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EFFECTS OF SOME PHENOLIC ACIDS UPON THE GERMINATION OF LETTUCE SEED

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ABSTRACT

Effects of 13 naturally occurring phenolic acids, lactones and flavonoids upon the germination of seed of *Lactuca sativa* L. var. *Black Seeded Simpson* were determined. The di-hydroxy phenolic acids, caffeic acid and chlorogenic acid, and the di-hydroxy flavonoids, quercetin and rutin, were completely non-inhibitory to germination. Mono-hydroxy compounds, isomers of coumaric acid, exhibited slight toxicity, while the lactones coumarin and unbelliferone showed the greatest degree of inhibition of any of the compounds tested.

INTRODUCTION

The hydroxylated cinnamic acids are widely distributed constituents of higher plants, occurring free or in combination with sugars, as glucosides or esters (Harborne and Simonds, 1964; Harborne, 1964). Mayer and Evenari (1953) and Mayer and Poljakoff-Mayber (1963) have studied the effects of a number of these compounds upon the germination of "seeds" of lettuce, *Lactuca sativa* L. var. *Progress*. The present studies were undertaken in order to test the germination response of lettuce seed to additional compounds of this type, as well as a number of structurally and biochemically related lactones and flavonoids of natural occurrence.

MATERIALS AND METHODS

Seed of *Lactuca sativa* L. var. *Black Seeded Simpson*, a light-insensitive variety, were obtained from Carolina Biological Supply Company, Burlington, N.C. 27215. Batches of **ca.** 100-150 seed were sown in petri dishes containing 2 sheets of filter paper, and moistened with 8.0 ml of water or experimental test solution. The dishes were then placed in a dark box, and the percentage of germination was scored after 2 days at 25°C. Concentrations of the test solutions were varied in order to determine the concentration resulting in 50% inhibition of germination. Germination of controls in distilled water exceeded 99 per cent in all instances.

RESULTS

Caffeic acid, chlorogenic acid, sinapic acid and the flavonoids quercetin and rutin were completely non-inhibitory to the germination of lettuce seed in concentrations equivalent to the upper limits of their solubilities in water (Table 1), while some degree of inhibition of germination was produced by all other compounds tested. The concentration resulting in 50% inhibition of germination was determined for each of the remaining compounds, with the exception of *m*-coumaric acid, a saturated solution of which produced only 15-20% inhibition. Since solubilities of a saturated solution of these acids are low, so that saturated solutions are still quite dilute, it does not appear that pH is an important factor in their toxicity (Mayer and Evenari, 1953).

TABLE 1: *Effects of Cinnamic Acid Derivatives upon Germination of Lettuce Seed.*

Compound	Concentration producing 50% inhibition of germination	
o-Coumaric Acid	0.05-0.1%	
m-Coumaric Acid	*	
p-Coumaric Acid	0.05-0.1%	0.08%***
Caffeic Acid	No inhibition**	>0.18%***
Chlorogenic Acid	No inhibition**	
Quinic Acid	0.2%	
Ferulic Acid	0.1-0.2%	0.09%***
Sinapic Acid	No inhibition**	
trans-Cinnamic Acid	0.05%	0.04%***
Umbelliferone	0.02%	
Quercetin	No inhibition**	
Rutin	No inhibition**	
Coumarin	0.01%	0.002-0.007%***

*15-20% inhibition of germination produced by a saturated solution.
**No inhibition produced by a saturated solution.
***Calculated from data of Mayer and Evenari (1953) and Mayer and Poljackoff-Mayber (1963).

Coumarin, the lactone of *cis-o*-hydroxy cinnamic acid, was the most inhibitory of the compounds tested, slightly exceeding the inhibition produced by exposure of the seed to the closely related compound, umbelliferone. The *o*- and *p*-isomers of coumaric acid and *trans*-cinnamic acid were somewhat more toxic than *m*-coumaric acid, while ferulic acid and quinic acid were only moderately inhibitory.

Discussion

It is of interest to compare the present data with those of Mayer and Evenari (1953) and Mayer and Poljakoff-Mayber (1963) which were reported on a molarity basis. Their figures for inhibitory concentrations of ferulic, caffeic, *p* (?)-coumaric acids, and coumarin (Mayer and Poljakoff-Mayber, 1963) have been converted to a percentage basis, and their result with cinnamic acid (Mayer and Evenari, 1953) has been estimated from their graph and likewise converted to a percentage basis (Table 1). When one considers that the findings of these workers were based upon a different variety, namely *Progress,* the degree of agreement is very close.

