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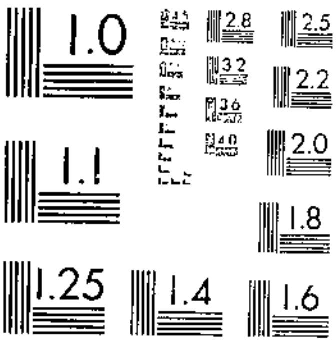
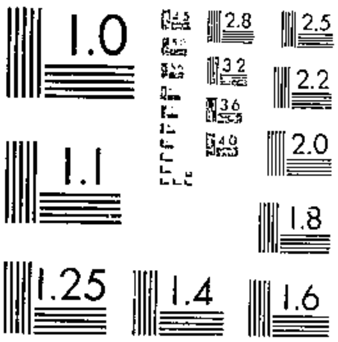


MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A



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STUDIES ON THE POSSIBILITIES OF DEVIL'S SHOESTRING (TEPHROSIA VIRGINIANA) AND OTHER NATIVE SPECIES OF TEPHROSIA AS COMMERCIAL SOURCES OF INSECTICIDES[1,2]

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United States Department of Agriculture, Bureau of Plant Industry, in Cooperation with the Texas Agricultural Experiment Station

CONTENTS

[1] Received for publication May 28, 1937.

[2] This bulletin is a report on the first phases of an investigation on the commercial possibilities of native species of *Tephrosia* as sources of insecticides in cooperation with the Texas Agricultural Experiment Station. The cultural plot of *Tephrosia virginiana* started by V. A. Little, of the Agricultural and Mechanical College of Texas, and the selected planting stock from that plot with all data pertaining thereto were made available for this purpose. With this substantial beginning the cooperative project was started in July 1934 under the local supervision of G. A. Russell, agent, Division of Drug and Related Plants, Bureau of Plant Industry, with the collaboration of Mr. Little. Through the cooperation of the Bureau of Entomology and Plant Quarantine the necessary biological tests were made by F. L. Campbell, formerly entomologist, and W. N. Sullivan, assistant entomologist, Division of Control Investigations, and the several rotenone determinations were made by Howard A. Jones, associate chemist, Division of Insecticide Investigations. Acknowledgment is also made of the helpful cooperation of the Forest Service in connection with experimental plantings of *Tephrosia* in the Choctawhatchee National Forest in western Florida and of the Georgia Coastal Plain Experiment Station, Tifton, Ga., and the South Carolina Sandhill Experiment Station, Pontiac, S. C., where facilities have been provided for cultural tests.

[3] E. D. Fowler, now senior soil scientist, Soil Conservation Service, made the studies on the possible relation of soil environments to toxicity, prepared the soil-profile figure, and furnished the substance of the discussions of that phase of the investigations. C. O. Erlanson made the taxonomic studies and prepared the figure showing their approximate northern ranges. Acknowledgment is made of the assistance of J. W. Kelly, junior biochemist, Division of Drug and Related Plants, in the collection of the root samples and their extraction and examination in the laboratory.

INTRODUCTION

The increasing demand for insecticides nonpoisonous to man for controlling insects on food plants has intensified the search for sources of such materials among the plants of the world. The use of rotenone and rotenone-containing products obtained commercially from several species of *Derris* and *Lonchocarpus* is perhaps the best example of the practical utilization as insecticides of plants used as fish poisons in tropical countries. The success that has attended the introduction of these insecticides has encouraged search for other species containing the same compounds in greater quantities or other substances of equal or greater toxicity to insects but harmless to man and animal.

Rotenone so far has been found only in members of the family Leguminosae and to date in six genera in that family, namely, *Derris*, *Lonchocarpus*, *Mundulea*, *Millettia*, *Spatholobus*, and *Tephrosia* (*Cracca*). All six genera are widely distributed in the Tropics, but only one, *Tephrosia*, has a distribution extending into temperate regions and is to be found in the United States. Little (*5*)[4] reported in 1931 that the root of *Tephrosia virginiana* L., occurring in eastern Texas and commonly known as "devil's shoestring", had insecticidal properties. Clark (*1*) subsequently isolated rotenone and related compounds from the roots. These findings suggested this species, which is widely distributed through the Southeastern and Southern States, as a readily available rotenone-containing plant with pronounced insecticidal properties.

The devil's shoestring, also known by such other names as rabbit bean, goats' rue, hoary pea, and turkey pea, was well known to the American Indians who used the roots as a vermifuge and for other medicinal purposes. They also utilized the root for poisoning fish, a practice that was apparently handed on to white settlers in regions where the plant was sufficiently toxic for the purpose. Roark[5] has summarized the published accounts concerning these early uses as a medicine and fish poison, but reports no reference to the insecticidal properties of its roots prior to the publication of Little's work. Jones, Campbell, and Sullivan (*3*), on the basis of their investigations, point out that the rotenone content of the samples of native species of *Tephrosia* examined by them are not on a par with that of derris (*Derris* sp.) and cube (*Lonchocarpus nicou* (Aubl.) DC.) now available, but suggest that more toxic individuals of these species may be found and that the rotenone content may perhaps be increased by selection, breeding, and cultivation.

The work on devil's shoestring here described was undertaken: (1) To determine the general distribution of *Tephrosia virginiana* and other native domestic species, their relative toxicity, and the relation of toxicity to geographic source; (2) to determine what factors are responsible for the wide differences in the contents of rotenone and other toxic substances; (3) to increase the amount of toxic material in the roots by selection and breeding or other means; and (4) to study the cultural requirements of the plant and determine the cost of its cultivation and its crop possibilities in general. This bulletin

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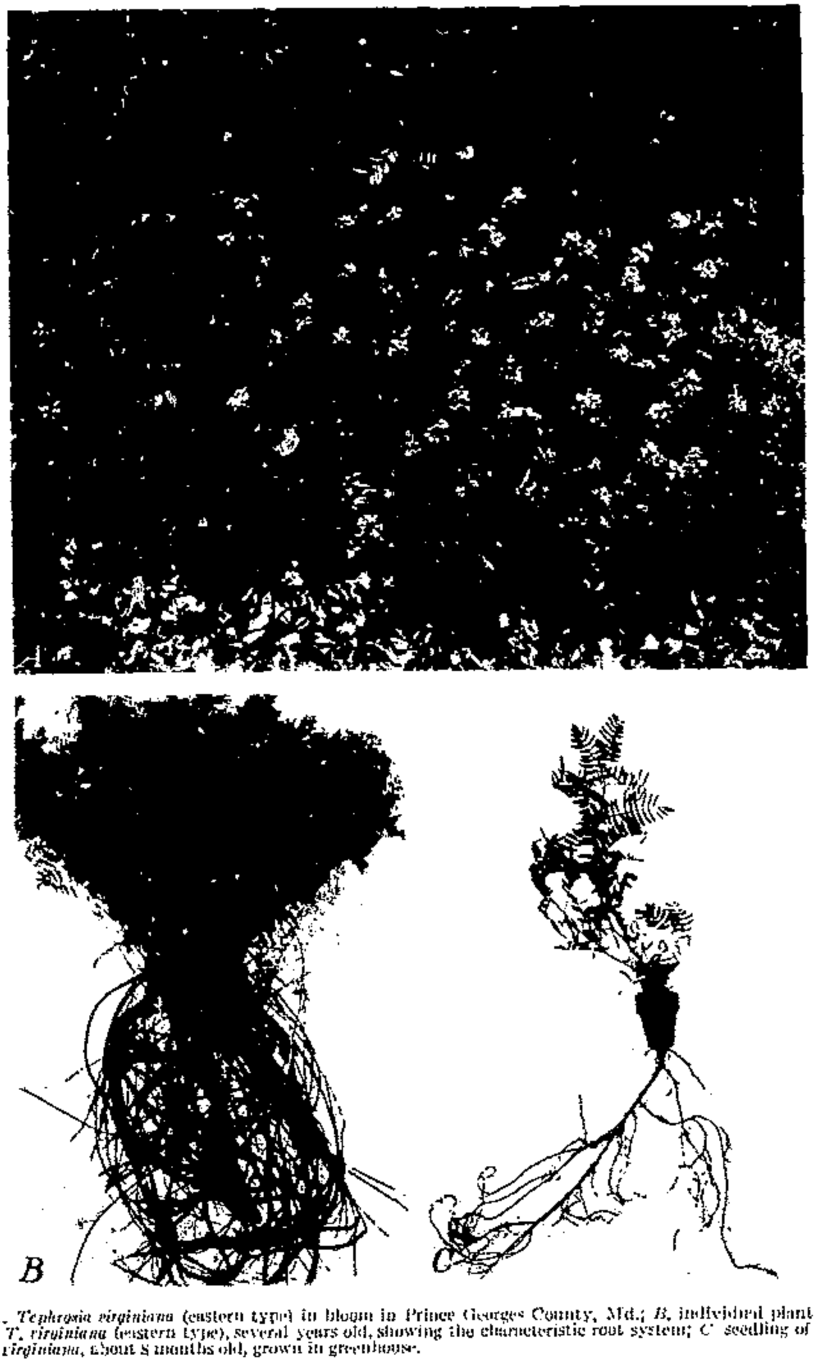
[4] Italic numbers in parentheses refer to Literature Cited, p. 40.

[5] ROARK, R. C. DEVIL'S SHOESTRING (*Cracca virginiana* L.) A POTENTIAL SOURCE OF ROTENONE AND RELATED INSECTICIDES. U. S. Bur. Chem. and Soils, 1934. [Mimeographed.]



A, *Tephrosia virginiana* (eastern type) in bloom in Prince Georges County, Md.; *B*, individual plant of *T. virginiana* (eastern type), several years old, showing the characteristic root system; *C* seedling of *T. virginiana*, about 8 months old, grown in greenhouse.

reports the results of field surveys and of some of the miscellaneous studies completed in connection with cultural studies that are under way.

DESCRIPTION OF TEPHROSIA VIRGINIANA

Tephrosia virginiana is a herbaceous perennial growing to a height of 1 to 2 feet with erect stems, which are leafy to their tops with compound leaves made up of numerous narrow-elliptic leaflets. The flowers, which appear in May and June, are comparatively large and numerous, a dull yellowish white in color with purple markings, and are arranged in a terminal cluster. The fruits are beanlike pods about 3 inches long, which normally contain an abundance of seed. However, over a considerable part of the South a species of blister beetle devours the reproductive organs of the flower just as it begins to open and prevents the development of seed. Furthermore, apparently in all the localities where the plant occurs, a weevil lays its eggs in the young pods, the larvae later eating the seed, so that collected seed should be fumigated with carbon disulphide promptly to prevent damage. The roots are numerous, long, slender, and tough. Normally there is a taproot which descends almost vertically and numerous side roots, which arise from the crown and spread more or less horizontally. There are no underground stems by which the plant can propagate itself by suckering, although in loose sandy soil the new crowns often branch widely from the parent clump, giving the appearance of suckering.

Of the species of *Tephrosia* in the United States, *virginiana* is the most common and widespread. It extends from the New England States to Minnesota, south to Texas and Florida. It prefers low altitudes and is rarely found in the Appalachian Range. Although occurring on widely different soils, it prefers well-drained sandy situations. It lacks the aggressiveness of a weed but is tolerant to new soils and is a characteristic plant of railway embankments and roadsides in the South (pl. 1, *A* and *B*).

The plant may be propagated from seed, but germination is sometimes slow and uneven unless it has been previously treated (pl. 1, *C*). It may be propagated vegetatively by dividing the crown. The roots alone cannot be used for propagating.

SURVEY OF 1934

The region surveyed in 1934 included the following States: Pennsylvania, New Jersey, Delaware, Maryland, West Virginia, Virginia, North Carolina, South Carolina, eastern Tennessee, Georgia, Florida, Alabama, Mississippi, Louisiana, Texas, Arkansas, and Oklahoma. Collections were made by J. W. Kelly, L. A. Whitford, Elbert Voss, G. A. Russell, and V. A. Little. In addition to the material collected directly, samples were obtained from a number of collaborators in the various States, whose assistance was much appreciated. The roots from New York, Connecticut, Indiana, and Kentucky gave no indication of containing rotenone and were not toxic to flies, and no further reference is made to them.

The root samples as collected in 1934 comprised a number of individual plants in a locality and thus represented approximately the quality of the plants in the particular localities in which they were collected. In some localities such composite samples have been found not always to be reasonably representative. For example, a sample showing moderate toxicity does not indicate whether all the component plants were moderately toxic or whether roots from both strongly toxic and nontoxic individuals were included in about equal proportions. The range in degree of toxicity in plants growing within a very small locality was later found to be much greater than expected. Nevertheless, these composite samples served to indicate quite clearly in which regions toxic plants occur.

METHODS OF TESTING

The root samples as received were thoroughly air-dried and then ground so that the coarsest particles would pass through a 20-mesh screen. Twenty grams were extracted with acetone for 7 hours in a Soxhlet extractor. The extract was made up to 100 cc of which 25 cc were used to determine the total acetone extractives. The remainder was available for the Durham test for rotenone and related substances and for biological tests to determine toxicity.

The samples were tested colorimetrically for the presence of rotenone by the method of Durham, as quoted by Gimlette (*2, p. 221*). Jones and Smith (*4*) point out that the characteristic blue color produced when rotenone is present in the acetone extract fades rapidly, and that deguelin, a constituent of some plants containing rotenone, also gives the color. The test, therefore, is of no value as an exact quantitative measure of the rotenone present. However, inasmuch as deguelin also possesses insecticidal properties, any plant material giving the blue color merits investigation as an insecticide. Their investigations suggest, therefore, that this color test may be useful for obtaining quickly a rough indication of the insecticidal quality of plant material insofar as such quality depends on rotenone and deguelin, and possibly on other related substances as yet unknown.

The results obtained by Jones, Campbell, and Sullivan (*3*) indicate a considerable correlation between the intensity of color obtained and the toxicity of the extract to flies. Nevertheless, all the samples collected during this survey also were tested directly for their toxicity to flies.

