax in contrast to the other waxes showed a marked increase in acid content in the early part of the growth period followed by a gradual decrease in the acid content during the later period of growth. There seemed to be the greatest lack of consistency in the changes in the ester content of the waxes, as indicated by the difference between the saponification number and the acid number. The ester content of the Western Blackhull grain wax gradually increased during the period studied. In the Atlas grain wax the ester content increased and decreased again almost to the starting level during the last week of growth. The amount of ester in the Western Blackhull plant wax increased rapidly in the first stages of growth and varied within 10 points of the highest level during the remaining period of growth. The Atlas plant wax showed only a very slight increase in the amount of ester present during the first part of the period studied followed by a decrease and another slight increase during the last week of growth. In spite of the changes in the amount of the wax and the changes in the chemical nature of the waxes, the melting ranges for the various samples showed little variation.

It was mentioned in the section on experimental procedures that the harvest cutting of each type of sorghum was divided for separate extraction of the leaf, stalk, and grain wax. The experimental data on these waxes and that on carnauba

wax are given in Table 2. It can be seen that the carnauba wax had a slightly higher melting range than the sorghum waxes which all melted in about the same range with the possible exception of the Atlas grain wax. The most apparent difference among the sorghum waxes was the differences in the acid numbers which were highest in the leaf wax and lowest in the grain wax. It should also be noted that while the sorghum leaf waxes and the carnauba wax had similar saponification numbers, the amount of ester present in the carnauba wax was much greater than in any of the sorghum waxes as evidenced by the low acid number of the carnauba wax. There was some variation in acetyl numbers which should not be overlooked. The grain wax in each case had the highest acetyl number whereas the stalk wax had the lowest acetyl number. The acetyl number of the carnauba wax was similar to that of the Western Blackhull grain wax, the Western Blackhull leaf wax, and the Atlas leaf wax.

The experimental data from the work done on the remaining lipid fraction, after wax extraction, are given in Table 3. This lipid fraction would contain mostly triglycerides, pigments, and possibly some long chain wax components which did not precipitate from cold Skellysolve B.

GENERAL DISCUSSION AND CONCLUSIONS

It appeared from the percentages by weight of the waxes that the wax was laid down throughout the growth of the plant and accumulated to a somewhat constant level which was reached about the time that the grain heads became apparent. Either the wax was synthesized at a constant rate after a certain stage in growth, or the wax in excess of a certain amount was brushed off by some physical means such as wind, hail, or rain. This finding is in contrast to that of Sahai and Chibnall (13) that brussel sprout wax accumulates throughout the life history of the plant. The contrast may be accounted for by the fact that they were dealing with a cytoplasmic wax which would not be subjected to the same physical conditions as a cuticle wax such as dealt with in these experiments.

In addition to the change in the amount of wax, the chemical constants indicate that there were definite chemical changes in the wax during growth. This would mean that the intermediate compounds and the final components of the wax were not in equilibrium with each other as concluded by Sahai and Chibnall (13). The apparent changes in the sorghum waxes were not necessarily similar in the plant and grain wax nor were they similar from one variety to another. Such a situation does not make the understanding of the metabolism of the wax components easier. However, it does emphasize the idea

that the wax of each part of the plant, though possibly serving the same protective function, is synthesized completely or in part by the cells at the site of its secretion. The character of the wax in this case would probably be determined by the type of cell in which the components were made and the age of the wax as well as by varying light, heat, and moisture received by the plant. This line of thought is compatible with the fact that water insoluble fatty and waxy substances are not translocated in the plant so that such substances would be characteristic of the cell in which they were made. It is also in harmony with the idea that probably the wax itself is synthesized outside the cell after components of lower viscosity and higher solubility in the fat phase of the cytoplasm have been secreted from the cell.

The conclusions of Chibnall and Piper (3) and the comments of Warth (14) on the metabolism of waxes indicate that considerable interconversion among the wax components is possible. Some of these possible relationships seemed to be illustrated by the changes in the chemical constants of the sorghum waxes. The changes in the Western Blackhull grain wax appeared to illustrate one of the most conventional chemical reactions in wax metabolism. The ester seemed to be formed continually at the expense of the alcohols, as represented by the acetyl numbers, and the acids. From the very slight decrease in the amount of the alcoholic components and the fact that the amount of free acidic components remained

almost constant, it would seem that they were being synthesized and used at about the same rate throughout the period studied. The slight decrease in the alcoholic components may mean that they were being used in the formation of acids as well as in the formation of the esters.