In attempting to interpret these results and relate them to the structure of the compounds concerned, it is apparent that the di-hydroxy phenolic acids, caffeic acid and chlorogenic acid, and their flavonoid derivatives, quercetin and rutin, are completely non-inhibitory to lettuce seed germination, while the mono-hydroxy cinnamic acid derivatives, the *o*-, *m*- and *p*-isomers of coumaric acid, show varying degrees of toxicity. It has been shown that mono-hydroxy phenolic acids are cofactors of IAA oxidase, resulting in increased rates of destruction of auxin and consequent decreased growth, while di-hydroxy phenolic acids inhibit IAA decarboxylation, resulting in an increase of growth (Nitsch and Nitsch, 1962; Henderson and Nitsch, 1962; Zenk and Müller, 1963; Tomaszewski and Thimann, 1966). While the mechanism of the effects of these compounds upon lettuce seed germination may be different, the present results can be correlated with those of other workers concerned with growth responses.

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DEPOSITIONAL ENVIRONMENT OF THE WILCOX-CLAIBORNE SEDIMENTS IN HENRY, WEAKLEY, AND CARROLL COUNTIES, TENNESSEE

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Abstract

The Wilcox-Claiborne sediments in Henry, Weakley, and Carroll counties were deposited in a fluvial environment. The values of the textural parameters, sorting and skewness, calculated from grain size analysis of 116 samples on a quarter phi interval basis, indicate fluvial sedimentation. Other physical characteristics that support a fluvial interpretation include cut-and-fill struc-

tures, kaolinite and lignite beds, leaf fossils and lenses of lignite in some clay beds. There is also an absence of features associated with a marine environment: no marine fossils, glauconite, or grains of calcite, dolomite or siderite were found in the study area.

INTRODUCTION

There are a few investigations of Eocene rocks in Tennessee and much of what has been done deals with the commercially valuable clay bodies enclosed by the sands. Very little work pertains to the environments in which the sands were deposited. Statements made in earlier studies about the sands are based on very little quantitative evidence. The purpose of this study is to provide a better understanding of the depositional environment of the sands, and to some extent the clays, in northwestern Tennessee (Fig. 1).

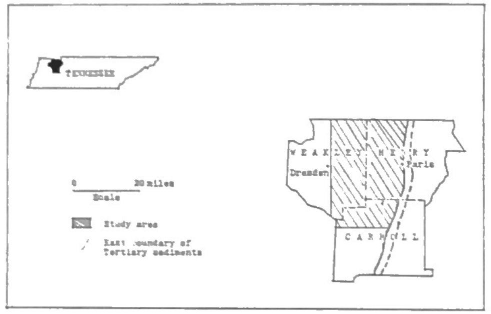


FIG. 1: *Location map of study area.*

The study area includes parts of Henry, Weakley, and Carroll counties. The area is rectangular, extending north-south about 35 miles and east-west about 25 miles. The northern boundary is the Tennessee-Kentucky state line; the southern boundary is the 36°N parallel; the eastern boundary is the outcrop of the Porters Creek Clay of Paleocene age; and the western boundary is State Highway 118 from the Tennessee-Kentucky state line to Dresden. The western boundary continues south as an imaginary line to the 36° N parallel.

GEOLOGIC SETTING

The area studied is in the northeastern part of the Mississippi embayment on the east limb of the Mississippi embayment syncline; formations strike approximately north-south and dip about 30 feet per mile to the west. The stratigraphic column in Fig. 2 shows the generalized relationships of the exposed geologic units present.

The Wilcox and Claiborne Formations are lithologically similar and, for the most part, lack distinguishing characteristics (Parks, 1971). They are varicolored, heterogeneous units which consist chiefly of sand, silt, and clay. These sediments are interbedded and interlensed and no lithology or sequence of lithologies is laterally persistent for any great distance. The sand consists chiefly of quartz and is sparsely to very micaceous. Bedding ranges from thick to very thin and is horizontal and irregular, crossbedded, and lenticular. Cut-and-fill structures are present. Iron-oxide concretions of various sizes and shapes and discontinuous iron-oxide layers one to several inches thick are abundant locally. Clay beds of these formations range from thick to laminated and ordinarily occur in discontinuous lenses which apparently are not related to a particular stratigraphic position within the formations. In some lenses, clay is separated from the overlying sands by lignite beds which range in thickness from several inches to several feet. Leaf fossils and lenses of lignite are present in some clay beds. Scattered clay pebbles and lenses of clay conglomerate occur at various horizons throughout the Wilcox-Claiborne sediments. No marine fossils, glauconite, or grains of calcite, dolomite or siderite were found in the study area.