The biological tests were made in the Division of Control Investigations, Bureau of Entomology and Plant Quarantine by the turntable method (*3*). Acetone extracts were used, 1 cc of which represented 0.02 g of root, or a ratio of root to solvent of 1:50. The samples were graded into those that did not kill flies in excess of the pure acetone control, those that killed more than the control but less than 50 percent of the flies, and those that killed more than 50 percent. The extracts in the third group were then tested in a further dilution, representing a ratio of root to extract of 1:125. Those that killed more than 50 percent of the flies in this concentration were then used in a dilution representing a ratio of 1 g of root to 250 cc of extract. The extracts that killed more than 50 percent of the flies in this dilution thus represented the samples that contained the roots of the greatest toxicity.

RESULTS AND CORRELATIONS

The results obtained are shown in table 1. It is evident that the roots of *Tephrosia virginiana* from over most of its principal range contained insufficient toxic substances to give them insecticidal value. Moreover, the small districts from within which toxic roots were obtained are restricted approximately to northeast Texas, northeast Florida, and southwest Georgia. This restriction of toxicity also is shown by the distribution of samples on the basis of toxicity by States in table 2.

TABLE 1.—*Comparative insecticidal value of roots of Tephrosia virginiana from various localities as determined by the Durham color test and the Campbell-Sullivan test on flies, using acetone extracts of the air-dried root, each cubic centimeter of which represented 0.2 g of root*

State and county	Locality	Laboratory no.	Degree of color with Durham test according to [1]—		Toxicity to flies as determined by Campbell-Sullivan method [2]
			M. S. L.	H. A. J.	
New Jersey:					
Gloucester	Malaga	94	0	0	—
Cumberland	Millville	95	2	1	—
Do	do	96	1	1	—
Atlantic	Mays Landing	97	1	1	—
Do	Egg Harbor City	98	2	1	—
Delaware:					
Sussex	Georgetown	93	0	0	—
West Virginia:					
Mercer	Princeton	233	0	0	—
Kanawha	Big Chimney	234	1	0	—
Randolph	Elkins	235	2	2	—
Hampshire	Romney	101	1	0	—
Maryland:					
Calvert	Prince Frederick	73	0	0	—
Do	Chesapeake Beach	69	2	1	—
Cecil	North East	90	1	0	—
Talbot	Wye Mills	91	1	0	—
Dorchester	East New Market	92	0	0	—
Carroll	Manchester	84	0	1	—
Do	Westminster	99	1	1	—
Frederick	Libertytown	102	1	1	—
Do	Ridgeville	112	0	0	—
Virginia:					
Prince William	Dumfries	54	0	0	—
Surry	Surry	55	0	0	—
Greensville	Emporia	56	1	1	—
Orange	Daniel	57	0	0	—
Lunenburg	Lunenburg	58	2	1	+
Prince Edward	Farmville	59	1	0	—
Charlotte	Keysville	60	1	0	—
Mecklenburg	Clarksville	61	1	0	—
Pittsylvania	Danville	62	1	0	—
Halifax	Halifax	63	0	0	—
Do	Clarkton	64	1	0	—
Do	Ingram	257	3	2	—
Campbell	Brookneal	65	0	0	—
Amherst	Amherst	66	3	2	+
Do	do	225	0	1	—
Do	Clifford	224	1	1	—
Buckingham	Buckingham	67	0	0	—
Powhatan	Tobaccoville	68	0	1	—
Hanover	Ashland	69	0	0	—
Louisa	Pendletons	70	0	1	—

[1] Explanation of symbols: Durham color test according to M. S. Lowman and Howard A. Jones, working independently: 0=no color; 1=mere trace to very pale blue; 2=pale blue; 3=blue; 4=deep blue; 5=dark blue.

[2] The effects on flies of the extract when used in 3 dilutions are indicated as follows: — =none killed in excess of control in dilution of 1 to 10; + =more killed than by control but less than 50 percent killed in dilution of 1 to 10; ++ =more than 50 percent killed in dilution of 1 to 10; +++ =more than 50 percent killed in dilution of 1 to 25; ++++ =more than 50 percent killed in dilution of 1 to 50.

TABLE 1.—*Comparative insecticidal value of roots of Tephrosia virginiana from various localities as determined by the Durham color test and the Campbell-Sullivan test on flies, using acetone extracts of the air-dried root, each cubic centimeter of which represented 0.2 g of root*—Continued

State and county	Locality	Laboratory no.	Degree of color with Durham test according to—		Toxicity to flies as determined by Campbell-Sullivan method
			M. S. L.	H. A. J.	
Virginia—Continued.					
Spotsylvania	Fredericksburg	71	1	1	—
Orange	Wilderness	72	0	0	—
Do	Gordonsville	215	1	1	—
Frederick	Gore	100	0	0	—
Culpeper	Culpeper	214	0	0	—
Fluvanna	Fork Union	216	1	1	—
Nelson	Lovingston	217	0	1	—
Bedford	Goodes	226	3	2	—
Do	Bedford	227	1	0	—
Do	Villamont	228	1	0	—
Montgomery	Christiansburg	229	2	1	—
Pulaski	Pulaski	230	3	2	—
Wythe	Wytheville	231	0	0	—
Do	do	232	0	0	—
Highland	McDowell	236	1	0	—
Augusta	West Augusta	237	0	0	—
Shenandoah	New Market	238	0	0	—
Rappahannock	Sperryville	239	2	1	—
Do	Amissville	240	0	0	—
Dinwiddie	Carson	241	0	0	—
North Carolina:					
Lenoir	Kinston	107	0	0	—
Johnston	Smithfield	36	0	0	—
Harnett	Dunn	37	0	0	—
Cumberland	Fayetteville	38	0	0	—
Robeson	Saint Pauls	39	2	1	—
Craven	Vanceboro	172	1	2	—
Beaufort	Washington	173	1	2	—
Martin	Williamston	174	0	1	—
Northampton	Jackson	176	2	2	—
Halifax	Littleton	177	0	1	—
Warren	Norlina	178	0	0	—
Vance	Henderson	179	0	1	—
Franklin	Louisburg	180	1	1	—
Wake	Wendell	181	0	0	—
Do	Cary	182	1	1	—
Do	Raleigh	162	1	0	—
Do	Morrisville	222	1	1	—
Chatham	Merry Oaks	183	1	0	—
Lee	Sanford	184	0	0	—
Moore	Aberdeen	185	0	0	—
Richmond	Rockingham	186	0	0	—
Anson	Wadesboro	153	1	1	—
Union	Monroe	187	0	0	—
Gaston	Gastonia	188	1	1	—
Cleveland	Kings Mountain	189	0	0	—
Rutherford	Lake Lure	196	1	1	—
Polk	Saluda	113	0	0	—
Henderson	East Flat Rock	197	0	0	—
Buncombe	Skyland	198	0	0	—
Do	Swannanoa	203	0	0	—
Swain	Whittier	200	0	0	—
Jackson	Dillsboro	201	1	1	—
Haywood	Waynesville	202	0	1	—
McDowell	Marion	204	1	2	—
Watauga	Blowing Rock	205	2	2	—
Caldwell	Whitnel	206	2	1	—
Catawba	Hickory	159	1	1	—
Do	Newton	160	0	1	—
Do	Catawba	161	1	0	—
Iredell	Statesville	163	1	1	—
Forsyth	Clemmons	207	0	0	—
Stokes	Walnut Cove	209	0	1	—
Rockingham	Wentworth	210	2	1	—
Harnett	Mamers	211	1	1	—
Guilford	Greensboro	212	0	0	—
Alamance	Burlington	218	1	0	—
Do	Haw River	219	0	0	—
Orange	Hillsboro	220	1	1	—
Durham	Durham	221	1	1	—

TABLE 1.—*Comparative insecticidal value of roots of Tephrosia virginiana from various localities as determined by the Durham color test and the Campbell-Sullivan test on flies, using acetone extracts of the air-dried root, each cubic centimeter of which represented 0.2 g of root*—Continued

State and county	Locality	Laboratory no.	Degree of color with Durham test according to—		Toxicity to flies as determined by Campbell-Sullivan method
			M. S. L.	H. A. J.	
South Carolina:					
Marion	Mullins	40	0	0	–
Do.	Marion	169	0	1	–
Williamsburg	Hemingway	41	1	0	–
Do.	Kingstree	42	0	1	–
Dorchester	Summerville	43	1	0	–
Colleton	Walterboro	44	0	0	–
Bamberg	Ehrhardt	45	1	0	–
Orangeburg	Neeses	110	0	0	–
Lexington	Batesburg	151	0	0	–
Newberry	Prosperity	156	0	0	–
Do.	Whitmire	164	1	1	–
Lancaster	Lancaster	166	0	0	–
Kershaw	Kershaw	167	1	1	–
Lee	Bishopville	168	0	0	–
Florence	Florence	152	1	0	–
Spartanburg	Spartanburg	190	0	0	–
Greenville	Greer	191	0	0	–
Oconee	Clemson	192	0	0	–
Pickens	Pickens	193	0	0	–
Tennessee:					
Washington	Johnson City	199	0	1	–
Hawkins	Surgoinsville	157	0	1	–
Knox	Knoxville	158	0	0	–
Georgia:					
Lee	Stocks	1	3	2	+
Tift	Tifton	2	3	2	++
Sumter	Americus	3	4	3	+++
Muscogee	Columbus	4	4	2	++
Charlton	Folkston	16	4	4	+++
Ware	Waycross	17	2	1	++
Wayne	Jesup	18	1	1	–
Jeff Davis	Hazlehurst	19	0	1	–
Telfair	McRae	20	0	0	–
Toombs	Vidalia	21	0	0	–
Tattnall	Reidsville	22	0	0	–
Evans	Claxton	23	0	0	–
Candler	Metter	24	0	0	–
Emanuel	Garfield	25	2	1	–
Do.	Swainsboro	26	0	0	–
Jefferson	Louisville	27	0	0	–
McDuffie	Thomson	28	0	0	–
Richmond	Augusta	29	0	0	–
Clarke	Athens	30	0	0	–
Do	Winterville	31	0	0	–
Do	Athens	32	0	0	–
Franklin	Franklin Springs	33	0	0	–
Stephens	Toccoa	34	0	0	–
Habersham	Cornelia	35	0	0	–
Hall	New Holland	46	0	1	–
Gwinnett	Lawrenceville	47	0	0	–
Dawson	Dawsonville	48	0	0	–
Pickens	Jasper	49	1	0	–
Bartow	Pine Log	50	0	0	–
Do	Cartersville	51	0	0	–
Gordon	Calhoun	52	0	1	–
Walker	LaFayette	74	1	1	–
Chattooga	Summerville	75	0	1	–
Polk	Cedartown	85	0	0	–
Cobb	Austell	76	0	0	–
Carroll	Carrollton	77	0	0	–
Coweta	Newnan	78	0	0	–
Clayton	Mountain View	79	0	0	–
Spalding	Griffin	80	0	0	–
Bibb	Macon	81	0	0	–
Crisp	Cordele	82	4	3	+++
Cook	Adel	83	4	4	+++
Lowndes	Valdosta	84	0	1	–
Thomas	Thomasville	86	0	0	–
Mitchell	Camilla	87	0	1	–

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State and county	Locality	Laboratory no.	Degree of color with Durham test according to—		Toxicity to flies as determined by Campbell-Sullivan method
			M. S. L.	H. A. J.	
Florida:					
Gadsden	Chattahoochee	8	0	0	—
Do	do	269	0	0	—
Leon	Tallahassee	9	1	0	—
Marion	Weirsdale	10	0	0	—
Duval	Jacksonville	15	4	3	++
Clay	Green Cove Springs	351	5	4	+++
Madison	Madison	354	1	2	+
Jackson	Cottondale	355	0	0	—
Putnam	Grandin	14	3	2	++
Alabama:					
Lee	Auburn	5	0	1	—
Pike	Troy	6	0	0	—
Houston	Dothan	7	0	0	—
Mobile	Citronelle	271	1	2	—
Russell	Crawford	270	3	2	+
Macon	Tuskegee	272	2	1	—
Mississippi:					
Pearl River	McNeill	263	1	1	—
Do	Poplarville	264	2	2	+
Do	Hillsdale	265	1	2	—
Stone	Wiggins	266	2	2	—
Do	do	267	2	2	+
George	Lucedale	268	1	1	—
Clarke	Pachuta	273	1	1	—
Jasper	Heidelberg	274	2	1	—
Jones	Mosolle	275	3	2	+
Forrest	Hattiesburg	276	2	2	+
Pike	McComb	277	1	1	—
Franklin	Meadville	278	0	0	—
Marion	Hub	103	2	1	—
Louisiana:					
Saint Tammany	Folsom	261	0	0	—
Washington	Franklinton	262	0	1	—
La Salle	Trout	279	0	0	—
Rapides	Alexandria	280	2	2	+
Beauregard	De Ridder	311	1	1	+
Vernon	Leesville	315	2	3	++
Caddo	Shreveport	316	1	1	+
Claiborne	Homer	317	1	1	—
Arkansas:					
Ouachita	Camden	318	5	5	+++
Garland	Hot Springs National Park	319	0	0	—
Do	do	320	0	0	—
Pulaski	Little Rock	321	0	0	—
Faulkner	Conway	322	0	0	—
Pope	Russellville	323	0	0	—
Crawford	Alma	324	1	1	+
Washington	Fayetteville	325	1	1	+
Benton	Bentonville	326	0	1	—
Oklahoma:					
Choctaw	Boswell	134	1	0	—
Bryan	Durant	135	1	1	—
Comanche	Lawton	137	3	2	+
Delaware	Jay	327	0	1	—
Mayes	Pryor	328	0	0	—
Tulsa	Tulsa	329	2	3	+
Lincoln	Stroud	330	0	0	—
Oklahoma	Luther	331	1	2	—
Grady	Chickasha	332	4	5	++
Do	do	333	2	2	+
Stephens	Comanche	334	4	3	++
Texas:					
Wilson	Stockdale	111	3	2	+
Caldwell	Luling	115	4	3	++
Do	do	339	4	3	++
Gonzales	Gonzales	116	3	2	+
Do	do	338	2	2	+
Austin	Sealy	117	5	3	+++
Waller	Hempstead	118	3	2	+
Do	Waller	243	0	0	—
Washington	Brenham	119	1	1	—
Robertson	Hearne	120	1	1	—
Do	do	121	3	2	++

