A Comparison of Plant and Grain Wax from Two Varieties of Sorghum

CYNTHIA CANNON [1] and FRED A. KUMMEROW,[2] Department of Chemistry,[3]
Kansas State College, Manhattan, Kansas

IT HAS BEEN SHOWN that as much as 0.5% wax may be obtained from whole sorghum grain (1, 2, 3). This wax appears to be similar in some respects to carnauba wax (4). In the course of these studies it was noted that the leaves and stalks of the plant were covered with a white, loosely bound wax. The present work was undertaken to compare this plant wax with the grain wax in respect to the amount of wax. The waxes used in these experiments were obtained from a forage (Atlas) and a grain type of sorghum (Western Blackhull), which were cut at various stages in plant growth so that the period of maximum deposition and changes in chemical composition of the wax during growth could be noted.

Experimental

Samples of a forage type of sorghum were cut at ground level at about one-week intervals from the ninth to the seventeenth week of growth, and samples of a grain type sorghum were cut in a similar manner from the eighth to the nineteenth week of growth. These cuttings were started about one week before the appearance of the grain head within the boot. The plants were dried at room temperature for one week and stored at 60°C. until they could be extracted.

Some of the same seed was planted, without fertilizer, in the greenhouse. The seedlings were cut at ground level after 15 days of growth, at which time they were about 3 or 4 in. tall. For comparison several stalks of corn (SM2) grown in the greenhouse were cut in the hard-dent stage, a stage in plant growth comparable to the last cutting of both types of sorghum.

The waxes from the sorghum grain heads and the remainder of the plant were extracted separately with hot Skellysolve B. The heads or stalks and leaves were chopped in a Wiley mill without a screen and extracted in percolators or a Soxhlet extractor having a capacity of 5 liters. Since there appeared to be a greater amount of wax under the leaf sheath than on the leaves, the leaves, including the leaf sheath, were separated from the stalks in the final cuttings of each type of sorghum and extracted separately. The mature corn was treated in the same manner. The last cuttings of sorghums, the corn, and the sorghum seedlings were dried at 65°C. overnight. After extraction the volume of the lipide solution was reduced to about 1/8 of the volume of solvent used in extraction. The extract was chilled to −20°C. and filtered cold. The material which crystallized from cold Skellysolve B was taken as wax. The waxes thus obtained were dried in a vacuum oven at about 65°C. over-night to remove the solvent.

The melting point range, acid, acetyl, saponification, and the ester number were determined on each wax sample. The melting-point range was determined by the method of Shriner and Fuson (5). The acid number was determined with a 0.2- to 0.5-g. sample, depending on the color intensity of the particular sample and using 10 ml. of toluene and 50 ml. of 95% ethanol as solvents. The mixture was heated to boiling in a two-neck flask, equipped with a burette and a reflux condenser, and titrated at about 75°C. with 0.05N NaOH. The saponification and the acetyl number were determined according to the method of Knight (6), Roberts and Schuette (7), respectively. For the acetyl number the reagents and reaction-tube size were adjusted for the use of a 0.5-g. instead of a 2.5- or 5.0-g. sample.

Results

The results indicated that the highest percentage of wax was present at an early stage in growth (Table I). The corn leaves showed a little over half as much wax as the sorghum leaves at the same stage in growth. The corn stalks, on the other hand, had only a fourth or a sixth as much wax as the sorghum stalks. In addition to the change in the amount of wax there were definite changes in the chemical constants of the

TABLE I

Characteristics of Waxes

Sample from planting time	Amount of wax	Melting-point range	Acetyl number	Ester number	Acid number
Plants	%	°C.			
Atlas-forage type of sorghum			58.0		
Seedlings	1.10	70.5–82.5	*	*	*
9th week	0.08	77.6–79.1	*	*	*
10th week	0.12	72.6–76.5	58.0	21.9	33.2
12th week	0.21	72.0–74.8	37.4	22.8	37.4
14th week	0.18	73.1–76.4	34.4	15.5	36.6
16th week	0.18	70.8–76.2	30.7	18.5	39.2
17th week					
Mature leaf	0.60	75.2–77.8	41.2	28.2	50.4
Mature stalk	0.33	68.9–77.2	26.1	23.7	22.8
Western Blackhull-grain type of sorghum					
Seedlings	1.24	73.1–82.5	*	*	*
8th week	0.22	77.2–79.1	50.8	8.4	24.1
10th week	0.31	73.1–76.2	48.6	28.3	48.2
13th week	0.34	71.8–74.1	37.2	38.8	40.9
15th week	0.34	72.7–77.2	35.6	29.9	39.9
17th week	0.25	67.7–76.7	34.4	34.4	38.5
19th week					
Mature leaf	0.56	73.1–77.2	37.7	28.3	56.6
Mature stalk	0.48	76.4–77.8	26.7	33.7	23.1
Grain					
Atlas-forage type of sorghum					
12th week	0.16	63.5–69.1	*	*	*
14th week	0.21	76.2–78.6	40.0	18.3	9.3
16th week	0.37	77.2–78.8	41.3	36.4	9.7
17th week	0.31	77.0–80.7	56.4	22.2	9.2
Western Blackhull-grain type of sorghum					
10th week	0.15	69.1–74.1	*	*	*
13th week	0.32	78.7–80.4	48.7	18.1	11.9
15th week	0.32	76.7–79.1	47.9	25.9	11.1
17th week	0.28	78.4–80.2	44.9	30.2	11.8
19th week	—	76.4–77.8	44.0	36.8	12.2
Corn SM2					
Mature leaf	0.35	68.0–76.0	*	*	*
Mature stalk	0.07	*	*	*	*
Carnauba–fatty gray	—	80.0–84.0	38.3	70.8	7.2

* Insufficient sample for further investigation.