SYSTEM	SERIES	FORMATION	THICKNESS IN FEET	LITHOLOGY
QUATERNARY	Pleistocene and Recent	Alluvium	Up to 25	Sand, silt, clay, and gravel.
QUATERNARY	Pleistocene	Loess	Up to 10	Clayey and sandy silt, gray to brown, massive.
QUATERNARY AND TERTIARY (?)	Pleistocene and Pliocene (?)	Fluvial Deposits	Up to 80	Iron-stained sand, gravelly sand, sandy gravel and silty and clayey sand.
	Lower and Middle Eocene	Claiborne and Wilcox Formations	More than 500	Irregularly bedded white, gray, brown and orange sand; locally interbedded with lenses and beds of gray, brown, black and white clay, silty clay, and lignite.
TERTIARY	Paleocene	Porters Creek Clay	Up to 170	Gray, black, sandy to silty micaceous clay; massive to bedded, breaks with conchoidal to hackly fracture; locally cut by sand dikes.

FIG. 2: *Generalized stratigraphic column of geologic units in study area.*

GRAIN SIZE ANALYSIS

Grain size analysis of the Wilcox-Claiborne sediments was performed to determine the textural parameters in order to discover the depositional agents and environments. The sampling techniques used are those outlined by Folk (1965, p. 16) as to sample size and method of collection. Samples were obtained from road cuts and overburden faces in clay pits. A field description of each sampled exposure was made to note any features that could be used in interpreting the environment. Each sample was tagged and numbered, and its location and tag number were recorded on the topographic map. Sample site locations were shown and given in Tennessee Rectangular Coordinates (Clark, 1973, Fig. 9 and Table 8). Commonly, a series of samples was taken at a particular site or location. These samples were grouped and shown as one sample. One hundred and sixteen samples from 57 sand sample sites were selected for grain size analysis.

Techniques utilized in sieve and pipette analyses are standard sedimentological procedures described by Folk (1965, p. 34-39). Sieve sizes used in this study ranged from −2.0 phi to 4.5 phi using quarter phi sieve intervals. In 15 samples the weight percent of the finer than 4.5 phi fraction (pan fraction) exceeded 5 percent and grain sizes of these pan fractions were measured by pipette analysis on a quarter phi interval basis from 4.75 to 8.75.

Graphic analysis of each sample following the technique of Folk (1965, p. 41-48) was completed and percentiles used in calculating the textural parameters of Graphic Mean (M_z), Inclusive Graphic Standard Deviation (σ_I), Inclusive Graphic Skewness (Sk_I), and Graphic Kurtosis (K_G) determined. These parameters were calculated and tabulated for all samples (Clark, 1973, Table 9).

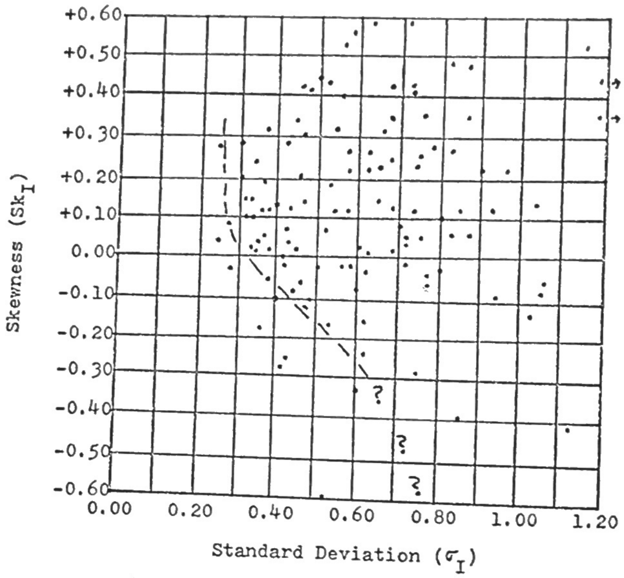
Results of the grain size analysis are summarized in Table 1. The average grain size of the samples is 2.13 phi, fine sand. Individual samples range from 0.90 phi, coarse sand, to 4.32 phi, coarse silt. The mean sorting value for the sediments is 0.61 phi, moderately well sorted. Sorting values range from 0.25 to 1.74 phi, very well sorted to poorly sorted, and place the sediments in the fluvial class using either Folk's (1965, p. 46) or Friedman's (1962, p. 749) classification. Sorting values suggest the Wilcox-Claiborne depositional environment was not a high energy environment like a beach but a lower energy environment such as a fluvio-deltaic complex that lacked sufficient current action to remove silt and clay. The average skewness valus is +0.12, fine skewed. Though skewness values range from −0.60 to +0.59, the sands are predominantly positively skewed, which supports a fluvial classification (Friedman, 1961, p. 519). The average Graphic Kurtosis value is 1.28 and the range of values extends from 0.80 to 2.47. A representative curve for the samples is leptokurtic, showing that the central portion of the curve is better sorted than are the "tails." Skewness and Graphic Kurtosis values indicate the presence of silt in the Wilcox-Claiborne sediments, which reinforces the idea that depositional currents were unable to remove effectively the finer grained material. Sorting and skewness values for the sand samples support a fluvial interpretation for the Wilcox-Claiborne sediments.