Instead of a continuous increase in the amount of ester, a sudden decrease was seen in the Atlas grain wax. This may be explained as being caused by a decarboxylation of the esters yielding hydrocarbons and carbon dioxide. Like the Western Blackhull grain wax acids, the Atlas grain wax acids seemed to be formed and used at the same rate. Since the alcoholic content went up as the ester content decreased, it would appear that the alcohols were continually synthesized, perhaps from the acidic components by reduction, even though they were no longer needed for ester formation.

The Atlas plant wax furnished another instance where decarboxylation of the ester might have taken place. In contrast to the Atlas grain wax, the amount of alcoholic components decreased. This may mean that the alcoholic components were not formed throughout growth and that they were used in ester formation and possibly in acid formation via hydroxy acids since there was some increase in the amount of free acid in the Atlas plant wax. Though the changes in the Western Blackhull plant wax appeared to be more irregular, they did seem to indicate that the acidic and alcoholic com-

ponents were made continuously throughout growth, but that at some periods they were used for ester formation more rapidly than they were made. It should be noted that any evaluation of the changes in the plant wax is even more speculatory in nature than that for the grain wax since the plant wax was composed of both stalk and leaf wax which are shown in Table 2 to be different and might be expected to exhibit different changes during growth.

Not only were the changes in the plant and grain wax different during growth, but the separate analysis of the leaf, grain, and stalk wax from the harvest cutting of the two varieties of sorghum indicated that the waxes from the different parts of the plant differed chemically and in amount of wax. Here again the type of cell at the site of the secretion of the wax components may in part determine the character of the wax. Another fact which may account for the difference between the leaf and stalk wax which have similar cells is that the stalk wax is partly covered by the leaf sheath and thus is not as exposed to atmospheric conditions which might influence the chemical changes in the wax. The matter of exposure to climatic conditions also may be used to rationalize the large amount of ester found in carnauba wax as compared with any of the sorghum waxes. The carnauba wax which is formed on a plant having a longer life and growing in a warmer climate than the sorghums might be expected to form a greater amount of some of the end products of wax metabolism.

From the foregoing discussions and conclusions it may be said that changes during growth and differences in waxes probably were governed by the cells from which their components were secreted, the age of the wax, the species of the plant, the variety of the plant, and the climatic conditions which affected the plant.

SUMMARY

1. A study was made of the plant and grain waxes of Atlas sorghum and Western Blackhull sorghum at different stages in plant growth in order to gain information leading to an understanding of the metabolism of the wax components. The leaf, grain, and stalk waxes were compared with each other and with carnauba wax.

2. The percent by weight of wax, the melting range, acid number, acetyl number, ester number, and saponification number were determined for samples of the waxes extracted with hot Skellysolve B from plants cut at intervals from about one week before the appearance of the grain head to harvest.

3. It was found that the grain and plant waxes of Atlas and Western Blackhull were laid down throughout the growth of the plant and accumulated to a somewhat constant level reached about the time that the grain heads became apparent.

4. There appeared to be definite chemical changes in the wax during the growth period studied.

5. The waxes laid down on the leaf, stalk, and grain differed chemically and in amount from each other.

6. The changes during growth and differences in the waxes probably were governed by the cells from which their components were secreted, the age of the wax, the species and variety of the plant, and the climatic conditions affecting the plant.

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APPENDIX

Table 1. Data on waxes taken from plants at various stages of plant growth.

Sample	Percent by weight	Melting range	Acid no.	Acetyl no.	Ester no.	Saponification no.
Plants						
Atlas I	0.08	77.6-79.1	*	*	*	*
II	0.12	72.6-76.5	33.18	60.64	21.95	55.13
IV	0.21	72.0-74.8	37.44	38.47	22.84	60.28
VI	0.18	73.1-76.4	36.59	35.38	15.55	52.14
VIII	0.18	70.8-76.2	39.23	31.45	18.50	57.73
Seedlings						
Dry weight	1.10	70.5-82.5	*	*	*	*
Fresh	0.17					
Western Blackhull						
I	0.22	77.2-79.1	24.13	52.82	8.44	32.57
III	0.31	73.1-76.2	48.24	50.47	28.36	76.60
V	0.34	71.8-74.1	40.86	38.44	38.82	79.30
VII	0.34	72.7-77.2	39.94	36.54	29.86	69.80
IX	0.25	67.7-76.7	38.54	33.23	34.39	72.93
Seedlings						
Dry weight	1.24	73.1-82.5	*	*	*	*
Fresh	0.20					
Grain						
Atlas II	**	**	**	**	**	**
IV	0.16	63.5-69.1	*	*	*	*
VI	0.21	76.2-78.6	9.26	41.20	18.31	27.57
VIII	0.37	77.2-78.8	9.68	42.63	36.36	46.04
IX	0.31	77.0-80.7	9.22	58.89	22.18	31.40
Western Blackhull						
I	**	**	**	**	**	**
III	0.15	69.1-74.1	*	*	*	*
V	0.32	78.7-80.4	11.89	50.58	18.07	29.96
VII	0.32	76.7-79.1	11.07	49.67	25.92	36.99
IX	0.28	78.4-80.2	11.75	46.44	30.23	41.98
X	-	76.4-77.8	12.25	45.46	36.76	49.01

