Leon	9Ap	3 m. E of Buffalo; roadside sands	15	—
Van Zandt	15Ap	2 m. W of Wills Point; old field pasture; sandy clay-loam	16	—
Van Zandt	15Ap	Northern outskirts of Ben Wheeler; old field; sandy soil	15	32
Van Zandt	16Ap	2.5 m. SE of Ben Wheeler; sandy clearing in oak-hickory woods	2	2
Smith	16Ap	[illegible] W of Tyler; old field; sandy-loam	20	18
			207	128

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A Laboratory Experiment Involving Paper-Chromatography and Statistics

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Many experiments have been suggested for the use of paper chromatography in the laboratory. These include the separations of amino acids and other biological materials (2,7,10,11,12); cations (4,5,6,9,13,14,15); and dyes (8). Some of these have the disadvantage that they cannot be completed in one laboratory period, and some call for the use of materials which are not found in the usual laboratory equipment.

This article suggests a paper chromatographic separation of the dyes in commercial black inks, which has been designed for completion in one 3-hour laboratory period with the use of equipment and solvents found in practically all laboratories. The experiment gives very satisfactory results, and allows the student to become familiar with the general field of paper chromatography and some of the factors which affect R_f values of constituents of mixtures. It also may be used to demonstrate the use of simple statistical tests, if desired.

Several commercial black inks are run by each student using water and two buffer solutions having different pH values. The equipment consists of a short-form 100-ml. Tuttle graduate (Kimble No. 200032) covered by an in-

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verted 800-ml. beaker. Each separation requires only 15 to 20 minutes, and 4 chromatograms may be run simultaneously.

Experimental

STRIPS: The chromatograms are run on 6x0.5-inch strips of Whatman No. 1 filter paper. Each student receives 18 strips which are used to run duplicate chromatograms of 3 different black inks in 3 different solvents. Each strip is marked with pencil 4 inches and 4.5 inches from one end, and is then folded at the 4.5-inch mark. The ink is placed on the strip at the 4-inch mark, using a fine pen-point. This ink mark is made perpendicular to the long edges, should not be more than 1/16-inch wide, and should not be closer than 1/16-inch to the edges of the strip. Each strip is identified by a penciled label and number at the end of the long portion. The ink should be allowed to dry several minutes before development.

SOLUTIONS:

pH 5.2 Solvent: Prepared by mixing 29.95 ml. of 0.200 N sodium hydroxide and 50.0 ml. of 0.200 M potassium acid phthallate and diluting to 200 ml. A solution prepared from certified buffer tablets may be used if desired.

pH 8.8 Solvent: Prepared by mixing 16.30 ml. of 0.200 N sodium hydroxide and 50.0 ml. of a 0.200 M solution of boric acid which is also 0.200 M in potassium chloride, and diluting to 200 ml. A solution prepared from certified buffer tablets may be used if desired.

Distilled water.

Various black inks. In this investigation Skrip Permanent Black, Skrip Washable Black, Sanford's Penit Jet Black and Quink New Permanent Black were used.

PROCEDURE: Fill the graduate to within ¼ to ⅛ of an inch of the top with the solution to be used. Invert the 800-ml. beaker over the graduate, and allow five to ten minutes for vapor-equilibrium to be obtained. Remove the beaker, always keeping it vertical, and place four strips on the graduate, immersing the short length of the fold in the solution. Immediately cover with the beaker.

Allow the chromatograms to develop until the leading edge of the solvent is approximately ½-inch from the end of the strip. This usually requires 15 to 20 minutes. Remove

the beaker and then remove the strips and place on absorbent paper towels to dry. If the leading edge of the solvent does not coincide with a color band, mark its position with pencil just before the strip is completely dry.

Run the remaining chromatograms in the same manner.

DETERMINATION OF R_f FACTORS: The R_f factor is defined as the ratio of the movement of the leading edge of a band to the movement of the leading edge of the developing solvent. After the chromatograms are dry, use a ruler to measure the distances in millimeters from the 4-inch mark to the leading edge of each color band and to the leading edge of the solvent. Calculate and report the R_f factors for each color on each chromatogram.

