

and 10 ml. of 5*N* nitric acid in hot water and diluting to 100 ml.

The bromide equivalent of the potassium thiocyanate solution is obtained by standardizing with 5 ml. of 0.0100*N* potassium bromide. Boiling under reflux has been found unnecessary in the blank and standardization. Then

$$\text{Mg. of ethylene dibromide} = 1.879 \times 5 \times \frac{T_B - T_D}{T_B - T_S}$$

and

$$\text{Mg. of ethylene chlorobromide} = 1.434 \times 5 \times \frac{T_B - T_D}{T_B - T_S}$$

where T_B , T_D , and T_S are the respective titers obtained in the blank, determination, and standardization.

Recovery Tests

Purified ethylene dibromide, boiling at 131.1–131.2° C., was obtained by fractional distillation through a spinning band column. Ethylene chlorobromide, boiling at 106–108° C., was prepared by the method of Calingaert *et al.* (7) and separated by distillation through a packed column of 10 theoretical plates.

The weighed sample was sealed in a thin-walled capillary tube which was introduced into the sampling bulb and broken by shaking. The bulb was warmed in the vicinity of the broken tube to ensure complete vaporization and subsequently cooled before introduction of the ethyl alcohol.

The mean per cent recovery was 99.5 ± 1.3 for ethylene dibromide and 99.4 ± 1.6 for ethylene chlorobromide. The results are given in Tables I and II.

Acknowledgment

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COTTONSEED CONTENTS VARIATION

Influence of Variety and Environment on Oil Content of Cottonseed Kernels

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The oil contents of moisture-free cottonseed kernels from the seed of 8 commercial varieties of cotton grown at 13 locations during 3 years ranged from 26.8 to 43.4%. Analysis of variance indicated that both variety and environment have a highly significant influence on the oil content. Correlations on a basis of years in locations between oil content of the kernels and temperature for six periods of boll and seed development showed that the highest correlation was obtained for mean maximum temperature during the maturation period. Similarly, the highest correlation between oil content and rainfall for rain-grown cottons was obtained for the same period. Rainfall had a significant influence on oil content even when temperature was held constant, and maximum temperature significantly influenced oil content when rainfall was held constant. Maximum temperature and rainfall appeared to be of nearly equal importance in influencing oil content under the conditions of the investigation. A considerable part of the environmental variation in oil content is due to other factors. There was no statistically significant difference in the response of any of the varieties to variations in temperature and rainfall.

AS COTTONSEED OIL is next to lint in economic importance among the products of the cotton plant, information on the factors influencing the oil content of the kernels is of both interest and value to oil mill operators, geneticists, agronomists, and others associated with the cottonseed industry. The present communication reports findings regarding the influence of variety of cotton and environment during seed development

on the oil content of cottonseed kernels, which are a portion of the results of a symmetrically planned investigation of the factors influencing the composition of cottonseed.

Though numerous reports pertaining to the oil content of cottonseed have appeared in the literature, only a few are cited here. Most of these have dealt with analyses of commercial samples from a limited geographic area (2, 8, 9)

or investigations at various experiment stations, where most of the samples of seed were from cottons grown in individual states (4, 6, 7, 15). Although such investigations allow certain trends to be observed, such as the increase in oil content when cotton receives an unusual amount of rain during the growing period, the data are difficult to interpret satisfactorily, because they are limited in geographic scope or

represent the composite effects of varietal and ecological factors. In addition, the oil determinations were usually made on ginned seed or acid-delinted seed, with the variable amounts of residual lint and hulls in the sample influencing the results.

Several investigators (11, 13, 16) have undertaken cottonseed studies of greater scope. The study of Tharp *et al.* (16) was principally concerned with yield and composition as influenced by fertilization. Of particular interest was their observation that increase in potassium supply to the cotton plants usually occasioned a marked increase in percentage of oil in the cottonseed, whereas an increase in nitrogen supply resulted in a slightly lowered percentage of oil. Sievers and Lowman (13) reported oil contents of moisture-free

Table I. Oil Content of Moisture Free Cottonseed Kernels

(8 varieties grown at 13 locations during 3 years)