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State and county	Locality	Laboratory no.	Degree of color with Durham test according to—		Toxicity to flies as determined by Campbell-Sullivan method
			M. S. L.	H. A. J.	
Texas—Continued.					
Robertson	Franklin	122	0	0	–
Do	Bremond	125	0	0	–
Milam	Milano	124	5	4	+++
Do	do	340	5	5	+++
Do	do	341	5	4	+++
Do	Cameron	246	4	3	++
Leon	Buffalo	126	0	0	–
Do	do	127	0	0	–
Do	Keechi	342	5	5	++++
Trinity	Trinity	128	5	4	+++
Anderson	Montalba	129	4	4	+++
Do	do	343	5	5	++++
Do	do	344	5	5	++++
Do	Blackfoot	345	5	5	++++
Smith	Tyler	130	5	5	+++
Do	do	346	5	5	+++
Do	do	347	5	5	++++
Harrison	Harleton	131	5	5	++++
Do	Marshall	330	0	0	–
Lamar	Paris	132	2	2	+
Do	do	248	1	1	–
Do	do	133	3	3	++
Montague	Nocona	136	3	2	++
Do	Bowie	335	1	1	–
Callahan	Cross Plains	138	4	3	++
Do	Clyde	139	3	3	+
Comanche	De Leon	140	4	3	++
Do	do	337	4	5	+++
Do	do	143	4	3	++
Do	Rucker	142	3	3	++
Do	Hasse	144	2	2	+
Upshur	Gilmer	348	1	2	+
Eastland	Carbon	141	4	3	++
Burleson	Chriesman	244	1	2	+
Newton	Newton	247	1	2	+
Nacogdoches	Nacogdoches	311	5	4	++++
Jasper	Jasper	312	0	0	–
Do	do	313	2	3	++

TABLE 2.—*Number of composite samples of roots of Tephrosia virginiana of varying degrees of toxicity obtained from the several States*

State	Samples which were given a toxicity rating [1] of—					Total samples
	–	+	++	+++	++++	
	Number	*Number*	*Number*	*Number*	*Number*	*Number*
New Jersey	5					5
Delaware	1					1
West Virginia	4					4
Maryland	9					9
Virginia	38	2				40
Tennessee	3					3
North Carolina	49					49
South Carolina	19					19
Georgia	37	1	3	4		45
Florida	5	1	2	1		9
Alabama	5	1				6
Mississippi	9	4				13
Louisiana	4	3	1			8
Arkansas	6	2		1		9
Oklahoma	6	3	2			11
Texas	11	10	12	9	7	49

[1] For explanation of symbols see footnotes of table 1.

The restriction of toxicity to specific localities is in accord with the results of Jones, Campbell, and Sullivan (*3*), who examined roots from a limited number of localities, ranging from Maryland to Texas. It also is supported by additional data obtained in 1935.

All of the samples from the States north of Georgia were negative except two which were +.[6] Samples with a maximum toxicity were obtained only from Texas. Only 22 percent of the samples from Texas were nontoxic in comparison with 50 percent or more of the samples from any other State.

Of 29 samples giving a rating of 2 by the Durham test (table 3), 16 were given a toxicity rating of −, 10 of +, and 3 of ++. Of the 12 samples from the States north of Georgia all except 1 were nontoxic and all except 2 of the 13 that were rated as somewhat toxic (+or ++) came from the western section of the plant's range. The data are insufficient to permit any conclusions. They suggest, however, that the color obtained with the samples from the States in the northern section of the plant's principal range was due mainly to the presence of substances which give the characteristic color but which are much less toxic than rotenone, whereas the samples from the southern region contained a larger proportion of the latter.

TABLE 3.—*Distribution by States of samples of roots of Tephrosia virginiana given a color rating of 2[1] to show their relative toxicity in the northern and southern part of the plant's principal range*

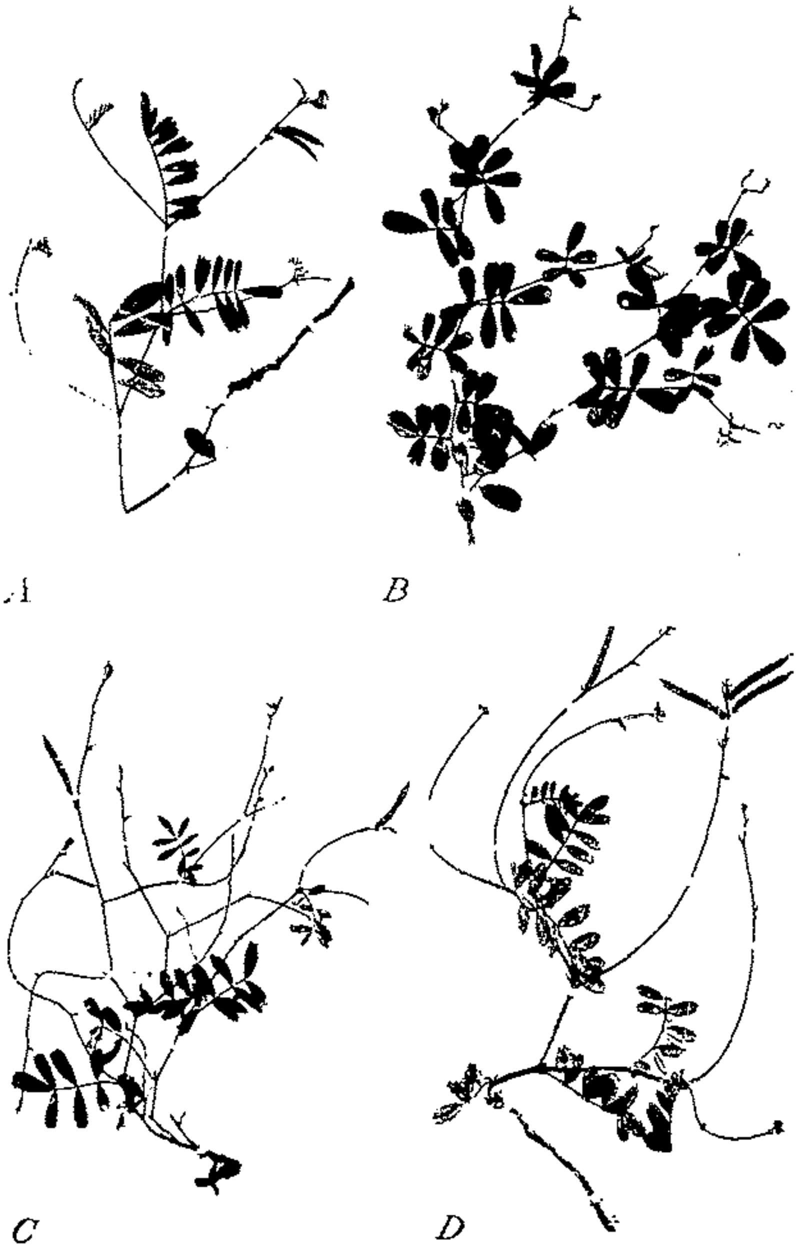
Region and State	Samples	Samples showing toxicity[1] of—			Region and State	Samples	Samples showing toxicity[1] of—		
		−	+	++			−	+	++
	Number	*Number*	*Number*	*Number*		*Number*	*Number*	*Number*	*Number*
Northern:					Southern:				
New Jersey	2	2			Georgia	2	1		1
Delaware	0				Florida	0			
West Virginia	1	1			Alabama	1	1		
Maryland	1	1			Mississippi	6	3	3	
Virginia	3	2	1		Louisiana	2		1	1
Tennessee	0				Arkansas	0			
North Carolina	5	5			Oklahoma	2		2	
South Carolina	0				Texas	4		3	1

[1] For explanation of symbols see footnotes of table 1.

The data of table 1 have been brought together in table 4 to show the relation between the results of the toxicity and the color tests. It is evident that the toxicity of the roots to flies is roughly but reliably indicated by the Durham test. This supports the results of previous investigators, but with data on many more samples. This correlation is of very great value in permitting the color test to be used as a quick method of distinguishing between toxic and nontoxic plants in any investigations that may be necessary to determine the actual possibilities of the plant. Moreover, the color test may be applied with reasonably reliable results to a small piece of root removed from a living plant, thus making it possible to study more effectively the influence of various environmental factors on the toxicity of the plant.

The total acetone extract of the roots apparently varies greatly. Since the roots were only air-dried and not entirely free from mois-

[6] For the explanation of this and other symbols used to indicate the toxicity to flies and the degree of color obtained with the Durham test, see footnote 1, table 1.



A, *Tephrosia ambigua* collected in Ware County, Ga.; *B*, *T. chrysophylla* collected in Jackson County, Fla.; *C*, *T. smallii* collected in Mobile County, Ala.; *D*, *T. spicata* collected in Dixie County, Fla.

ture, the variations in the amount of moisture present may have been responsible to some extent for some of the variations in the total extract. However, these variations were much too wide to be thus accounted for completely and they occurred without any relation to the toxicity or geographic source of the roots.

The rotenone content of a few toxic samples was determined by Howard A. Jones, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine. The data are presented in table 5 with those for the color test and toxicity. They indicate only a general relation between toxicity, as measured, and rotenone content. Thus, both samples rating ++ for toxicity had less than 0.40 percent of rotenone, whereas the seven samples rating + + + + ranged from 0.79 to 1.80 percent. On the other hand, sample no. 130, with a rotenone content of 1.80 percent, was rated a grade lower in toxicity than no. 131, which contained the same percentage of rotenone, and no. 351, with the same toxicity rating as no. 130, contained only 0.25 percent of rotenone. The number of samples is too small to permit any conclusions. Moreover, the method used at the time the determinations were made is now known to be unreliable when the rotenone content of the sample is low.

TABLE 4.—*Insecticidal value of roots of Tephrosia virginiana as tested with flies in relation to results with the Durham color test of the roots*

Degree of toxicity [1]	Distribution of samples showing the Durham color test [1] of 0	1	2	3	4	5	Total samples
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
−	128	64	16	3			211
+		8	10	9			27
++			3	6	11		20
+++					6	9	15
++++						7	7
Total	128	72	29	18	17	16	280

[1] For explanation of symbols see footnotes of table 1.

TABLE 5.—*Color obtained with the Durham test, toxicity to flies, and percentage of rotenone present in 17 root samples of Tephrosia virginiana from various localities*

Laboratory no.	Source	Degree of color by Durham test [1]	Toxicity to flies [1]	Rotenone by tetrachloride extraction [2]
				Percent
15	Jacksonville, Fla.	4	++	0.37
351	Green Cove Springs, Fla.	5	+++	.25
82	Cordele, Ga.	4	+++	.42
318	Camden, Ark.	5	+++	1.40
130	Tyler, Tex.	5	+++	1.80
346	do.	5	+++	.65
347	do.	5	++++	1.40
131	Harleton, Tex.	5	++++	1.80
143	De Leon, Tex.	4	++	.24
337	do.	4	+++	.42
311	Nacogdoches, Tex.	5	++++	.83
340	Milano, Tex.	5	+++	1.10
341	do.	5	+++	.54
342	Kechi, Tex.	5	++++	.99
343	Montalba, Tex.	5	++++	1.20
344	do.	5	++++	1.40
345	Blackfoot, Tex.	5	++++	.79

[1] For explanation of symbols see footnotes of table 1. [2] Determinations by Howard A. Jones.

SURVEY OF 1935

The differences in the toxicity of the plants in 1934 and the fact that toxic plants appeared to occur only in certain general districts indicated that a similar but more detailed survey was necessary. This was undertaken in 1935 with particular attention to (1) examination of individual plants; (2) a critical botanical study of the species occurring in the South and Southeast, with special consideration of the botanical variations in *Tephrosia virginiana* in relation to toxicity and of the toxicity and possible commercial value of other species of *Tephrosia*; (3) a study of the relation of soil types to toxicity and of certain soil and vegetative environments as a possible explanation of variations in toxicity; and (4) determination of the regions, if any, in which commercial growing would be most likely to succeed.[7] The areas surveyed are indicated in figure 1.

Some of the root of more than 300 individual plants was tested, and the soil and vegetative environments of the plants and their location with reference to other tested plants were noted. Herbarium material was collected and special attention given to the distribution of the recognized forms of *T. virginiana* generally designated as the eastern and western types. The species found, the number of each tested, and the degree of color obtained with the Durham test are shown in table 6.

TABLE 6.—*Number of individual plants of various species of Tephrosia giving the several degrees of blue color obtained with the Durham test*

Species	Plants tested	Dark blue	Deep blue	Blue	Light blue	Pale blue	Trace	No color
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
T. ambigua (M. A. Curtis)[1]	24						7	17
T. carpenteri (Rydb.) Killip	2							2
T. chrysophylla (Pursh)[2]	15	1			1			13
T. gracillima (Robinson) Killip	1			1				
T. hispidula (Michx.) Pers.	7					1	3	3
T. smallii (Vail) Robinson[3]	5						1	4
T. latidens (Small) Standl.	29	10	4	7	2			6
T. lindheimeri A. Gray	5					1		4
T. onobrychoides Nutt.	7					1	1	5
T. onobrychoides var. *texana* (Rydb.) Macbride	5							5
T. spicata (Walt.) Torr. & Gray[4]	32					1	9	22
T. virginiana (L.) Pers.	202	46	10	21	16	9	29	161

[1] Pl. 2, *A*. [2] Pl. 2, *B*. [3] Pl. 2, *C*. [4] Pl. 2, *D*.

Toxicity of the material collected in 1935 was not determined by biological methods. In view of the close correlation between the results of the Durham and toxicity tests, however, the writers will use "toxicity" as measured by the Durham test. Thus, plants giving no color, a moderate blue, or a dark blue will be classed as nontoxic, moderately toxic, or distinctly toxic, respectively.

DISTRICTS IN WHICH TOXIC AND NONTOXIC PLANTS OCCUR

The localities from which plants have been examined are shown in figure 2. The extensive areas in which practically all plants are nontoxic are not designated on the map (fig. 2) and may be referred to conveniently as nontoxic districts.

[7] The botanical studies were made by C. O. Erlanson, of the Division of Plant Exploration and Introduction, the soil studies by E. D. Fowler, at that time of the Division of Soil Fertility. The Durham tests in Texas and the Gulf States were made by G. A. Russell and in the rest of the area by M. S. Lowman, both of the Division of Drug and Related Plants, all of the Bureau of Plant Industry.