TABLE I

R_f FACTORS OF SKRIP PERMANENT BLACK INK

COLOR BANDS

	Yellow		Blue		Red		Blue	
pH	R_f*	S.D.	R_f*	S.D.	R_f*	S.D.	R_f*	S.D.
5.2	0.89	0.016	0.31	0.027	0.15	0.018	0.06	0.011
7.0	0.95	0.018	0.89	0.029	0.64	0.029	0.36	0.041
8.8	0.97	0.017	0.92	0.027	0.49	0.022	0.17	0.024

*average of thirteen duplicate determinations

TABLE II

R_f FACTORS OF SKRIP WASHABLE BLACK INK

COLOR BANDS

	Blue		Yellow		Red	
pH	R_f*	S.D.	R_f*	S.D.	R_f*	S.D.
5.2	0.98	0.010	0.88	0.012	0.18	0.021
7.0	0.99	0.009	0.96	0.014	0.81	0.042
8.8	0.99	0.004	0.96	0.012	0.60	0.021

*average of fifteen duplicate determinations

TABLE III

R_f FACTORS OF SANFORD'S PENIT JET BLACK INK

COLOR BANDS

	Blue		Red		Yellow	
pH	R_f*	S.D.	R_f*	S.D.	R_f*	S.D.
5.2	0.97	0.017	0.58	0.024	0.43	0.017
7.0	0.98	0.013	0.91	0.031	0.89	0.032
8.8	0.98	0.018	0.91	0.026	0.87	0.023

*average of ten duplicate determinations

TABLE IV

R_f FACTORS OF QUINK NEW PERMANENT BLACK INK

COLOR BANDS

	Yellow		Red		Blue	
pH	R_f*	S.D.	R_f*	S.D.	R_f*	S.D.
5.2	0.74	0.019	0.57	0.027	0.16	0.020
7.0	0.91	0.019	0.90	0.021	0.84	0.043
8.8	0.93	0.016	0.92	0.015	0.48	0.029

*average of eleven duplicate determinations

Results

The results of a class of 20 students, each of whom ran 3 of 4 different inks, are summarized in Tables I-IV. The standard deviations in these tables were calculated by the difference of duplicate method in which the standard deviation is defined as the square root of the sum of the squares of the differences between duplicates divided by twice the number of duplicate pairs (16).

Comparison of the R_f values of similar colors in these different inks indicates that some identical dyes may be used

in both the permanent and washable inks of the same manufacturer or in inks of different manufacturers. Colors which have the same R_f factors at all three pH's, within the limit of experimental error, include the yellow of Skrip Permanent Black and Skrip Washable Black, the blue of Skrip Washable Black and Sanford's Penit Jet Black, and the red of Sanford's Penit Jet Black and Quink New Permanent Black. It should be noted however, that reasonable agreement at only one pH is not a satisfactory criterion for specifying that two dyes are identical. The R_f values of the red dye of the two Skrip inks agree within limits at pH 5.2, but definitely disagree at both the other pH values. A few simple statistical tests may be applied to the data to prove, within a certain probability, that these observations are correct.

Statistical Analysis

The R_f factors obtained by five different students on the yellow and red dyes contained in the two Skrip black inks at pH 5.2 and the statistical analysis of these results are shown in Table V. Calculation of the standard deviation and confidence factors from the range for sets of measurements up to ten as suggested by Dean & Dixon (3) greatly simplifies the calculations involved in the application of statistical tests to small sets of data.

The standard deviation is taken as the product of the range (w) and the deviation factor (K_w) and the confidence interval is the product of the range and the confidence factor (t_w). Values for these factors are given in the original article (3) for sets of measurements up to ten. The variance is obtained by squaring the standard deviation.

The variances are used to compare the precision of two sets of data on two materials or by two methods (17) by use of the F test. The ratio F of the variances of two sets has been studied and the limits are known within which the value will normally vary if there is no significant difference in precision. In performing this test, the smaller of the two variances is placed in the denominator and the calculated value of the ratio compared to the critical value for the desired probability and the number of degrees of freedom involved. If the calculated value is larger than the critical value, it is evidence that the variances differ significantly and that the two materials or methods being compared are

not identical. If the calculated value is smaller than the critical value, it is evidence that the materials or methods being compared are identical. If the calculated value is close to the critical value, this test should be verified by further