Variety	Oil in Moisture-Free Kernels, %			Standard deviation
	Low	High	Mean	
Acala 4-42	27.6	41.2	35.4	2.8
Acala 1517W	32.4	42.7	37.7	2.4
Rowden 41B	29.4	43.1	36.5	2.8
Mebane,				
Watson's	28.8	41.0	35.8	2.5
Stoneville 2B	28.4	43.4	37.4	3.0
Deltapine 15	26.8	42.4	35.6	3.0
Coker 100W	28.3	43.3	36.5	3.4
Coker Wilds	29.6	42.6	35.9	3.3
All samples	26.8	43.4	36.4	3.0

kernels (calculated from seed analyses) ranging from 24.10 to 47.46%, and pointed out that the oil content of the seed depended upon two factors—the percentage of kernels in the seed and the percentage of oil in the kernels. These investigators concluded that oil content of the kernels is undoubtedly a varietal characteristic, as it appeared to have no relationship to the geographic source of the seed. Pope and Ware (11), on the other hand, analyzed delinted seed from 16 varieties grown for 3 years at from 11 to 14 locations and observed that oil content was generally more significantly affected by location of growth than by variety, although the ranking of varieties with respect to oil content tended to be rather consistent when the same group was grown under a wide range of environmental conditions.

Samples and Methods of Analysis

The cottonseed used in this investigation were from eight commercial varieties of cotton grown experimentally at 13

Table II. Mean Oil Contents of Moisture-Free Cottonseed Kernels

(8 varieties grown at specified locations during 3 years)

Location	Mean Oil Content of Moisture-Free Kernels, %			
	1947	1948	1949	3 years
Statesville, N. C.	34.4	29.9	38.0	34.1
Florence, S. C.	41.5	38.5	37.9	39.3
Tifton, Ga.	33.1	37.3	38.6	36.3
Auburn, Ala.	37.4	37.6	37.6	37.5
Jackson, Tenn.	34.8	32.5	39.8	35.7
Stoneville, Miss.	35.1	37.1	36.4	36.2
St. Joseph, La.	39.9	38.2	42.0	40.0
Chickasha, Okla.	33.8	33.5	38.4	35.2
Greenville, Tex.	33.9	33.2	36.9	34.7
College Station, Tex.	38.3	33.2	34.8	35.4
State College, N. M.	37.2	36.2	37.7	37.0
Sacaton, Ariz.	35.2	35.1	37.4	35.9
Shafter, Calif.	37.0	30.2	38.2	35.1

locations during 1947, 1948, and 1949. The samples were obtained through the cooperation of the Section of Cotton and Other Fibers of the Field Crops Research Branch. The varieties and locations are listed in Tables I and II. The plantings were irrigated at State College, N. M., Sacaton, Ariz., and Shafter, Calif. All the cottons were grown in replicated plots and representative samples of seed cotton were picked from recently opened bolls and air-dried under cover. After ginning, the seed were stored in sealed containers at 0° F., previous work having shown that under these storage conditions there is no significant change in chemical composition (10, 14).

The kernels were mechanically separated from the hulls and residual lint, and ground to pass a 2-mm. screen prior to analysis. The ground kernels were analyzed for moisture and oil, using the American Oil Chemists' Society official methods (7).

Results and Discussion

The oil contents of the moisture-free kernels, summarized in Tables I and II, show considerable variation, the values ranging from 26.8 to 43.4%. The oil value for the sample of Acala 1517W

Table III. Analysis of Variance of Oil Content of Moisture-Free Cottonseed Kernels

Source	D.F.	Mean Square	F Value
Varieties	7	27.62	22.64 ^a
Location-years	38	59.13	48.47 ^a
Error ^b	265	1.22	
Total ^b	310		

^a Highly significant, 1% level.

^b One degree of freedom subtracted for missing plot.

seed which was missing at Jackson, Tenn., for 1949 was calculated by Yates' method for missing plots. Examination of the varietal means shows that Acala 1517W variety is highest in oil content and Stoneville 2B variety ranks second in oil content for each of the 3 years. The average oil contents of Deltapine 15 and Acala 4-42 varieties were relatively low and the other varieties were intermediate in rank. The varieties tended to hold approximately the same rank from year to year. However, the order (rank) of location means (Table II) was in most cases very variable for the 3 years. The samples grown at St. Joseph, La., were an exception, in that they ranked first or second in average oil content for each