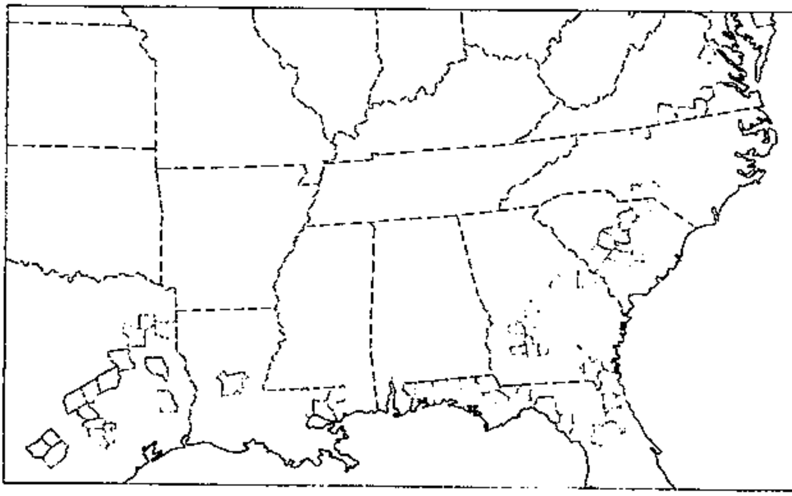


FIGURE 1.—Counties in which the principal studies were made during the survey of 1935.

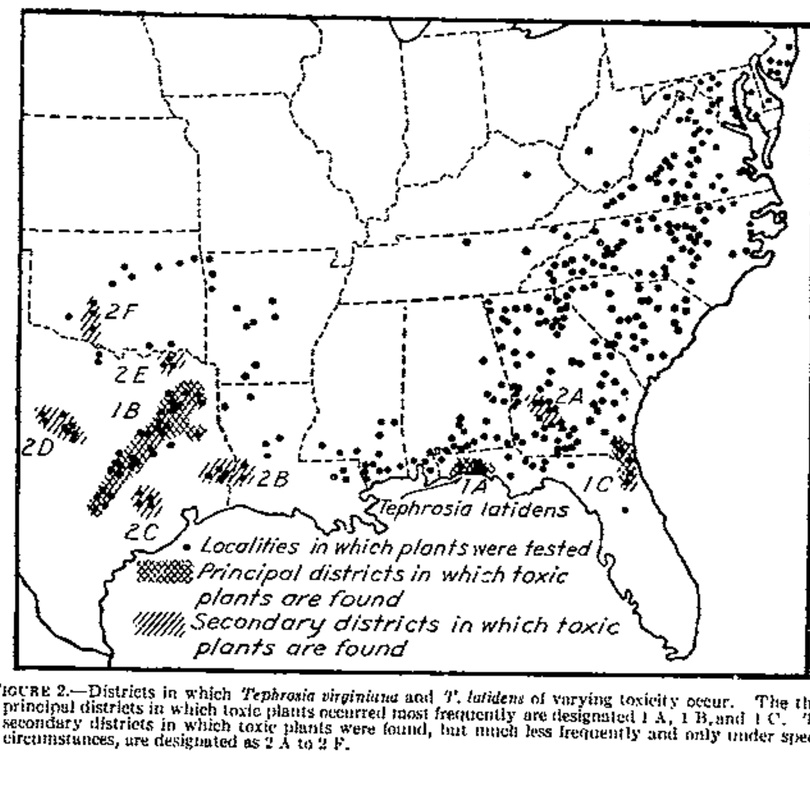


FIGURE 2.—Districts in which *Tephrosia virginiana* and *T. latidens* of varying toxicity occur. The three principal districts in which toxic plants occurred most frequently are designated 1 A, 1 B, and 1 C. The secondary districts in which toxic plants were found, but much less frequently and only under special circumstances, are designated as 2 A to 2 F.

Of the principal districts, district 1 A, almost entirely within the Choctawhatchee National Forest, in western Florida, extends about 25 miles from the eastern part of Santa Rosa County through Okaloosa County into the western edge of Walton County and about 10 miles from some distance south of the Yellow River to the Choctawhatchee Bay. *Tephrosia latidens* occurs in this district, but *T. virginiana* has not so far been found. District 1 B is a narrow belt in northeast Texas extending approximately 300 miles from Luling in Caldwell County northeasterly to Marshall in Harrison County. It is wider in the northern half where it extends into Nacogdoches County to the east. The relatively small district 1 C in northeast Florida includes Clay County and the western portions of Duval and Nassau Counties and extends across the St. Marys River to Folkston, Ga. It is approximately 60 miles long and 25 miles wide.

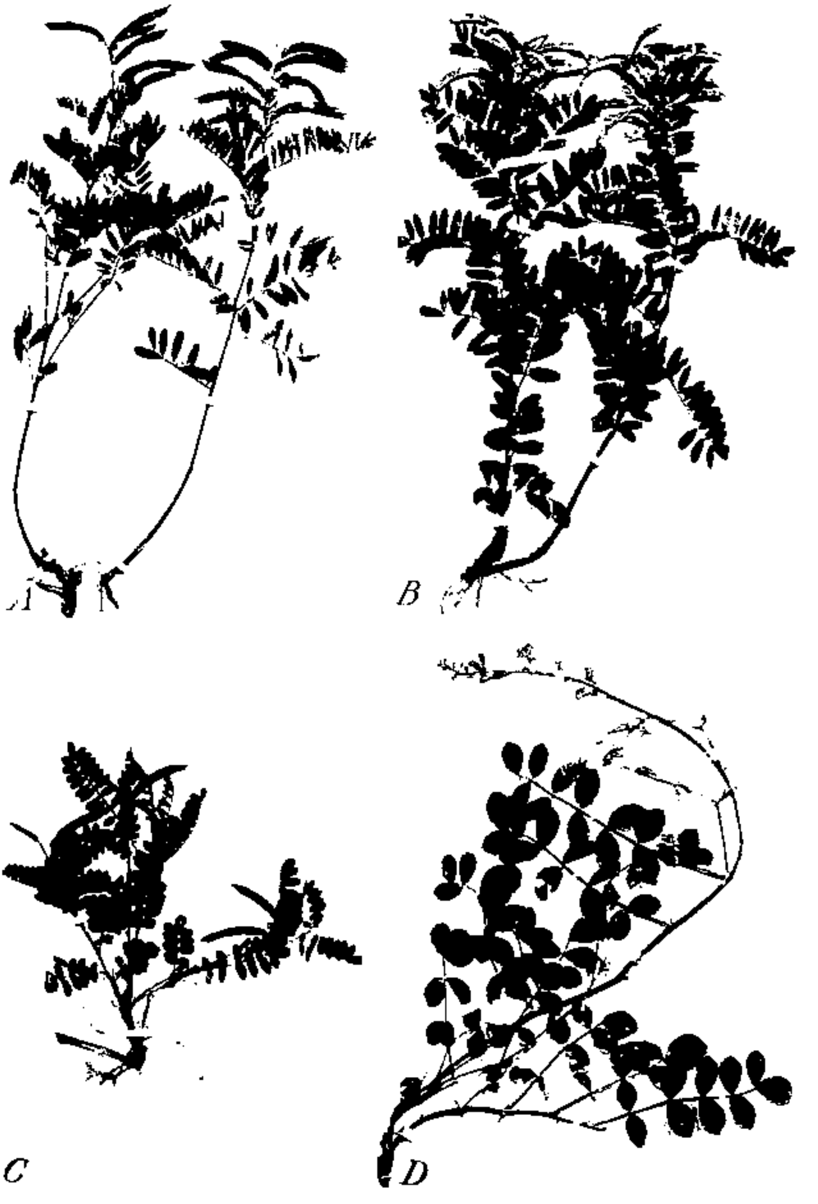
One of the larger secondary districts, 2 A, is located in southwest Georgia, extending approximately from Adel in Cook County northwesterly to Cordele and Americus, and including Albany, Sylvester, and Tifton. District 2 B is about 75 miles square, includes Newton, Jasper, and part of Tyler Counties in east Texas, and extends into the western portions of Vernon and Beauregard Counties, La. District 2 C includes parts of Washington, Austin, and Waller Counties, Tex. District 2 D consists of parts of Callahan, Eastland, Comanche, and Brown Counties, Tex. District 2 E in north Texas is very small, including only most of Lamar County. District 2 F includes most of Jefferson, Stephens, and Grady Counties, Okla.

If collection or cultivation of toxic plants is practical, the principal districts, one in northeast Texas, one in northwest Florida, and one in northeast Florida, would be those in which it would be promising. Even within these districts, however, there are many small localities in which only nontoxic or mildly toxic plants are found. Individual plants in certain localities and environments in the secondary districts may be as toxic as those in the principal districts. Indiscriminate collection of roots in the secondary districts, however, would yield material of little insecticidal value because of the preponderance of nontoxic roots. Collection in the nontoxic districts would be futile. Neither should its cultivation be undertaken in these areas unless later experiments should develop methods for obtaining toxicity.

SPECIES OF NATIVE TEPHROSIA

According to Rydberg (7) there are 24 species of *Tephrosia* native to the United States. Of these, 21 occur east of the Great Plains, the other 3, which have not been examined, being northern extensions of Mexican species into the Southwest. Of the 21 eastern species 9 have not been found north of Florida and only *Tephrosia virginiana* (pl. 3, *A* and *B*) ranges far from the southeastern part of the United States, probably occurring in all States east of the Great Plains. The approximate northern limits of the range of nine species of *Tephrosia* are shown in figure 3.

All species native to the United States are perennial and herbaceous, although in some the crown and roots become slightly woody. In habit they are either erect, ascending, or procumbent, never growing higher than 2 to 3 feet. The leaves are compound, of a varying number of leaflets. The inflorescence is either terminal or lateral and the flowers numerous or few, depending on the species. The flowers are



A, *Tephrosia virginiana* (eastern type) collected in Crisp County, Ga.; B, *T. virginiana* (western type) collected near Milano, Tex.; C, *T. latidens* collected in Okaloosa County, Fla.; D, *T. lindheimeri* collected in Guadalupe County, Tex.

A B C D

A, *Tephrosia hispidula* collected in Taylor County, Fla.; B, *T. curtissii* collected in Santa Rosa County, Fla.; C, *T. onobrychoides* var. *texana* collected in Tangipahoa Parish, La.; D, *T. onobrychoides* collected in Leon County, Tex.

usually yellow, but in some species are red tinged or whitish. In some species the roots become thickened and little developed, as in *T. hispidula* (pl. 4, *A*), while in others the root system becomes greatly developed, with taproot and long lateral roots 5 or 6 feet in length, as in *T. virginiana.* It is because of the long, tough, and flexible lateral roots that this species gets its name, "devil's shoestring." Most of the species are rather particular as to habitat, the widely distributed *T. virginiana* being exceptional in tolerance.

Within the area examined 11 species of Tephrosia were tested and studied in addition to *T. virginiana* (table 6), to determine whether they could be eliminated from further consideration. Of these, *T. latidens* (pl. 3, *C*), a very close relative of *T. virginiana*, showed considerable promise.

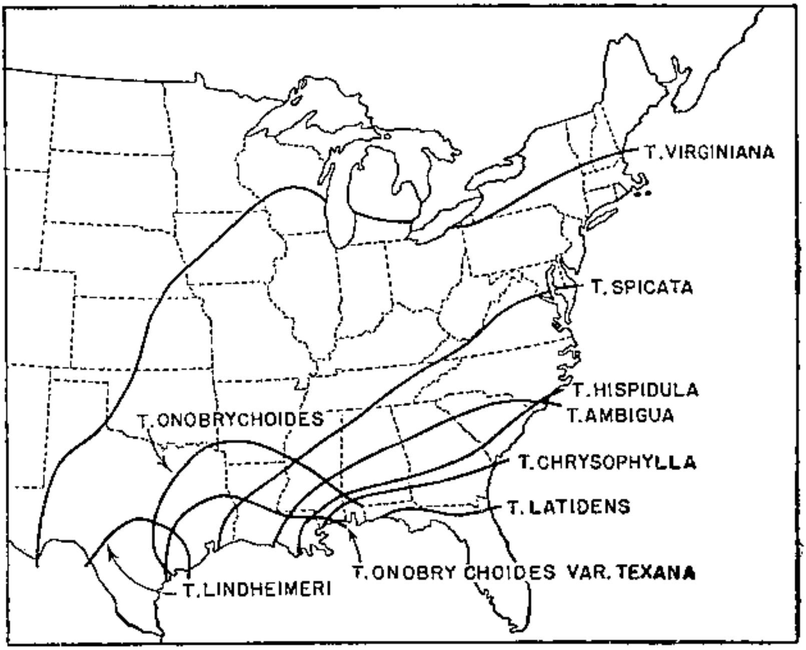


FIGURE 3.—Approximate northern limits of the range of nine species of *Tephrosia* in the eastern and southeastern United States.

The possible commercial usefulness of the several species must be judged on the basis of their insecticidal value and growth habits. Only with *T. carpenteri* (pl. 4, *B*) and *T. onobrychoides* var. *texana* (pl. 4, *C*) were the tests all negative, but very few plants of these species were tested, and in some localities it is likely that individual plants of all species studied may contain some rotenone or related substance in the root. It is unlikely, however, that any of the species other than *T. virginiana* and *T. latidens* warrant further study as practical sources of insecticidal material from the standpoint of toxicity. Both of these species, moreover, have sufficiently well-developed root systems to promise fairly good acre yields of roots under cultivation. To what extent this promise may be fulfilled will depend, not only on the ultimate size of the roots, but also on the rate of growth, on which point sufficient information is as yet not available.

In addition to *T. virginiana* and *T. latidens*, *T. onobrychoides* (pl. 4, *D*), *T. lindheimeri* (p. 3, *D*), and *T. onobrychoides* var. *texana* also have reasonably well-developed root systems. The possibility of using these and perhaps some of the other species in a breeding program should be kept in mind.

The foliage of the several species in various localities also was tested, but with negative results in all cases.

One of the special objectives of the field study was to note whether any correlation could be found between the varying taxonomic characters in individual plants of *T. virginiana* and the presence or absence of rotenone in their roots. No such evidence could be found. If the capacity to produce rotenone is a hereditary characteristic, there is no outward manifestation by which it may be recognized.