* No chemical constants determined because of small amount of wax obtained. It was included in the table to show the weight relationship.

** No grain head apparent at this cutting.

Table 2. Comparative data on sorghum waxes and carnauba wax.

Sample	Percent by weight	Melting range	Acid no.	Acetyl no.	Ester no.	Saponification no.
Atlas leaf	0.60	75.2-77.8	50.37	42.09	28.20	78.57
Atlas stalk	0.14	68.9-77.2	22.75	26.64	23.65	46.40
Atlas grain	0.31	77.0-80.7	9.22	58.89	22.18	31.40
Western Blackhull leaf	0.41	73.1-77.2	56.39	38.76	28.30	84.69
Western Blackhull stalk	0.13	74.1-77.2	23.09	27.21	33.66	56.75
Western Blackhull grain	-	76.4-77.8	12.25	45.46-	36.76	49.01
Carnauba	-	80.0-84.0	7.18	39.40	70.82	78.00
			± 1.13	± 5.4		

Table 3. Data on sorghum lipid residue at various stages in plant growth.

Sample	Percent by weight	Acid no.	Acetyl no.	Ester no.	Saponification no.
Plants					
Atlas II	0.17	72.45	22.72	51.89	124.34
IV	0.10	88.79	11.64	54.84	143.63
VI	0.10	78.38	22.92	55.32	134.75
VIII	0.10	51.68	21.14	35.58	87.26
Stalk IX	0.03	47.09	no sample	35.30	82.39
Leaf IX	0.75	33.60	38.36	44.09	77.69
Seedlings Dry weight	0.85				
Western Blackhull					
I	0.29	61.10	36.46	41.75	102.85
III	0.18	68.27	22.62	29.82	98.09
V	0.21	78.05	22.22	39.38	117.43
VII	0.19	48.49	28.66	58.00	106.49
IX	0.05	61.30	34.86	37.60	98.90
Leaf X	0.38	29.38	37.06	85.33	116.33
Stalk X	0.09	31.32	31.36	80.79	112.11
Seedlings Dry weight	0.90				
Grain					
Atlas II	**	**	**	**	**
IV	0.25	*	*	*	*
VI	0.21	24.24	17.02	92.99	117.23
VIII	0.11	19.37	19.16	116.34	135.71
IX	0.19	14.91	10.63	114.45	129.36
Western Blackhull					
I	**	**	**	**	**
III	0.29	*	*	*	*
V	0.22	30.37	23.24	63.57	93.94
VII	0.07	19.47	13.15	118.93	138.40
IX	0.33	19.33	16.72	122.73	142.06
X	-	22.29	17.84	126.09	148.38

* Insufficient sample at this cutting.
** No grain head apparent at this cutting.

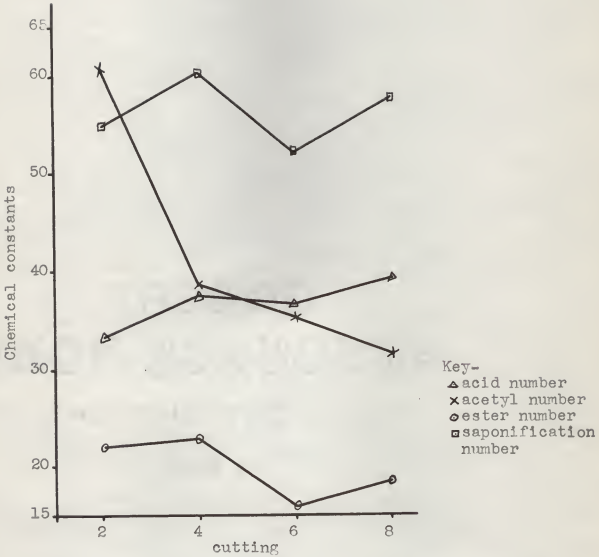

Fig. 1. Chemical constants of Atlas plant wax at various stages in plant growth.