Table IV. Correlations Between Oil Content of Moisture-Free Cottonseed Kernels and Temperatures

(Years-in-locations basis)

Period	Range in Mean Temperature, ° F.			Correlation Coefficients, r		
	Maximum	Minimum	Mean	Max. temp.	Min. temp.	Mean temp.
	Rain-grown and irrigated cottons					
1	85.2-99.8	60.0-71.9	75.4-84.4	-0.15	+0.21	+0.04
2	85.3-107.2	62.2-80.0	75.2-91.6	-0.16	+0.02	-0.10
3	83.6-107.6	60.2-75.4	71.9-91.4	-0.57 ^a	-0.14	-0.45 ^b
4	86.0-101.9	61.0-73.1	75.6-84.2	-0.22	+0.13	-0.07
5	86.1-106.0	61.6-76.8	74.6-89.1	-0.49 ^a	-0.06	-0.38 ^b
6	85.9-103.8	61.5-73.9	76.9-86.6	-0.51 ^a	-0.08	-0.38 ^b
	Rain-grown cottons					
3	83.6-99.8			-0.56 ^a		

^a Highly significant, 1% level.

^b Significant, 5% level.

Table V. Correlations Between Oil Content of Moisture-Free Cottonseed Kernels and Rainfall for Rain-Grown Cottons

(Years-in-locations basis)

Period	Total Rainfall, Inches		Correlation Coefficients, r	Regression Coefficients, b_{yz}
	Range	Average		
1	0.00- 8.81	2.19	+0.35	+0.53
2	0.00-11.12	2.86	+0.09	+0.09
3	0.00- 8.38	3.76	+0.59 ^a	+0.82
4	0.33-13.58	5.04	+0.29	+0.27
5	0.86-17.30	6.62	+0.41	+0.33
6	1.63-19.76	8.81	+0.53 ^b	+0.37

^a Highly significant, 1% level.

^b Significant, 5% level.

Table VI. Analyses of Covariance Between Oil Content of Moisture-Free Cottonseed Kernels and Two Weather Properties During Maturation Period

(No. 3)

Source	Rain-Grown Cottons					
	D.F.	% Oil in Kernels, y , vs. Inches of Rainfall, x_1				
		$ss_{x_1^2}$	ss_{x_1y}	ss_{y^2}	b_{yx_1}	r
Locations	9	61.0691	22.342	101.97	+0.37	+0.28
Years-in-locations	20	68.8932	56.149	130.52	+0.82	+0.59 ^a
Total (location-years)	29	129.9623	78.491	232.49	+0.60	+0.45 ^b
% Oil in Kernels, y , vs. Mean Maximum Temperature, ° F., x_2						
		$ss_{x_2^2}$	ss_{x_2y}	ss_{y^2}	b_{yx_2}	r
Locations	9	216.39	4.56	101.97	+0.02	+0.04
Years-in-locations	20	181.12	-86.54	130.52	-0.48	-0.56 ^a
Total (location-years)	29	397.51	-81.98	232.49	-0.21	-0.27
Rain-Grown and Irrigated Cottons						
% Oil in Kernels, y , vs. Mean Maximum Temperature, ° F., x						
		ss_x^2	ss_{xy}	ss_y^2	b_{yz}	r
Locations	12	787.45	-26.89	108.75	-0.03	-0.09
Years-in-locations	26	206.69	-107.40	172.29	-0.52	-0.57 ^a
Total (location-years)	38	994.14	-134.29	281.04	-0.14	-0.25

^a Highly significant, 1% level.

^b Significant, 5% level.

Table VII. Correlations of Oil Content of Moisture-Free Kernels with Rainfall and Maximum Temperature During Maturation Period (No. 3)

(8 varieties of cotton, years-in-locations basis)