DISTRIBUTION OF TOXIC PLANTS IN RELATION TO SOIL AND VEGETATIVE ENVIRONMENTS

The possible relation of environment to toxicity in the plant roots was studied in detail in all districts except secondary districts 2 D, 2 E, and 2 F (fig. 2).

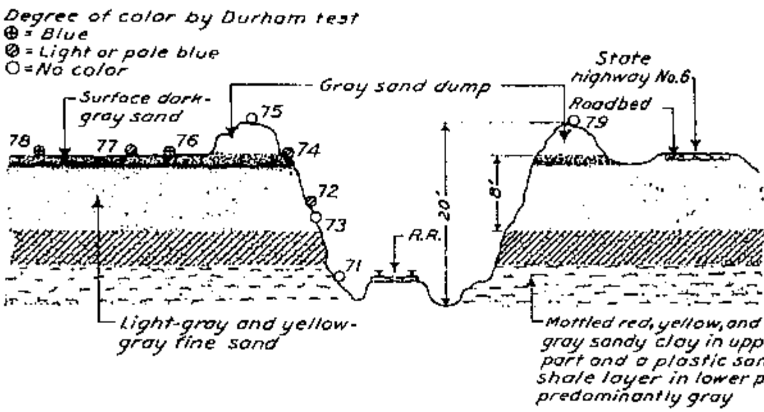


FIGURE 4.—Profile of Norfolk sand in principal district of toxic plants, 1 B, near Bryan, Tex., showing toxic plants nos. 76, 77, and 78 growing on the normal surface soil and nos. 72 and 74 on slumped sections of it. Nontoxic plants nos. 75 and 79 grow on disturbed soil material and nos. 71 and 73 on subsoil layers not favorable to toxic plants.

The principal data pertaining to particular situations that appear significant in their indication of possible relationships between environment and toxicity are assembled in table 7. A typical soil profile relation is illustrated in figure 4. Reference is to *T. virginiana* unless otherwise indicated.

The principal districts producing toxic plants, designated 1 A to 1 C, have a large proportion of toxic plants growing on the virgin and normally developed surface soils of the region. These soils are chiefly the more sandy types of the Norfolk and Blanton series. The districts designated 2 A to 2 F are considered secondary districts producing toxic plants. Of the plants examined in these districts most of the toxic ones grew in situations or associations that do not predominate in the localities in which they are found. Toxic plants are, therefore, exceptional rather than common. They occur in these secondary districts mostly on soils of the Susquehanna, Tifton, Norfolk, Ruston, and Orangeburg series.

4800°—38——3

TABLE 7.—*Data on the general environments of Tephrosia plants in a number of situations*

Situation no.	Location			General soil type in locality	Species	Plant no.	Degree of color by Durham test	Acidity of soil in which plants grow (pH)	General environments
	State	County	Locality						
1	Texas	Anderson	1 mile northwest of Montalba.	Norfolk sand (sandhill phase).	*T. virginiana*	42	No color	7.0 at 3 inches, 6.7 at 7 inches.	Vigorous plant on slope where decomposed oak log provided thick dark organic layer.
					do	43	Dark blue	6.2 at 1 inch, 4.6 at 8 inches.	Lower down on slope from no. 42. Thin organic layer and generally lighter colored soil apparently thoroughly leached.
					do	43A to 43G	One no color; others dark blue.		Down slope from no. 43. 6 inches dark sandy layer over light-gray sandy subsoil and substratum.
2	do	Robertson	About 7 miles northwest of Bryan.	Kirvin very fine sandy loam with 6-inch reddish-brown surface layer.	do	67	No color	6.7 at 2 inches, 5.5 at 6 inches.	On normal profile.
					T. onobrychoides	68	do	6.6	In bottom of cut on ditchbank. Mixed soil conditions, chiefly yellow very fine sandy loam.
					T. virginiana	69	do	4.6	Near no. 67, but growing on matted red and gray subsoil (predominantly red).
					do	70	do	4.5	Young plant growing on scraped subsoil of the highway gutter.
3[1]	do	do	About 7½ miles northwest of Bryan.	Norfolk sand	do	71	do		Chiefly shales parent material.
					do	72	Light blue		4 inches dark gray, then 2 inches light gray, then clay and shale.
					do	73	No color		Very little dark soil.
					do	74	Light blue		Root partly in old surface below.
					do	75	No color		Sand dump; neutral reaction.
					do	76	Blue		Virgin soil; nearly level, dark, acid surface.
					do	77	Light blue		Near no. 76; less acid and lighter gray.
					do	78	Blue		Similar to no. 77.

[1]Illustrated in fig. 4.

TABLE 7.—*Data on the general environments of Tephrosia plants in a number of situations*—Continued

Situation no.	Location			General soil type in locality	Species	Plant no.	Degree of color by Durham test	Acidity of soil in which plants grow (pH)	General environments
	State	County	Locality						
4	Texas	Milam	About 4½ miles southwest of Gause.	Susquehanna fine sandy loam, nearly flat surface but well drained.	*T. virginiana*	86	Trace	7.0 organic layer, 6.8 subsurface.	On road-cut face. Surface soil a light yellowish gray, loamy, fine sand with little organic accumulation. Subsoil predominantly gray clay, but matted with red and yellow splotches.
					do	87	No color	7.0 at 3 inches (dark material), 7.0 at 6 inches (lighter gray material).	Growing on the normal surface soil layer.
					do	88	Light blue	5.5 in clay	On mottled sandy clay layer near bottom of road ditch with 2 to 4 inches of slump soil on top.
					do	89	do	7.0 (dark layer), 6.0 (mottled layer).	On opposite slope from no. 86.
5	do	Wilson	Half mile south of Guadalupe County line.	Kirvin stony, sandy loam; high iron content; red shale, sandstone and iron crusts numerous.	do	106	No color		On crest of hill.
					do	107	do	4.2 at 5 inches	On slope of hill about 5 feet below crest.
					do	108	do	4.5 (leaf litter)	At same altitude as no. 107, but in less stony soil with more leaf litter.
					do	109	Trace		About 35 feet down at base of slope; in reddish stony sand washed down from hill, but roots grow in dark, sandy surface soil of Norfolk sand.
6	Florida	Okaloosa	About 6 miles northeast of Fort Walton.	Norfolk sand; extensive, nearly level and uniform area with rather thick growth scrubby oak.	*T. latidens*	219	Blue	4.8 at 0–3 inches 6.8 at 3–48 inches (loose yellow sand), 6.8 at 48–60 inches (almost white, quartz sand).	Surface soil of pepper-and-salt gray sand about 3 inches deep underlain by deep layer of loose yellow sand about 4 feet deep.
					do	220	Deep blue		Growing on soil layer corresponding to second layer mentioned above.

					T. virginiana	322	No color		On slope in normal surface soil layer.
					do	323	Dark blue		On normal soil, but near hickory bark under 2 large oak trees.
7	Georgia	Sumter	1½ miles north of Americus.	Ruston sandy loam (deep phase).	do	324	No color		On normal soil about 12 feet from no. 323, but free from tree-root influence.
					do	325	Dark blue		Along roadside where red clay was thrown on normal surface soil. Roots grew alongside of roots of large oak tree.

PRINCIPAL DISTRICTS IN WHICH TOXIC PLANTS OCCUR

District 1 A is characterized by the uniformity of its soil and vegetative characteristics with a corresponding uniformity in the toxicity of the *Tephrosia* plants so far tested. It may be found later, of course, that in this district plants with little or no toxicity occur in situations where environmental factors vary from the average. Nevertheless, it is believed that on the whole this area is probably the largest area of uniformly toxic plants. As previously stated, the predominant species is *T. latidens*, *T. virginiana* not occurring in this district.

The soil is classed as Norfolk sand, although it is recognized that the parent material is a comparatively recent deposit of the lower Coastal Plain. Decomposition and leaching processes, therefore, have not progressed to the degree that they have in the older geological deposits in districts 1 B and 1 C or in the sand-hill section of Georgia and the Carolinas. This latter area is included in those of nontoxic plants.

This district is believed to offer promise for the commercial growing of *T. latidens* in view of the extensive occurrence and the uniform development of a soil which normally produces toxic plants on either its surface or subsoil layers. However, more information is needed regarding the toxicity and root growth of this species. Whether *T. virginiana* can be grown in this district must be determined by trial.

In district 1 B it is significant that all toxic plants grew on a soil classed as Norfolk sand and in situations where the surface soil had not been disturbed in its normal development. On the other hand, all nontoxic plants on this same soil type grew in situations where the feeding zone of their roots was in soil from other layers of Norfolk sand or in a deeper less-acid surface soil. This is illustrated in figure 4.

This correlation between soil environment and toxicity of the plants occurred in several localities in district 1 B, namely, near Montalba, Stockdale, Bryan, Harleton, Nacogdoches, Milano, and Winona, Tex. The locality near Winona is locally known as Sandflat, within which is a nearly level, quite uniform area of Norfolk sand. Most of the tested plants from this area were toxic in some degree. Outside of this area, where low-lying ridges and knolls, shallow valleys, and seepage spots occurred, more of the plants examined were nontoxic or only slightly toxic. There evidently is some relation between environmental factors and the toxicity of the plants. Within the boundaries of district 1 B are many thrifty plants growing on other than Norfolk soils. Examination of such plants showed that these are, as a rule, nontoxic, and that the ones that are toxic usually grow on a substratum layer and not on the normal surface soil. These soils are the same as those occurring in the secondary districts 2 A and 2 B and in parts of the areas of nontoxic plants, chiefly members of the Kirvin, Ruston, and Orangeburg series. They are characterized by the red color of either or both the surface or subsoil and by their heavier textures. As a rule in these reddish soils less leaching of soluble plant-food material has taken place. Such soils as these occur in close proximity to all localities in which toxic plants occur in district 1 B. They occur at Harleton, Nacogdoches, Sandflat, Montalba, Bryan, and Milano and also in the southern limits in the district near Luling.

In district 1 C the plants occur predominantly on soils of the Blanton series, but also on those of the Leon and Bladen series. These soils, like those in district 1 A, are developed from a comparatively recent geological deposit of the lower Coastal Plain, but they are apparently more thoroughly leached than the sands of that district. The Blanton soils are light-gray sandy soils occurring typically on low-lying knolls within the so-called flatwoods of the lower Coastal Plain. The Leon or Bladen soils are more poorly drained and commonly adjoin the Blanton. Scrub oaks, some pine, wire grass, and scattered palmetto are the common types of vegetation. On the Blanton soils the oaks, and on the Leon and Bladen soils the pines and palmetto, predominate. The probability that vegetative environment exerts some influence on the toxicity of plants in this district is strongly suggested by the fact that a large number of toxic plants were found to have their roots in contact with roots of oaks. Two plants which were several feet away from oaks and the roots of which did not appear to be in close proximity to oak roots were nontoxic. However, in the northern part of this district near Folkston this relationship was not so common, and toxic plants were found growing on the normal surface soil and also on the exposed subsoils.

SECONDARY DISTRICTS IN WHICH TOXIC PLANTS OCCUR

Many toxic plants occur in district 2 A, but they apparently occur only in those situations where some factor has prevented the development of the normal soil profile of the region or where vegetative influences are apparently active. The former are chiefly eroded soils or so-called bald spots, or they are exposed substratum layers occurring along road ditches, stream banks, and railroad cuts. On the other hand, when toxic plants are found on the normally developed surface soil they occur chiefly in those places where the plant roots are in close proximity to, or in direct contact with, roots of other plants such as oaks, hickories, or poison-ivy.

Observations on the apparent influence of vegetative environment were made near Weirsdale, Fla. This is an area of Blanton fine sand. The surface of the soil was more or less barren except for the cover provided by scattered palmetto plants and a rather open forest growth of scrub oak and scattered pines. Here 37 plants were tested in a tract of about 2 acres. Only two of these plants were toxic. One of these toxic plants had its roots in direct contact with the roots of an oak tree. With this in mind, seven more plants were collected, every effort being made to secure them from similar situations. Although it was difficult to duplicate the exact environment in all cases, it is significant that of the seven plants, five were toxic.

In district 2 A toxic plants were found growing on Susquehanna clay and clay loam. This soil results from the removal of the former upper layers of the soil profile, thus exposing the original substratum layer or the partially altered parent material layer. As a rule, plant-food elements in this layer are not readily available, and, therefore, plants are not commonly established on it. It is significant, however, that all plants growing on the less-altered layer that were tested were toxic in some degree, the degree of toxicity being less in those plants growing in situations where a thin organic surface layer and one less acid had developed. On the lower slope of these clay knolls, where a definite surface soil layer had accumulated, only nontoxic plants were found.

In districts 2 B and 2 C observations suggest similar environmental influences to those found in district 2 A. In districts 2 D, 2 E, and 2 F such studies were not made, but a knowledge of the character of the soils in these districts, gained on other occasions, leads to the assumption that toxic plants in these localities would be found to be less common than nontoxic plants, and that they would occur in situations similar to those in which they are found in the other secondary districts.

In district 1 B near Milano, Tex., an example of conditions common in secondary districts of toxic plants, but occurring more rarely in a principal district, was noted. Here plants varying in degree of toxicity were located on the subsoil or substratum layers of Susquehanna fine sandy loam, whereas others located on the normal surface of this soil were nontoxic. This occurrence is contrasted with the previously noted situation on the Norfolk sand in this district where the toxic plants occur on the normal surface soil and the nontoxic plants on subsoil or substratum.

AREAS OF NONTOXIC PLANTS

It has already been mentioned that in certain wide areas, with few exceptions, only nontoxic plants are found. Within these areas the relatively few plants that were somewhat toxic in all instances were growing on a deep substratum layer or their roots were in contact with the roots of other plants. However, in the nontoxic areas, even these environmental factors, which appear to favor the development of toxicity in other districts, fail to produce any widespread or extreme toxicity in any of the plants.