TABLE V

R_f FACTORS OF FIVE STUDENTS FOR YELLOW AND RED COLORS OF SKRIP PERMANENT AND SKRIP WASHABLE BLACK INKS AT pH 5.2

Student	R_f FACTORS			
	Yellow of Skrip		Red of Skrip	
	Permanent	Washable	Permanent	Washable
1	0.91	0.86	0.15	0.14
	0.91	0.87	0.17	0.15
2	0.91	0.93	0.15	0.15
	0.91	0.93	0.15	0.19
3	0.89	0.86	0.16	0.17
	0.89	0.86	0.15	0.15
4	0.90	0.91	0.10	0.17
	0.92	0.90	0.14	0.16
5	0.86	0.89	0.13	0.16
	0.90	0.87	0.15	0.15
average	0.9000	0.888	0.145	0.159
Range (w)	0.06	0.07	0.07	0.04
Standard Deviation (wK_w)(K_w = 0.33)	0.0198	0.0231	0.0231	0.0132
99% confidence limits (wt_w) (t_w = 0.33)	0.90 + 0.02	0.89 + 0.02	0.15 + 0.02	0.16 + 0.01
Variance (SD^2)	0.000392	0.000534	0.000534	0.000174
F (ratio of variance)	1.36		3.07	
F (critical 5%)	3.18		3.18	
Pooled Standard Deviation	0.0215		0.0188	
Stan.Dev. of difference between averages	0.00962		0.00668	
t (Pooled SD/SD of diff.)	1.24		2.10	
t (Critical - 5%)	2.10		2.10	

tests or determinations before a conclusion is made concerning the two materials or methods.

The application of this test to the yellow and red dyes of the two Skrip inks is shown in Table V. Since the calculated value of F for the yellow dyes is significantly less than the

critical value for 5% limits, this is evidence that the two dyes are identical. Since the calculated value for the red dyes is very close to the critical value, this test should not be used by itself to make a decision as to whether these dyes are identical or different.

A second statistical test which may be applied to the data to determine whether two dyes of the same color are identical or different is the "t" test which is used to compare averages of sets of determinations. The value "t" is the ratio of the difference between the averages of two sets of data to the standard deviation of this difference (18). The standard deviation of the difference may be calculated by dividing the pooled standard deviation of the two sets by the square root of the value obtained by dividing the product of the numbers of determinations in each set by the sum of these numbers. The pooled standard deviation of two sets which contain equal numbers of determinations is the average of the two standard deviations.

If the calculated value of "t" is greater than the critical value for the desired probability and the number of degrees of freedom involved, it is evidence that the two materials or methods are different while a calculated value less than the critical is evidence that the materials or methods are identical. A calculated value of "t" which is close to the critical value indicates that further tests or determinations should be made before a decision is reached as to whether or not the two materials or methods are identical or different.

The application of this test to the yellow and red dyes of the two Skrip inks is shown in Table V. Since the calculated value of "t" for the yellow dyes is significantly less than the critical value of "t" for 5% limits, it is evidence that the two dyes are identical. In the case of the red dyes, the calculated value of "t" equals the critical value, so other tests or determinations should be made before a decision is reached that they are identical or different.

Both these statistical tests as applied to this set of data give the same evidence that the two yellow dyes are identical and that the red dyes may or may not be identical. Thus more determinations should be made on the red dyes in order to determine whether or not they are identical. In-

spection of the R_f values of the red dyes in Tables I and II show that these two dyes are in reality not identical since the R_f values are significantly different at the higher pH values. It should also be noted that the R_f values of the yellow dyes are essentially the same at each pH value.

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Parapholis incurva and Chloris polydactyla in Texas

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The establishment of the grass *Parapholis incurva* (L.) C. E. Hubb. along the Gulf Coast of Texas has been brought to the attention of the writer by recent collections at Port Arthur, Jefferson County (*W. J. Waldrip 139*), and High Island, Chambers County (*F. W. Gould 6770*). By coincidence the two collections were made the same day, April 19, 1955, on independent field excursions by members of the Texas A. & M. College, Department of Range and Forestry. A check of the Herbarium of Southern Methodist University by Dr. Lloyd Shinners revealed two earlier Texas collections, one from near Bayside, Refugio County (*Eula Whitehouse 21209*, in 1949), and one from near Texas City, Galveston County (*B. L. Turner 1813*, in 1950). Dr. Turner states that a specimen of the latter collection is also in the University of Texas Herbarium.

Parapholis, commonly known as "sickle grass," has been introduced into this country from Europe. The 1950 edition of Hitchcock's "Manual of the Grasses of the United States" reports its occurrence on mud flats and salt marshes along the Atlantic Coast from New Jersey and Pennsylvania to Virginia, and on the Pacific Coast in California and Oregon.