Variety	r	Regression Equation	s_{yz}
% Oil in Kernels, y , vs. Rainfall, x^a			
Acala 4-42	+0.67 ^b	$y = 31.6 + 1.02x$	1.9
Acala 1517W	+0.50 ^c	$y = 35.5 + 0.58x$	1.1
Rowden 41B	+0.53 ^c	$y = 33.9 + 0.68x$	1.3
Mebane, Watson's	+0.56 ^b	$y = 33.3 + 0.67x$	1.2
Stoneville 2B	+0.53 ^c	$y = 34.4 + 0.81x$	1.5
Deltapine 15	+0.55 ^b	$y = 32.2 + 0.90x$	1.7
Coker 100W	+0.45 ^c	$y = 33.6 + 0.77x$	1.4
Coker Wilds	+0.81 ^b	$y = 30.6 + 1.41x$	2.6
% Oil in Kernels, y , vs. Maximum Temperature, x^d			
Acala 4-42	-0.58 ^b	$y = 89.1 - 0.58x$	1.6
Acala 1517W	-0.59 ^b	$y = 81.2 - 0.47x$	1.3
Rowden 41B	-0.60 ^b	$y = 85.5 - 0.53x$	1.5
Mebane, Watson's	-0.58 ^b	$y = 80.2 - 0.48x$	1.4
Stoneville 2B	-0.40 ^c	$y = 73.5 - 0.39x$	1.1
Deltapine 15	-0.50 ^b	$y = 82.8 - 0.51x$	1.4
Coker 100W	-0.46 ^c	$y = 83.7 - 0.51x$	1.4
Coker Wilds	-0.63 ^b	$y = 104.4 - 0.74x$	2.1

^a D.F. = 20 (10 rain-grown locations).

^b Highly significant, 1% level.

^c Significant, 5% level.

^d D.F. = 26 (13 locations, both irrigated and rain-grown).

year. The low average oil content (30.2%) at Shafter, Calif., in 1948 may have been due partly to a heavy infection of *Verticillium* wilt at that station.

The analysis of variance of the oil values (Table III) indicates that both variety and environment have a highly significant influence on the oil content of the kernels. Because environment

(location-years) has a statistically significant effect on oil content, correlations were determined between oil content of the kernels and temperature and rainfall during several selected periods of growth of the cotton plant:

Period 1. Squaring, 21 days prior to average blooming date, which is considered as 10 days after first bloom.

Period 2. Fiber length development, 17 days after average blooming date.

Period 3. Maturation, 35 days following period 2 to average boll opening.

Period 4. Combination of periods 1 and 2.

Period 5. Combination of periods 2 and 3.

Period 6. Combination of periods 1, 2, and 3.

In view of the findings of Tharp *et al.* (76), it seemed desirable to remove main effects for locations, especially as soil fertility differences from location to location are involved in the present study. Therefore, the calculations were made on a basis of "years-in-locations"—i.e., variation within a location—by covariance analysis (as illustrated in Table VI), rather than on an over-all location basis.

Correlation coefficients for the relationships between oil content of moisture-free cottonseed kernels (rain-grown and irrigated cottons) and mean maximum, minimum, and mean temperatures for each of the six periods are given in Table IV. Highest correlation was obtained for mean maximum temperature in the maturation period (No. 3). Correlations for periods 1 and 2 were non-significant. Combinations of periods resulted in no higher correlation than that for period 3 alone. The correlation coefficient (-0.56) obtained for the relationship between oil content and mean maximum temperature during period 3 for rain-grown cottons—i.e., samples grown at the first 10 locations—was almost identical with the -0.57 value for rain-grown and irrigated cottons.

In the case of correlations between oil content of the kernels and total rainfall for each of the six periods for rain-grown cottons (Table V), positive correlation coefficients were obtained, indicating an increase of oil with increase in rainfall. Statistically significant coefficients were obtained for periods 3 and 6, the highest value (+0.59) being for the maturation period (No. 3). This period also gave a considerably larger regression coefficient (+0.82) than did the other five periods, indicating a greater change in oil content per unit change in rainfall. The importance of period 3 is explainable as it has been observed by several workers (3, 5, 72) that the oil is synthesized during this period.

Analyses of covariance between oil content of moisture-free cottonseed kernels and rainfall, and between oil content and mean maximum tempera-