The areas in which *Tephrosia virginiana* is commonly found, yet where it is rarely toxic, are the sand-hill districts of Georgia and the Carolinas, the Gum Pond district of south-central Mississippi, the southern part of Alabama, northern-central Florida, and parts of Oklahoma, Arkansas, Virginia, and Maryland. The soils in these localities fall naturally into two general groups. The first consists of the deep sands of the sand hills of the upper Coastal Plains occurring typically in the vicinity of Columbia, S. C. The second group consists of sandy loams or heavier types of more fertile soils chiefly of the Tifton, Orangeburg, Ruston, Kirvin, Greenville, and Cecil series. In general, these latter soils are more abundantly supplied with all the elements essential for plant growth than are the deep sands of the sand-hill section or the sandy soils in the principal districts 1 A to 1 C. Other soil types on which toxic plants have not been found in all cases are similar in character to one or the other of the two groups mentioned.

POSSIBLE SIGNIFICANCE OF OBSERVATIONS

When detailed studies of *Tephrosia* species in the field were undertaken, it was hoped to obtain definite information on the causes of variation in toxicity. No relation was found between toxicity and noticeable variations in visible botanical characters. On the other hand, soil studies furnish indications that soil and vegetative environments play some role in determining the extent to which toxic substances will form in the plant. Some evidence, also, was obtained which suggests that genetic strains of *T. virginiana* may differ in ability to develop toxicity. If strains are found which prove capable of developing toxicity when grown on soils and under environments that now appear unfavorable to the development of toxicity, then the studies on environment naturally become secondary. Even so, certain environments may tend to make toxic plants more toxic, in which case an accurate understanding of such relations is of practical value in developing possible commercial production.

In discussing the significance of observations, the writers wish to emphasize that interpretations are tentative and that subsequent studies may change them.

Tephrosia virginiana and *T. latidens*, and to a less extent other species of *Tephrosia*, thrive on a large number of well-drained virgin soils. They are rarely found in wet situations or on cultivated lands and never on permanently waterlogged soils. Toxic plants of the two species mentioned were found growing on natural undisturbed surface soil only on sandy types of the Norfolk, Blanton, Leon, and Bladen series. On the other hand, toxic plants were found growing on subsoil or substratum layers of a much larger number of soils, including not only Norfolk and Blanton soils but also Ruston, Orangeburg, Tifton, Susquehanna, Luverne, Tabor, Crockett, and Alamance series. In a very few instances toxic plants were also found growing on the normal surface soils of the Ruston, Tifton, and Orangeburg series. In such cases, however, either mixtures of soil layers or close association with other plant roots altered the environment, so that the latter was unlike that of the normal surface soil nearby on which nontoxic plants were growing.

Soil characteristics of the several habitats offer a number of interesting comparisons. The sandy soils of the Norfolk, Blanton, Bladen, and Leon series are derived from comparatively recent geological deposits of the lower Coastal Plain, except that the Norfolk sand of the sand-hill section is derived from an older geological deposit of the upper Coastal Plain. In the case of the more recently formed soils of these series, toxic plants were found growing on the normal undisturbed surface, whereas on the older soil of the sand hills, the plants while growing extensively on the undisturbed surface were, with rare exceptions, nontoxic. This suggests that some substance absorbed from the soil solution may be essential for the production of toxicity in the plant. If this is true then this substance apparently has been removed from the surface soils derived from the older deposits by leaching and weathering and apparently is not available in sufficient quantities to produce the toxic effect except in the less-altered layers of the deeper substratum.

The fact that on soils such as Susquehanna, Tifton, Orangeburg, and Ruston the toxic plants are, as a rule, found growing only on exposed, bare, subsoil layers or on the deep substratum and not on the more thoroughly leached surface soil layers may or may not have similar significance.

The tenability of the above hypothesis is supported by the observation that in a number of instances plants growing on Norfolk, Blanton, and Leon soils show more pronounced toxicity under certain special environments. Proximity of the plant's roots to other vegetation, particularly oak roots, seems to have such effect. In these situations the soil was more acid than elsewhere in the surface layers. Also, in most instances, plants growing on the less-leached lower layers of these soils were more toxic than those plants growing on the surface soils. Where the surface soil had been changed from acid to neutral or alkaline through the decomposition of oak logs or the burning of logs or brush or through other influences, either the necessary substance was less available or conditions were unfavorable in other respects because in such situations the plants were found to be less toxic or entirely without toxicity.

Toxic plants have never been found on Greenville, Blakely, Houston, Cecil, Pheba, or any of the heavier and more fertile soils occurring in the region where the plants grow, nor have toxic plants been found on heavy soils with high iron content and high acidity, such as the Kirvin stony clay loam. In these soils the degree of the soil acidity does not appear to be related to toxicity, although in the case of soils where toxicity does occur, acidity and low organic content are associated with toxic plants.

Considering origin and composition, it is not reasonable to assume that the soils that produce nontoxic plants are deficient in an element which has leached from the Norfolk sand of the sand-hill section but which is plentiful in Norfolk sand of the lower Coastal Plain. Many of these more fertile soils are derived from geological deposits similar to those from which come the soils on which toxic plants commonly grow and should contain similar materials. It is assumed that if such soils contain any toxicity-producing substance that may be contained in the soils where plant toxicity occurs, then their failure to produce toxic plants must be due to other substances antagonistic to toxicity. On such a basis it might be further assumed the inhibiting substance has been leached from the soils producing toxic plants.

Similarly it might be assumed that while the inhibiting substance may have been largely removed from both the Norfolk sand of the sand-hill section and that of the younger lower Coastal Plain, the substance essential for toxicity has also been largely removed from the sand-hill section. On such a basis, toxic plants should be found only rarely in the sand-hill section but quite commonly on the Norfolk sand of the lower Coastal Plain. This is actually the case. Proof of the theory rests on additional study.

The chemical analyses of the soils on which toxic and nontoxic plants grow is naturally suggested as a means of determining which elements, if any, are responsible for inducing or inhibiting rotenone production, but this is not a practical approach to the problem. All observations suggest that, if any element plays such a role, it is probably one that is present in very small quantities. The soil analyses would, therefore, have to be very complete and made on many samples. Sampling would be difficult because the roots usually extend considerable distances and frequently penetrate into several soil strata differing in composition. The possibility that the elements absorbed by the roots might give a clue has been considered but the analyses of the root ash of a number of toxic and nontoxic plants revealed nothing significant with respect to the quantities of the more common elements present. Spectographic examination [8] of the ground roots of six toxic and six nontoxic plants also failed to show any correlation between toxicity and the elements present, special

[8] The writers gratefully acknowledge the collaboration of O. R. Wulf and E. H. Melvin, Bureau of Chemistry and Soils, who made the analyses.

attention being given to those elements that are generally present in small quantities.

GENETIC CHARACTER A POSSIBLE FACTOR DETERMINING TOXICITY

The fact that distinctly toxic plants of *Tephrosia virginiana* are rarely, if ever, found in the northern section of the plant's range suggests that, in a broad sense, climate may have some relation to toxicity, but climate cannot be a factor by itself because toxic and nontoxic plants occur side by side in some southern localities. The possible effects of soil and vegetative environment on toxicity have been discussed. Another possibility is that the capacity to produce rotenone and related toxic substances in varying degrees is a genetic character. It has been pointed out that toxicity apparently has no relation to visible botanical characters, but toxicity, or the capacity to produce rotenone and related products, may be inherent in certain strains of the plant and lacking in others. If the capacity to produce rotenone is due entirely to such a genetic character and is not influenced by other factors, all the plants possessing such character should exhibit it, regardless of the locality in which they grow, which is not the case. On the other hand, if certain environments interfere with the performance of this function, nontoxic individuals of the same strain would be found in some localities. The logical approach to an understanding of this question is to grow plants of known quality in localities in which the quality of plants naturally occurring there has been determined. In this way it may be learned whether toxic plants from Texas can be transferred without effect on their toxic properties to localities in the Southeast in which no toxic plants have been found. Also, it may be learned whether nontoxic plants from the Southeast will remain so when transferred to a locality in Texas where toxic plants naturally occur. Such experiments are under way in several localities. If soil environment is the determining factor, then the commercial growing of the plant must be restricted to certain limited areas unless some practical means of modifying such environment is found. On the other hand, if a genetic factor is entirely responsible for the variability in the plant's toxicity its commercial culture may be possible in those regions in which the plants grow best and which are not well adapted to other crops by providing planting stock of acceptable quality. It is possible that both genetics and environment are important factors and some evidence supporting such a theory is at hand, but much additional study is necessary before it may be accepted or discarded.

The highest rotenone content recorded in table 5—namely, 1.8 percent—was found in composite root samples from Tyler and Harleton, Tex. Inasmuch as nontoxic plants also occur in these districts these composite samples undoubtedly included some roots containing little or no rotenone and others containing a percentage of rotenone considerably higher than that of the composite sample. It seems probable, therefore, that by an examination of many individual plants in such a district some would be found with a considerably higher rotenone content than heretofore noted. Several plants containing over 3 percent of rotenone have already been found in the Sandflat district in Texas, and these are being propagated. Whether such plants and those propagated from them by crown division will have an equally high rotenone content when grown in other districts is not known, but they can at least be used for gradually increasing planting stock and thus provide the means for more extensive field experiments.

Preliminary experiments on the possibility of developing toxic strains of *Tephrosia* have been undertaken. Unfortunately, however, attempts to insure self-pollination by enclosing plants in insect-proof cages were not successful, practically no seed pods or seed being obtained. Further studies of controlled pollination are planned. In the meantime, 125 plants from open-pollinated seed from 9 parent plants have been subjected to the Durham test, as have 29 plants propagated by crown division from the same parent plants. The results of these tests are shown in table 8.

TABLE 8.—*Results of the Durham test on plants of Tephrosia virginiana propagated from seed and by crown division*

Parent plant no.	Plants giving indicated color reaction										
	Plants from crown division						Plants from seed, 1934				
	1934			1935							
	Pale blue	Blue	Deep blue	Pale blue	Blue	Deep blue	No color	Trace	Pale blue	Blue	Deep blue
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
L-15		3			3					5	
L-21		3			4		1		1	15	4
L-22					3				1	8	----
L-23			5		3				1	6	----
L-32		2			2			1		5	----
L-34	1				2			1	8	14	----
L-59					4					3	----
L-64				5				2	1	3	----
L-73	1					3	5	4	10	7	18

The plants from crown division tested in 1934 and 1935 were the same so far as possible. Thus the three plants from L-21 tested in 1934 were included among the four plants tested in 1935. The results in these 2 years then indicate something of the variation that may be expected in the same plants from year to year due either to environment, method, or both. Nevertheless, and in spite of the small numbers, it appears that the variation among the seedlings indicates hereditary variability in toxicity. Furthermore, the data suggest (though too meager to do more) that plants tending to be more toxic tend also to have fewer seedlings in the slightly toxic or nontoxic groups and thereby warrant further experiments along this line.

MISCELLANEOUS STUDIES OF TEPHROSIA VIRGINIANA

The studies that have so far been made and the results obtained have naturally suggested numerous points regarding which information is necessary. It is desirable to know to what degree individual plants in a small area differ in toxicity, especially in a locality where their commercial collection would be most logical on the basis of information already at hand. The distribution of the toxic substances in individual roots of varying size and in the several root tissues must be determined. Information on toxicity in relation to the plant's age and to the time of the year when the roots are collected is important. Considerable data on these questions already obtained will be presented here, but additional information is expected to be developed as the investigations proceed.

EXAMINATION OF ALL WILD PLANTS OF TEPHROSIA VIRGINIANA ON 1 ACRE IN TEXAS

To determine the variation in toxicity of all individual plants of wild *Tephrosia virginiana* growing in a district in Texas where toxic

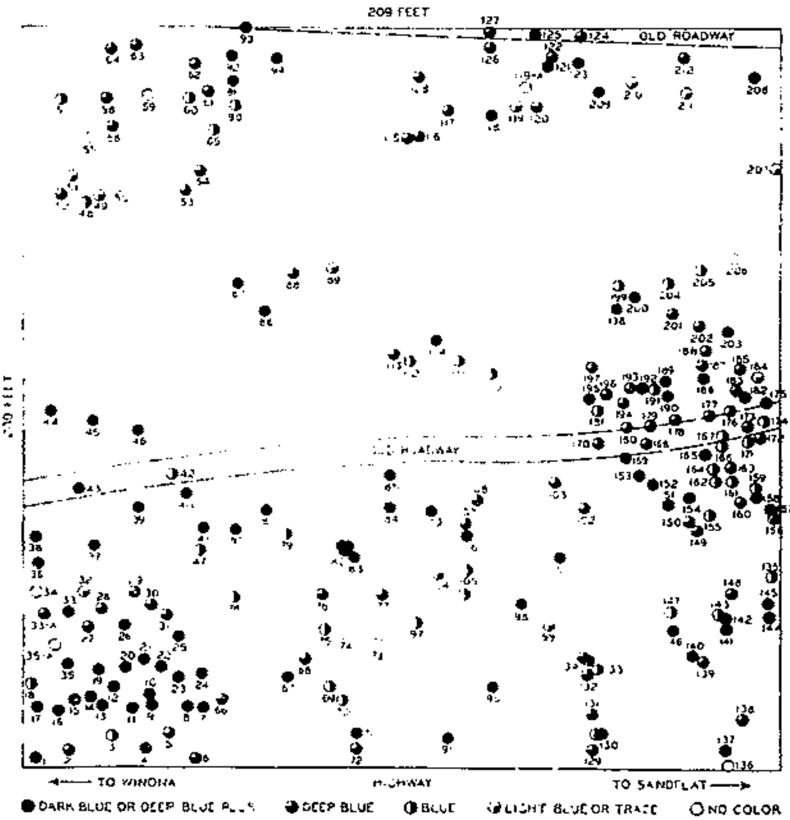


FIGURE 5.—Toxicity of individual plants of wild *Tephrosia virginiana* on 1 square acre of Norfolk sand near Sandflat, Smith County, Tex., as indicated by the degree of color obtained with the Durham test on acetone extracts of the roots.

plants predominate and where soil conditions are reasonably uniform, the roots of all the individual plants on 1 square acre in such a district were tested. The soil acidity at 3-inch and 6-inch levels under each plant was determined with the La Motte Duplex indicator and environments noted. The acre selected is on a level tract, known locally as Sandflat, between Lindale and Winona, about 6.5 miles due north of Tyler in Smith County. The soil is classified as Norfolk sand with the surface soil undisturbed in its normal development. It was cut over some years ago and now has a second growth, principally of scrub oak with scattered pine. Broomsedge, poison-ivy, bullnettle, some yucca, and an occasional cactus constitute the small vegetation. The plot has never been under cultivation but at one time was pastured. Two hundred and twenty thrifty plants of *T. virginiana* were found on this plot.