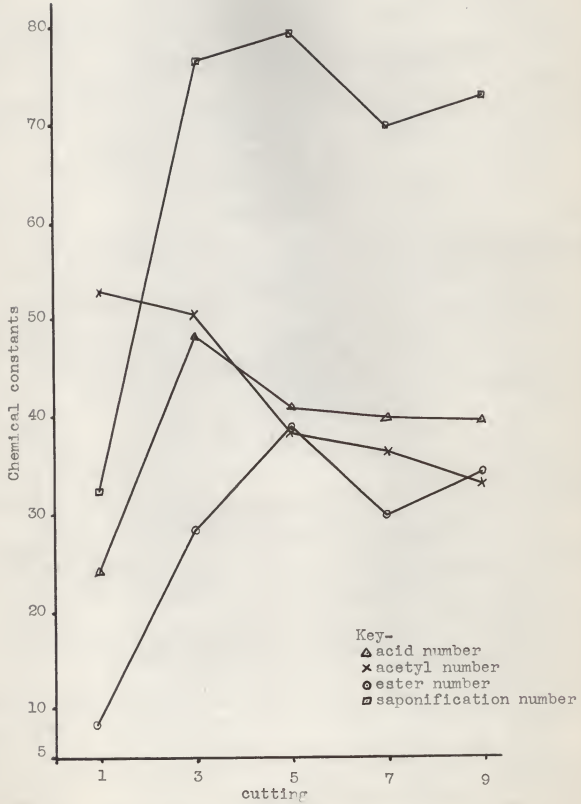

Fig. 2. Chemical constants of Western Blackhull plant wax at various stages in plant growth.

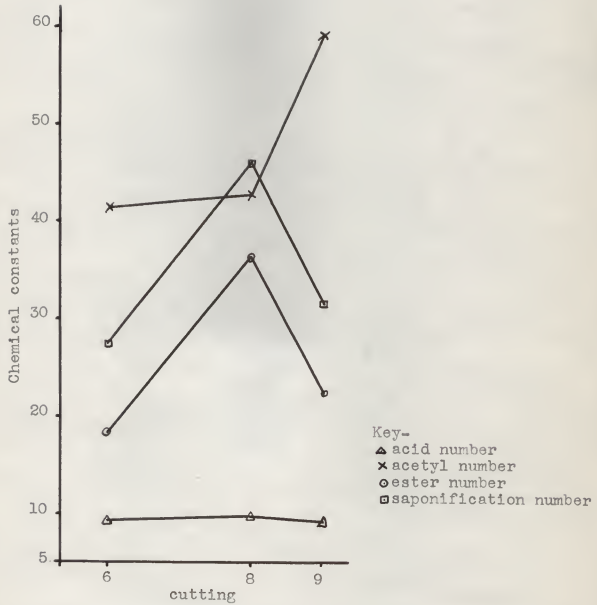

Fig. 3. Chemical constants of Atlas grain wax at different stages in plant growth.

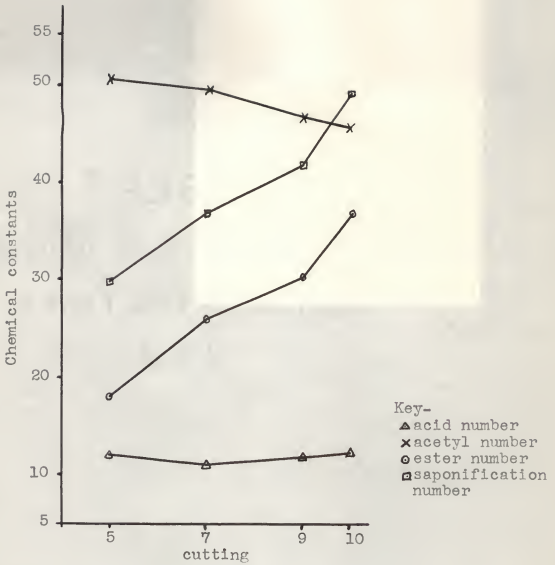

Fig. 4. Chemical constants of Western Blackhull grain wax at various stages in plant growth.