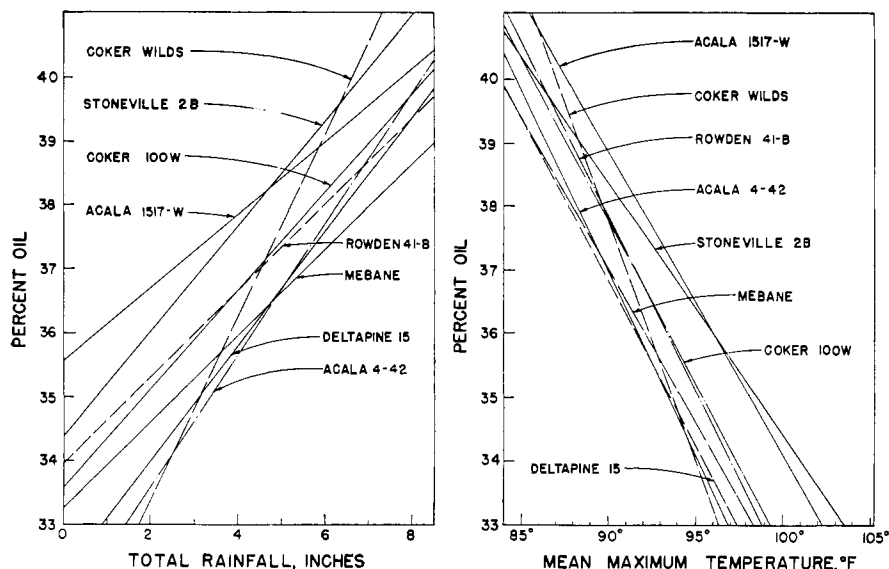


Figure 1. Relation between oil content of moisture-free kernels of eight varieties of cotton and rainfall (rain-grown cottons) and mean maximum temperature (rain-grown and irrigated cottons) during seed maturation

ture during the maturation period (No. 3), are presented in Table VI. It can be seen that the associations involving "locations" are different from those for "years - in - locations"—i.e., variation within a location—the former being non-significant in all three analyses whereas the years-in-locations correlation coefficients are highly significant in all three cases.

Correlation coefficients, regression equations, and standard errors of estimate ($s_{y\cdot x}$) were calculated for the relationships between oil content and rainfall (period 3) and between oil content and mean maximum temperature (period 3) for each of the eight varieties of cotton, using the same covariance procedure illustrated in Table VI. These data are given in Table VII. The correlation coefficients for both the rainfall and the temperature relationships are significant for each of the varieties. In order to determine whether or not the eight varieties behaved differently with respect to rainfall and with respect to maximum temperature, tests of significance for the eight variety regressions for each of the two relationships were made. No significant difference could be demonstrated for either variety regressions on rainfall or variety regressions on maximum temperature.

A summary of the relationships among oil content, rainfall, and mean maximum temperature during the maturation period (No. 3) for rain-grown cottons is given in Table VIII. The simple correlation coefficients show significance in all cases. In view of the fact that a statistically significant negative correlation coefficient (-0.44) was found for the relationship between mean maximum temperature and rainfall for period 3, partial correlation coefficients were

also calculated in order to evaluate the influence of rainfall on oil content independent of temperature, and the influence of temperature independent of rainfall. Because the partial correlation coefficients are also statistically significant, it is evident that rainfall had a significant influence on oil content, holding temperature constant, while temperature had a significant influence on oil content when rainfall was held constant. Maximum temperature and rainfall appear to be of very nearly equal importance in influencing oil content under the conditions of the present experiment. A generalized prediction equation for per cent oil in cottonseed kernels was calculated, based on the years-in-locations variation, and is given in the lower part of Table VIII. Because of

the relatively high standard error of estimate (1.97), the equation would probably be of more value in showing expected fluctuation in oil content with changes in weather rather than in making precise predictions.

As analysis of variance (Table III) showed that both variety and environment significantly influenced oil content, calculations were made (Table IX), which give a breakdown of the total environmental variation (232.49) into that due to association with rainfall and mean maximum temperature during the maturation period (R^2sy^2) and that due to other environmental factors [$(1 - R^2)(sy^2)$]. If each of the four values in the last two columns of Table IX is expressed as a percentage of the total environmental sums of squares (232.49), the following percentage contributions of various environmental factors influencing oil content of the kernels are obtained:

- 6.88%, due to rainfall and maximum temperature differences between locations
- 36.98%, due to other environmental differences between locations
- 26.07%, due to rainfall and maximum temperature differences within a location
- 30.07%, due to other environmental differences within a location

It is evident, therefore, that although rainfall and maximum temperature during the maturation period are important causes of variation in the oil content, a considerable part of the environmental variation in oil content is also due to other factors not considered in this study.