A binary plot was used to make evident the fluvial nature of the Wilcox-Claiborne sediments. Binary plots, constructed by plotting one textural parameter against another, have been used to determine depositional environments of samples taken from unknown environments. If the samples, indicated by points, group to form a pattern or trend, they can be compared with characteristic patterns established by plots of samples taken from known depositional environments. Friedman (1967) tried scatter plots of two parameters each and found 12 different combinations useful for distinguishing river and beach deposits. All of the 12 appeared to be equally effective.

One of Friedman's 12 plots was selected as representative of the method for use in plotting data derived from grain size analysis: Inclusive Graphic Skewness (Folk, 1965) versus Inclusive Graphic Standard Deviation (Folk, 1965). These parameters were selected because, as individual statistical measures, they appear to be more significant than the others in this study; furthermore, they are generally considered to be among the most environmentally sensitive parameters. These parameters of the Wilcox-Claiborne

TABLE 1: *Summary of Grain Size Analysis of the Wilcox-Claiborne Sediments.*

Graphic Grain Size Parameter	Total Range	Average Value	Standard Deviation	95 Percent Confidence	Verbal Designation
Mean Size (M_z)	0.90-4.32 phi	2.13 phi	.75 phi	.14 phi	fine sand
Sorting (σ_I)	0.25-1.74 phi	0.61 phi	.25 phi	.05 phi	moderately well sorted
Skewness (Sk_I)	−0.60 to +0.59	+0.12	.20	.04	fine skewed
Kurtosis (K_G)	0.80-2.47	1.28	.26	.05	leptokurtic



• Sand sample
--- River-beach boundary line. River sands to right of dashed line, beach sands to left. Based on data of Folk (1965) and Friedman (1962).

FIG. 3: *Plot of Inclusive Graphic Skewness and Inclusive Graphic Standard Deviation.*

samples were used in a binary plot (Fig. 3) to determine the depositional environment of the Wilcox-Claiborne sediments. A beach-river boundary line of known beach and river samples is superimposed on the Wilcox-Claiborne samples. The location of this line is based on data from Folk (1965) and Friedman (1962). Most of the Wilcox-Claiborne samples plot as river sands. The binary plot indicates that the Wilcox-Claiborne sediments represent fluvial deposits.

CLAY MINERAL ANALYSIS

Nineteen clay samples from 19 sites (Clark, 1973, Table 8 and Fig. 26) were used to study the depositional environment. Eleven of these sites are clay pits and the other eight are exposures in road cuts. One site is approximately 7 miles west of the western boundary of the study area, near Bradford in Gibson County, but is included because it represents the westernmost pit dug in Eocene clay in western Tennessee.

Oriented aggregates of the minus 8-micron fraction were made and X-ray diffractograms obtained for each sample. Four clay samples previously X-rayed were heated to 500°C for 12 hours and X-rayed again to determine whether the 7-angstrom peak was kaolinite or septechlorite (Warshaw and Roy, 1961, p. 1484). Heat treatment collapsed the 7-angstrom peaks, indicating the mineral represented by the peaks is kaolinite. The clay minerals, listed in order of most common occurrence, are kaolinite, illite, mixed-layer clay and montmorillonite. Only kaolinite occurs in all 19 samples; illite is present in all but two samples in various amounts. Judging from the asymmetry of the illite peaks, a little mixed-layer clay is present in most of the samples. Only one sample, taken immediately above the Porters Creek Clay, contains montmorillonite.

The ratio of kaolinite to illite was estimated for the samples using a semi-quantitative method proposed by Weaver (1958, p. 270). The ratios range from 4/1 to 1/1 with the average value 2.3/1. Kaolinite was dominant in 17 of the 19 samples and equal in abundance to illite in the other two samples.

Kaolinite is the dominant clay mineral in the samples. In a study of Cretaceous and Tertiary clays of the upper Mississippi embayment, Pryor and Glass (1961) found kaolinite to be the dominant clay mineral in a fluvial environment. Their criteria for the fluvial environment included fluvial and deltaic sands, cut-and-fill structures, leaf fossils, and lignite beds in the clay strata or in adjacent beds, and the general absence of features associated with a marine environment. All of these criteria are evident in the study area. The Wilcox-Claiborne clays support a fluvial origin for the Wilcox-Claiborne sediments.

CONCLUSIONS

The outcropping Wilcox-Claiborne sediments were deposited in a fluvial environment on the basis of the following physical characteristics of the study area: grain distributions characteristic of fluvial sands, cut-and-fill structures, kaolinite and lignite beds, leaf fossils and lenses of lignite in some clay beds. There is also an absence of features associated with a marine environment. There are no marine fossils, glauconite, or grains of calcite, dolomite or siderite.

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