The plants were dug in mid-September 1935. The roots from each plant were dried, ground, and extracted for 7 hours with acetone and the extracts made to volume so that 1 cc represented 0.4 g of the root. These were then tested by the Durham method in several dilutions. The data obtained are assembled in table 9. The locations of the individual plants in the plot and the degree of color obtained by the Durham test on 0.25 cc of the acetone extract are shown in figure 5. The tests were made at greater dilution than usual in order to differentiate between those samples that give a dark bluish-black opaque color when the test is made on extracts of greater concentration. At the dilution used the most toxic extracts still give an opaque color, whereas those somewhat less toxic give a deep but transparent blue.

Table 9.—*Degree of color obtained by the Durham test on individual plants of wild Tephrosia virginiana on 1 acre in the Sandflat district in Smith County, Tex., and the environment of such plants*

Plant no.	Degree of color[1] obtained by Durham test on acetone extracts of the roots (0.4 g to 1 cc) using—						Soil acidity at—		Environment
	1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	1 drop	3-inch level	6-inch level	
							pH	pH	
1	5	5	4+	4	3	2	6.7	5.7	In open.
2	5	5	4	3	2	1	5.7	5.7	Do.
3	5	4	3	2-	2-	0	6.7	5.7	In leaves and brush.
4	5	5	4+	4	3	2	4.9	4.7	Under oak tree.
5	5	5	4	3	2	1	6.2	5.9	In poison-ivy.
6	5	5	4	2	2-	1	5.5	5.8	Under oak tree.
7	5	5	5	4	3	2	6.4	4.2	Do.
8	5	5	4+	3	2	1	4.7	4.7	Do.
9	5	5	5	4	3	2	6.7	4.2	In open.
10	5	5	5	3	2	1	5.2	4.2	Do.
11	5	5	4+	3	2	1	5.2	4.2	In leaf litter and broomsedge.
12	5	5	4+	3	2+	2	4.2	4.2	In broomsedge.
13	5	5	5	4+	3	2+	6.2	4.7	Do.
14	5	5	5	4+	3	2+	4.2	4.9	In open.
15	5	5	5	4+	3	2+	6.0	4.7	Do.
16	5	5	5	4+	3	2+	6.2	5.2	Do.
17	5	5	5	4+	3	2+	6.0	6.0	Do.
18	5	4	3	2	2-	0	6.7	6.5	In poison-ivy under oak tree.
19	5	5	5	4+	3	2	6.2	6.2	In open.
20	5	5	5	4+	3	2	6.5	6.2	Do.
21	5	5	4+	4	3	2	5.7	4.2	Do.
22	5	5	4	3	2	1	6.0	5.2	Do.
23	5	5	4	3	2	1	4.7	4.7	Close to pine seedling.
24	5	5	4+	4	3	2	6.7	6.7	Next to yucca.
25	5	5	4+	4	3	2	5.2	4.9	In open.
26	5	5	5	4	3	2	6.2	6.2	Do.
27	5	5	4	3	2	2-	6.0	5.2	Do.
28	5	5	4	3+	3	2	5.2	4.7	Do.
29	5	5	4	3+	3	2	5.2	5.2	In broomsedge.
30	5	5	4	3+	3	2-	4.7	4.7	Do.
31	5	5	4	3+	3	2	5.2	4.9	Do.
32	3	3-	2+	2	2-	1	5.2	5.2	In open.
33	5	5	4+	4	3	2	5.0	5.2	In poison-ivy.
33-A	5	5	4	3	2	2-	5.2	5.0	In open.
34	0						5.7	5.7	Do.
35	5	5	4+	4	3	2	6.0	6.0	Do.
35-A	0						6.7	6.2	In open; lower soil layer dark.

[1] The numerals refer to the degree of color obtained as follows: 5=dark blue; 4+=deep blue plus; 4=deep blue; 3+=blue plus; 3=blue; 3-=blue minus; 2=light blue; 1=trace; 0=no color.

TABLE 9.—*Degree of color obtained by the Durham test on individual plants of wild Tephrosia virginiana on 1 acre in the Sandflat district in Smith County, Tex., and the environment of such plants*

Plant no.	Degree of color obtained by Durham test on acetone extracts of the roots (0.4 g to 1 cc) using—						Soil acidity at—		Environment
	1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	1 drop	3-inch level	6-inch level	
							pH	*pH*	
36	5	5	4+	4	3	2	6.2	6.2	In open.
37	5	5	4-	4	3	2	5.7	5.2	Do.
38	5	5	5	4+	3	2+	6.2	5.9	Do.
39	5	5	5	4+	3	2+	6.2	6.2	In broomsedge.
40	5	5	5	4+	3	2+	6.0	6.0	Do.
41	5	5	5	4+	3	2+	6.2	6.0	In open.
42	5	4	3	2+	2	1	5.7	5.7	In poison-ivy on old roadway.
43	5	5	5	4+	3	2+	5.2	5.2	On old roadway.
44	5	5	4+	4	3	2	6.2	6.2	In open.
45	5	5	4+	4	3	2	6.2	5.7	Do.
46	5	5	4+	4	3	2	6.2	6.2	In broomsedge.
47	5	4	3	2	2-	1	5.7	5.2	Do.
48	5	4	3	2	2-	1	5.2	5.2	Beside old pine stump.
49	3	3-	2	1	0		6.2	4.7	Under scrub oak.
50	0						5.4	5.4	In open.
51	3	3-	2	1	0		5.7	5.7	Do.
52	5	5	4	3+	3	2	5.2	5.2	Near scrub oak.
53	5	5	4	3	2	1	6.0	6.0	In open.
54	5	5	4	3	2	1	6.2	6.2	Do.
55	0						6.0	5.2	Do.
56	5	5	4	3+	3	2	5.2	5.7	Do.
57	5	4	3	2	1	0	6.2	6.2	Do.
58	5	5	4	3	2	1	6.2	6.2	Do.
59	0						6.2	5.7	Under scrub oak.
60	5	4	3	2	1+	1	6.6	5.7	Do.
61	5	5	4	3+	3	2	5.2	5.2	Close to pine tree.
62	5	5	4	3	2	1	6.2	5.2	In open.
63	5	5	4	3+	3	2	6.2	6.2	In broomsedge.
64	5	5	4	3+	3	2	5.2	5.2	Under wild grapevine.
65	5	5	3+	3	2	1	6.4	6.2	In open.
66	5	5	4	3+	3	2	6.6	6.6	Do.
67	5	5	4-	4	3	2	6.4	5.7	Do.
68	5	5	4	3+	3	2	6.4	6.4	Adjacent to rotting log.
69	5	5	3	2+	2+	2	7.0	6.2	In open.
70	5	5	3+	3	2	1+	6.7	6.0	In broomsedge.
71	5	5	4+	4	3	2+	7.0	7.0	Do.
72	5	5	4	3+	3	1+	5.7	5.2	In open.
73	0						6.2	6.2	Do.
74	0						5.7	6.2	Under scrub oak.
75	5	5	3+	3	2	1	6.4	6.4	In poison-ivy.
76	5	5	4	3+	3	2	6.6	6.2	Adjacent to rotting log.
77	5	5	4	3+	3	2	6.7	6.7	In open.
78	5	5	3+	3	2	1	7.0	6.7	Do.
79	5	5	3	3-	2	1	5.7	6.7	In open; lower soil layer dark.
80	5	5	5	4	3	2	6.4	6.4	In open.
81	5	5	5	4	3	2	6.2	6.2	In brush and fallen tree.
82-1	5	5	5	4	3	2			
82-2	5	5	5	4	3	2	6.2	7.0	In open; lower soil layer dark.
82-3	5	5	5	4	3	2			
83	5	5	5	4	3	2	6.6	6.6	Under a pine tree.
84-1	5	5	4+	4	3	2	6.5	6.5	In open.
84-2	0						6.5	6.5	Do.
85	5	5	5	4	3	2	4.7	6.2	At edge of wood; lower soil layer dark.
86	5	5	5	4+	3	2+	6.6	6.6	Under a pine tree.
87	5	5	5	4	3	2+	6.6	5.2	In broomsedge.
88	5	5	4	3	2	1+	6.2	6.2	Do.
89	3+	3	2	2-	1	0	6.2	6.2	Do.
90	5	4+	3	2	2-	1	6.8	6.2	In open.
91	5	5	5	4	3	2+	6.2	6.2	Do.
92	5	5	5	4	3	2	6.7	6.2	Do.
93	5	5	5	4	3	2+	6.2	6.2	On old roadway.
94	5	5	5	4	3	2+	6.2	6.2	Under wild grapevine.
95	5	5	5	4	3+	2+	5.5	5.2	In open.
96	5	5	4-	4	3	2	6.8	6.2	In broomsedge.
97	5	4	3	2	2	1	6.8	6.2	In open.
98	5	5	5	4	3+	2	6.2	5.2	Do.
99	3+	3	2	0			6.6	5.7	Do.
100	5	4	3	2	2-	1	6.4	6.2	Do.

TABLE 9.—*Degree of color obtained by the Durham test on individual plants of wild Tephrosia virginiana on 1 acre in the Sandflat district in Smith County, Tex., and the environment of such plants*

Plant no.	Degree of color obtained by Durham test on acetone extracts of the roots (0.4 g to 1 cc) using—						Soil acidity at—		Environment
	1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	1 drop	3-inch level	6-inch level	
							pH	*pH*	
101	5	5	5	4	3+	3	5.2	5.2	Adjacent to yucca.
102	5	5	4	3+	3	2	6.2	6.2	In open.
103	5	5	4	3+	3	2	6.2	6.2	Do.
104	4	3	3−	2	1+	0	5.7	6.2	In rotting log.
105	5	4	3	2+	2	1	6.2	5.2	Under scrub oak.
106	5	5	5	4	3+	2+	6.2	5.2	In open.
107	5	5	4	3+	3	2	6.7	6.2	Do.
108	5	5	4	3+	3	2	7.0	7.0	Do.
109	5	4+	4	3+	3	2	6.7	6.2	Do.
110	5	4	3	2	2−	1	6.2	5.7	Do.
111	5	4	3	2	2−	1	6.2	6.2	Do.
112	5	4	3	2	2−	1	6.2	5.7	In broomsedge.
113	5	5	4	4+	3	2	6.2	5.7	In oak brush.
114	5	5	5	4	3+	2+	6.2	6.2	In open.
115	5	5	4	3+	3	2	6.4	6.0	Do.
116	5	5	4	3+	3	2	4.7	4.7	Do.
117	5	5	4	3+	3	2	5.0	5.0	Do.
118	5	5	5	4	3+	3	4.7	5.4	Do.
119	3+	2+	2	1	0		4.7	4.7	Adjacent to scrub oak.
119-A	0						6.2	6.0	Adjacent to bullnettle.
120	5	5	4	3+	3	2	5.0	4.7	In open.
121	5	5	5	4	3+	3	6.0	6.0	Do.
122	5	5	4	3+	3	2	6.7	6.2	Adjacent to scrub oak.
123	5	5	5	4	3+	3	6.2	5.7	In open.
124	5	5	4	3+	3	2	6.7	6.7	In broomsedge on old roadway.
125	5	5	5	4	3+	3	6.2	6.0	Do.
126	5	5	4	3+	3	2	6.2	6.2	In oak brush.
127	5	5	4	3+	3	2	6.2	6.0	In broomsedge on old roadway.
128	5	5	4	3+	3	2	6.6	6.0	In open.
129	5	5	4	3+	3	2	6.7	6.2	Do.
130-1	5	4	3	2	1	0	6.2	6.2	Adjacent to pine tree.
130-2	5	4	3	2	2	1	6.2	6.2	Do.
131	5	5	4	3+	3	2	6.7	6.2	In open.
132	5	5	4	3+	3	2	6.2	5.2	Do.
133	5	4	3	2	1	0	5.6	5.6	In poison-ivy adjacent to oak.
134-1	5	4+	3	2	1+	1	6.0	6.0	Do.
134-2	3	2+	2	1	0		6.0	6.0	Do.
135	5	4+	3+	3	1+	1	4.2	6.2	In broomsedge.
136	0						6.4	6.2	In open.
137	5	5	5	4	3+	3	6.8	6.6	Do.
138	5	5	4	3+	3	2	6.6	6.2	Do.
139	5	5	4	3+	3	2	6.6	6.2	Do.
140	5	5	4+	3+	3	2	6.6	6.2	Do.
141	5	5	4+	3+	3	2	4.7	6.0	Adjacent to small pine.
142	5	5	5	3+	3	2	6.0	6.6	Do.
143	5	4	3	2+	2	2	6.6	6.0	Do.
144	5	5	5	4	3	2	5.2	6.6	In open, upper soil layer black.
145	5	5	4+	3+	3	2	6.7	6.7	In broomsedge.
146	5	5	4	3+	3	2	6.6	6.0	In open.
147	5	4	3	2+	2	1	6.2	6.2	Do.
148	5	5	4	3	2	1+	6.0	6.6	Adjacent to pine tree.
149	5	5	4	3	2	1+	5.2	5.2	In open.
150	5	5	4	3	2	1	5.2	5.2	Do.
151	5	5	5	4	3+	3	4.7	4.5	Do.
152	5	5	5	4	3+	3	4.7	4.5	Do.
153	5	5	5	4	3+	3	4.7	6.2	Adjacent to rotting pine stump.
154	5	4	4	3+	3	2	5.2	4.7	In open.
155	5	4	3+	3	2	1	5.2	4.7	Adjacent to scrub oak.
156	5	5	4	3+	3	2	5.2	4.7	In broomsedge.
157	5	5	4+	3+	3	2	5.2	4.7	In open.
158	5	5	4+	3+	3	2	6.2	5.2	Do.
159	5	4	3+	3	2	1	5.2	4.7	Do.
160	5	5	4	3+	3	2	5.2	4.7	Do.
161	5	5	3+	3	2	1	5.2	4.7	Do.
162	5	4	3	2	1	0	5.2	4.7	Do.