Varietal relationships between oil content and rainfall for rain-grown cottons and average maximum temperature for both irrigated and rain-grown cottons (period 3) on the years-in-locations basis are reported in Table VII. The re-

Table VIII. Summary of Relationships Among Oil Content, Rainfall, and Mean Maximum Temperature During Maturation Period (No. 3)

(Rain-grown cottons, years-in-locations basis)

Combination	Regression Coefficients, $b_{y\cdot x}$	Standard Partial Regression Coefficients, $b'_{y\cdot x}$	Correlation Coefficients, r_{\cdot}	Partial Correlation Coefficients, r'	Standard Error of Estimate
% oil vs. inches of rainfall	+0.82	+0.43	+0.59 ^a	+0.46 ^b	1.97
% oil vs. mean max. temp., ° F.	-0.25	-0.38	-0.56 ^a	-0.42 ^b	
Inches of rainfall vs. mean max. temp., ° F.			-0.44 ^b		
Years-in-Locations Multiple Regression (Prediction) Equation					
% oil in kernels, $y = \bar{y} + 0.5884x_1 - 0.3185x_2 + 26.76$					
where					
\bar{y} = variety mean oil content for the particular location over a period of years ^c					
x_1 = total inches of rainfall during maturation period					
x_2 = mean maximum temperature (° F.) during maturation period					
^a Highly significant, 1% level.					
^b Significant, 5% level.					
^c If the mean oil content of the variety and/or the location is unknown, an estimated mean value must be substituted for \bar{y} .					

Table IX. Breakdown of Total Environmental Variation in Oil Content

(Into that due to association with rainfall and mean maximum temperature during maturation period, No. 3, expressed as $R^2(\text{sy}^2)$, and that due to all other environmental factors)

Source	D.F.	sy ²	$R_{y,x_1x_2}^a$	$R^2(\text{sy}^2)$	$(1 - R^2)(\text{sy}^2)$
Locations	9	101.97	0.3960	15.99	85.98
Years-in-locations	20	130.52	0.6814 ^b	60.60	69.92
Total (location-years)	29	232.49			

^a $y = \%$ oil in kernels; $x_1 =$ inches of rainfall (period 3); $x_2 =$ mean maximum temperature, °F. (period 3).

^b Highly significant, 1% level.

gression equations for these relationships are shown graphically in Figure 1. Since the standard errors of estimate are rather high, the regression equations are not suitable for prediction purposes. However, the plot of the equations illustrates those varieties which average highest, lowest, or intermediate in oil content (Table I).

The results of this investigation suggest that it may be possible to increase oil content of cottonseed kernels as cotton varieties are improved, provided this character is considered in selection. However, rainfall and mean maximum temperature during the maturation period seem to play important roles in elaboration of oil in the kernel, as do other environmental factors.

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FORAGE CROP CONSTITUENTS

The Isolation and Analysis of Hemicelluloses From Orchard Grass

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THE HEMICELLULOSES OF NONWOODY PLANTS have been only sporadically investigated. These structural polysaccharides often amount to 30% of the dry weight of forage plants and supply a major portion of the caloric value of these plants to ruminant animals. Elucidation of their chemical composition would be valuable in determining their nutritive value and their role in plant physiology.

In modern usage hemicelluloses are designated as those cell-wall polysac-

charides which contain, in addition to pentoses and hexoses, some uronic acids, and which are soluble in alkali. They are also referred to as polyuronide hemicelluloses (23). The nomenclature used in this report for hemicelluloses and for related polysaccharides, follows that adopted by Norman (23) and Wise and Ratliffe (29). Natural cellulose is considered as being composed of a mixture of true cellulose, a polymer of D-glucose, and the cellulosans (xylans, galactans, arabans, etc.). Holocellulose is the mixture of lignin-free, structural components of the plant cell. This report is

concerned principally with the polyuronide hemicelluloses which are designated only as hemicelluloses.

Recent interest in possible commercial utilization of many soft plant materials—e.g., sugar cane bagasse, wheat straw, and corn cobs and stalks—has given impetus to investigations of the hemicelluloses from these sources. Since the early investigations of Schulze (26) and Shryver and his associates (11, 12) modern methods have been developed, such as chlorite delignification to produce holocellulose from which hemicelluloses may be obtained more readily (28), the use of mild extracting agents to avoid solu-

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