[1] Submitted by Cynthia Cannon as partial fulfillment for the M.S. degree in chemistry while an assistant at Kansas State College, Manhattan, Kans.

[2] Present address: Department of Food Technology, University of Illinois, Urbana, Ill.

[3] Contribution No. 388.

waxes. The amount of free acids remained almost constant for both types of grain wax. There was a slight increase in the free-acid content of the forage type of plant wax and a marked increase in the free-acid content followed by a gradual decrease during the later period of growth in the grain type of plant wax.

The acetyl number of both the plant and grain wax of the grain type of sorghum and the plant wax of the forage type of sorghum decreased from 5 to 30 points during growth. The acetyl number of the forage type of grain wax, on the other hand, increased from 40.0 during the same period to a value almost equal to that at which the acetyl number of the forage type of plant wax started to decrease.

The grain waxes had the highest acetyl numbers with 56.4 for the forage type and 44.0 for the grain type of sorghum, and the stalk waxes had the lowest acetyl numbers, or approximately 26, for both types of sorghum. The acetyl number of carnauba wax, which was 38.3, was similar to that of the grain and leaf wax of the grain type of sorghum and the leaf wax of the forage type of sorghum. It is interesting to note also that the sorghum leaf waxes had saponification numbers equivalent to that of carnauba wax (78.0) but had much less ester than the carnauba wax, as evidenced by the fact that the carnauba wax had a much lower acid number than the sorghum leaf waxes.

The ester content of the grain wax from the grain type of sorghum gradually increased from 18.1 to 36.7 during the period studied. The ester content of the grain wax from the forage type of sorghum increased to a similar extent and then decreased almost to the starting level during the last week of growth. The amount of ester in the plant wax from the grain type of sorghum increased rapidly from 8.4 to 38.8 and then varied within 10 points of the highest level during the remaining period of growth. The plant wax from the forage type of sorghum showed only a slight increase from 21.9 to 22.8 in the amount of ester present during the first part of the period studied, followed by a decrease to 15.5 and another slight increase to 18.5 during the last week of growth. Even though the amount of wax and its chemical composition showed changes during growth, there was little variation in the melting-point ranges. The corn leaf wax, which was sticky and more resinous than the sorghum waxes, melted in about the same range as the sorghum waxes.

Most apparent among the sorghum leaf, stalk, and grain waxes of the final cutting were the differences in the acid numbers, which were highest in the leaf waxes at 50.4 for the forage type and 56.4 for the grain type of sorghum and lowest in the grain waxes at 9.2 and 12.3, respectively.

Discussion

Wax was synthesized at a constant rate, which was proportional to the rate of growth after the appearance of the grain heads or after the wax in excess of a certain amount was brushed off by physical means. This finding does not entirely agree with that of Sahai and Chibnall (8), who found that brussels sprout leaf wax accumulates throughout the life history of the plant. This difference may be accounted for by the fact that Sahai and Chibnall were dealing with a cytoplasmic wax which would not have the same function or be subjected to the same physical conditions as a cuticle wax such as sorghum wax.

The chemical changes in the wax indicated that the intermediate compounds and the final components of the wax were not in equilibrium with each other, as suggested by Sahai and Chibnall (8). 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. The chemical changes in the waxes and the differences in the waxes from the various parts of the plant seem to emphasize that the wax is synthesized by the cells at the site of its secretion. The character of the wax is determined by the cell in which the components were made, the age of the secreted components, and the climatic conditions affecting the plant.

Summary

A study was made of the wax from a forage and a grain type of sorghum taken at different stages in plant growth in order to compare the amount and the chemical composition of the wax laid down at different stages in the development of the plant. The sorghum leaf, grain, and stalk waxes were compared to each other and to carnauba and corn wax.

It was found that the grain and plant waxes of both types of sorghum were laid down throughout the growth of the plant. A constant level was reached about the time that the grain heads became apparent. There appeared to be definite chemical changes in the wax during the growth period studied. These changes were not necessarily similar from one part of the plant to another or from one variety to another. The waxes laid down on the leaf, grain, and stalk differed from each other in quantity and in chemical composition.

Acknowledgment

Financial support for this work was furnished by the Kansas Industrial Development Commission. The authors wish to express their appreciation to L. A. Tatum and D. E. Weibel for their help in providing the sorghums and corn and to C. O. Johnston for his help in growing the sorghum seedlings.

REFERENCES

1. Kummerow, F. A., Oil and Soap, *23*, 167–170 (1946).
2. Kummerow, F. A., Oil and Soap, *23*, 273–275 (1946).
3. Kummerow, F. A., Barham, H. N., and King, H. H., The American Miller and Processor, *75*, 94–95, 143 (1947).
4. Bunger, W. B., and Kummerow, F. A., J. Am. Oil Chem., *28*, 121–123 (1951).
5. Shriner, R. L., and Fuson, R. C., 3rd ed., John Wiley and Sons Inc., New York (1948).
6. Knight, B. H., Ind. Eng. Chem., Anal. Ed., *19*, 359 (1947).
7. Roberts, W. L., and Schuette, H. A., Ind. Eng. Chem., Anal. Ed., *4*, 257–259 (1932).
8. Sahai, P. N., and Chibnall, A. C., Biochem. J., *26*, 403–412 (1932).

[Received March 11, 1957]