TABLE 9.—*Degree of color obtained by the Durham test on individual plants of wild Tephrosia virginiana on 1 acre in the Sandflat district in Smith County, Tex., and the environment of such plants*

Plant no.	Degree of color obtained by Durham test on acetone extracts of the roots (0.4 g to 1 cc) using—						Soil acidity at—		Environment
	1 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	1 drop	3-inch level	6-inch level	
							pH	*pH*	
163	5	5	4	3+	3	2	5.2	4.7	In open.
164	5	4	3	2	2−	1	5.2	4.7	Do.
165	5	5	5	4	3	3−	5.2	4.7	Do.
166	5	4	3+	3	2	1	4.7	4.2	Beside old roadway.
167	5	4	3+	3	2	1	6.2	5.2	On old roadway.
168	5	5	4	3+	3	2	6.0	5.2	Do.
169	5	5	5	4	3	3−	5.2	5.2	Beside an old roadway.
170	5	5	4	3	2+	1+	6.2	5.2	On old roadway.
171	5	4	3+	3	2	1	5.2	5.2	Beside an old roadway.
172	5	5	4	3	2	2−	5.2	4.7	Do.
173	5	5	4	3	2	2−	6.2	5.2	On old roadway.
174	5	4	3	2	1+	1	5.7	5.7	Do.
175	5	5	5	4	3+	3	6.2	4.7	Beside old roadway.
176	5	4	3	2	1+	1	6.2	4.7	Do.
177	5	5	4	3	2	2−	5.2	4.7	Do.
178	5	5	4	3	2	2−	5.7	5.2	Do.
179	5	5	4	3	2	2+	6.2	5.2	Do.
180	5	5	4	3	2	2−	5.7	5.0	Do.
181	5	4	3	2	1−	1	6.2	5.7	In open.
182	5	5	4+	4	3	3−	6.2	5.2	Under scrub oak.
183	5	5	4	3	2	2−	6.2	5.2	Do.
184	5	3	2+	2	1+	1	6.2	6.2	Do.
185	5	5	4	3	2	2−	7.0	7.0	In open.
186	5	5	5	4	3	2	6.6	6.0	Do.
187	5	5	4	3	2	2−	7.0	6.2	Do.
188	5	5	4	3	2	2−	6.7	6.7	In broomsedge.
189	5	5	4+	4	3	2−	6.2	5.7	Adjacent to scrub oak.
190	5	5	4+	4	3	2−	6.2	5.2	In open.
191	5	4	3	2	1+	1	6.0	4.7	Do.
192	5	5	4+	4	3	2+	6.7	4.2	Do.
193	5	5	4	3	2	2−	6.2	6.2	Do.
194	5	5	4	3	2	2−	6.7	6.2	In broomsedge.
195	5	5	5	4	3+	3	6.7	6.7	In open.
196	5	5	4	3	2	2−	6.7	6.7	Do.
197	5	5	4	3	2	2−	7.0	7.0	In broomsedge.
198	5	5	4+	3+	3	2	5.2	5.7	In open.
199	5	4	3	2	1+	1	6.7	6.2	Do.
200	5	5	4+	4	3	2	6.7	6.2	Do.
201	5	5	4	3	2	2−	6.7	6.2	Do.
202	5	5	4	3	2	2−	6.7	6.7	Do.
203	5	5	5	4	3+	3	6.7	6.7	Do.
204	5	4	3	2	1+	1	7.0	6.7	Do.
205	5	4	3	2	1+	1	6.2	6.2	Do.
206	1	0	...	...	...	...	6.7	6.0	Do.
207	2	1+	1	0	...	...	6.7	6.7	Do.
208	5	5	4+	4	3	2+	6.0	5.2	Do.
209	5	5	4+	4	3	2−	5.2	6.2	Do.
210	2	1+	1	0			5.2	6.2	Do.
211	2	1+	1	0			6.2	5.2	Do.
212	5	5	4	3+	2	2−	6.6	6.2	Do.

Of the 220 plants in the plot, 11 were nontoxic and 83 were included in the group of maximum toxicity. Thus only 5 percent of the plants were apparently without toxicity, another 5 percent were practically so, approximately 20 percent were of moderate toxicity, and almost 70 percent can be regarded as decidedly toxic. As this small plot is apparently typical of the level portions of the Sandflat district, it seems reasonably certain that throughout this district a similar preponderance of toxic plants occurs, and that cultivation of the plant there will yield a root crop of good quality. There are probably few, if any, other districts of similar size where plants of this species with roots of equal quality can be found in such proportion.

An inspection of figure 5 suggests that the plants are to some extent grouped with respect to location and toxicity, although this indication is hardly definite enough to be significant. Thus, in the lower-left corner there seems to be a preponderance of toxic plants; in the upper-left corner is a much smaller proportion of such plants; whereas, in the right center medium-toxic plants predominate.

No correlation can be noted between the toxicity of the plant and the acidity of the soil. Eight plants grew on soil the top layer of which was practically neutral (pH 7.0 at 3-inch level). None of these received a color rating below 3 and only three of them a rating below 4, using 0.25 cc for the test. These may be compared with another group of eight growing on very acid soil (pH 4.2 to 4.7 at 3-inch level) which also included none with a color rating below 3. As evidence of the apparent lack of correlation between soil acidity and toxicity in this locality, none of the 11 plants giving no color nor of the 11 giving only a light blue or trace is included in the two groups mentioned growing respectively in soils of maximum and minimum acidity at the 3-inch level. The 15 plants that ranked highest in toxicity grew on soils showing a pH value ranging from 4.7 to 6.7 and from 4.5 to 6.7 at the 3-inch and 6-inch levels, respectively. The 11 plants that gave no color, even with the concentrated extracts, grew on soils that at the same two levels ranged in pH value from 5.4 to 6.7 and from 5.2 to 6.5, respectively. Although no significant evidence of correlation can be found in these several comparisons, it cannot be held that no relation between soil acidity and toxicity exists under all conditions. In fact, rather definite indications of some relation between the two on some soil types have already been referred to in a preceding section.

The relative toxicity of plants growing close together should be especially noted. It is shown in table 9 that the three plants 82–1, 82–2, and 82–3 gave identical results with the Durham test, and the same was practically true in the case of plants 130–1 and 130–2. However, plants 134–1 and 134–2 differed somewhat in that respect, and in the case of plants 84–1 and 84–2 one gave about the maximum blue color and the other gave no color. This is an extreme variation in adjacent plants that is no doubt unusual, but if a sufficient search were made similar instances could probably be found in localities where toxic plants occur.

RELATIVE TOXICITY OF THE PRINCIPAL PARTS OF THE PLANT

The writers and other investigators have found that in *Tephrosia virginiana* the toxic substances are located mostly in the roots, and that no other part of the plant is of value for insecticidal purposes. The herbage has been repeatedly tested with negative results. Positive results have been obtained with the seed in the few tests that have been made, even in the case of plants growing in Virginia the roots of which were entirely lacking in color-giving substances. Further investigations on that point will be made as opportunity affords, but the seed, regardless of its toxicity, has no commercial possibilities as an insecticide.

On several occasions the Durham test when applied to more than one piece of root from the same plant has given different results, suggesting that not all parts of the root system of an individual plant are equally toxic, but in such instances the tests were not sufficiently quantitative with respect to the amount of material used to be especially significant.

The question of the distribution of the toxic substances in the various parts of the root system is important in connection with the testing of a living plant by using a small portion of one of its roots without disturbing the plant's growth; a procedure that, if reliable, is exceedingly useful for making successive tests of the same plant as it grows older and larger. It is also useful in making selections and in connection with numerous other steps necessary in studying variations or changes in toxicity resulting from known and controlled conditions.

In 1935, five plants grown under cultivation at Milano, Tex., were separated into their several parts and tested with the results shown in table 10. The leaves and stems gave no color. Plant R-1010 gave negative results throughout. The crown and fibrous roots of the others gave a trace or light-blue color. The upper part of the taproots in two instances gave as much color as the remainder of the root system and nearly so in the other two cases. The important point is that all the roots from an individual plant give the same degree of color regardless of their diameter. It may reasonably be concluded from this that the toxicity of a plant, so far as this is indicated by the Durham test, can be determined by applying this test to any part of the root system, and that the result obtained with a small piece of root is indicative of the quality of all the roots of that plant.

TABLE 10.—*Comparison of the results obtained with the Durham test on various parts of 5 plants of Tephrosia virginiana*

Part of plant tested	Degree of color [1] obtained with the Durham test				
	Plant no. R-1010	Plant no. R-1018	Plant no. R-1119	Plant no. R-1122	Plant no. R-1118
Leaves [2]	0	0	0	0	0
Stems [2]	0	0	0	0	0
Crown [2]	0	1	2	2	2
Roots:					
Upper part of taproot [3]	0	3	3	2	2
Fibrous roots [2]	0	2	2	2	2
1 mm in diameter [3]	0		3	3	3
1 to 2 mm in diameter [3]	0	3	3	3	3
3 mm in diameter [3]		3	3	4	3
4 mm in diameter [3]	0	3	(4)	4	3
5 mm in diameter [3]	0		(4)	3	3

[1] For explanation of symbols see footnote 1 of table 1.
[2] Test made on 1 cc of acetone extract representing 0.4 g of root.
[3] Test made on 0.5 cc of the extract.
[4] Included with the roots of 3 mm diameter.

RELATIVE TOXICITY OF THE BARK AND WOOD OF THE ROOTS

It is important to know in which of the root tissues the color-giving substances are located, because the accuracy of the test when used in the field must depend greatly on the method of obtaining the sample of shredded root unless there is a uniform distribution of these substances. Five cultivated plants of *Tephrosia virginiana* from Milano, Tex., were dug in the fall of 1935, and in each case the root bark was removed by carefully scraping it from the wood. The bark is very thin and represents only a small portion of the weight of the root. The bark and wood were thoroughly air-dried and the wood ground to the extent necessary for extraction with acetone. The bark was obtained in such condition when it was scraped from the wood that grinding was not necessary. Both materials were extracted with acetone for 7 hours, and the volume of the extracts so adjusted that 1 cc represented 0.4 g of the material. These extracts were then tested in a number of dilutions. The results are shown in table 11. The same five plants had been tested in the fall of 1934 by using a small piece of root from each and scraping enough material from it for a field test without quantitative extraction. The results of those tests are included in the table for comparison.

TABLE 11.—*Results obtained with the Durham test on the bark and wood of the roots of several plants of Tephrosia virginiana*

Plant no.	Color given by root in fall of 1934 [3]	Degree of color [1] obtained by using acetone extracts [2] of the root bark and root wood in the quantities indicated											
		Root bark						Root wood					
		1.0 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	1 drop	1.0 cc	0.5 cc	0.25 cc	0.1 cc	0.05 cc	1 drop
R-1132	1	1	0	0	0	0	0	5	5	4+	4	3	2
R-1144	0	0	0	0	0	0	0	2	2−	1	0	0	0
R-1146	3	2	1	0	0	0	0	4	3+	3	2	2−	1
R-1159	3	2	1	0	0	0	0	4	3+	3	2	2−	1
R-1176	4	2	1+	T	0	0	0	4	3+	3	2	2	1

[1] For explanation of symbols see footnote 1 of table 9.
[2] 1 cc of extract represented 0.4 g of material.
[3] These tests in 1934 were made in the field on material scraped from the roots with a knife.

It is obvious that practically all the color-giving substances are located in the wood. In fact, the small amount of color obtained with the extracts of the root bark may have been due to the particles of wood that were undoubtedly included with the bark because a perfect separation is impossible with the method used.[9]

The almost total absence of color-giving substances in the bark of the roots suggests at once that field tests made by scraping the root may give misleading results unless the sample secured consists of bark and wood in approximately the proportions in which these occur in the root. Merely scraping the root's surface would furnish a sample containing more than the normal proportion of bark, and for that reason would give less color than the root as a whole would give. However, if a piece of root is scraped or shredded completely the sample obtained is undoubtedly representative of the whole root. Inasmuch as the bark contains practically no color-giving substances and represents such a small proportion of the root, it is only necessary to avoid using largely surface scrapings in order to get a true indication of the root's value. In the case of the last three plants in table 11, the results obtained by the field tests in 1934 are in accord with those obtained by the more quantitative tests in 1935. The second plant, which gave negative tests with the bark, contained so little color-giving substances in the wood that a field test would very likely be negative as was the case in 1934. The first plant, however, when tested in the field in 1934 should have given a result equal to or better

[9] Since this paper was submitted Worsley and Nutman have reported that in *Derris elliptica* roots more than 8 weeks old the rotenone occurs throughout the xylem, parenchyma, and cortex. WORSLEY, R. R. LE G., and NUTMAN, F. J. BIOCHEMICAL STUDIES OF DERRIS AND MUNDULEA. I. THE HISTOLOGY OF ROTENONE IN DERRIS ELLIPTICA. The Annals of Applied Biology. Vol. XXIV, No. 4. Nov. 1937. pp. 696-702, illus.

than that obtained with the last three. In this case, the test made in 1934 was probably performed with insufficient care, with the result that mostly bark was used.

TOXICITY OF THE CULTIVATED PLANT OVER A 3-YEAR PERIOD

The changes taking place in the toxicity of the growing roots of *Tephrosia virginiana* from year to year have a direct bearing on the cultural methods to be followed. Thus, if it is found that the quantity of toxic substances present declines as the plant gets older, the roots should be harvested accordingly, whereas, if no changes occur in this respect, or if the quantity of these substances increases with the age of the plant, harvesting may be deferred in accordance with other considerations. This question involves not only changes that take place from one season to another but variations within a season. Biological tests of the roots from individual plants from successive years have not been made, but some data on the amount of color-giving substances present from year to year have been obtained. It is recognized that changes in toxicity taking place as the plants get older would not necessarily be revealed by the Durham test and that this test though adequate to indicate fairly pronounced changes in toxicity cannot be relied upon to do more.

A number of plants in the cultural plot established by Little were tested by him in 1932 with the Durham test and were made available for further tests in 1933 and 1934. Several pieces of roots taken from each plant without disturbing their growth were extracted with acetone, and the tests made on these extracts. The eight plants under observation gave the same results in all 3 years. Two of the plants gave only a trace of color, four a light blue, one a blue, and one a dark blue. Although the data so far obtained are limited, they indicate that these eight plants growing under the prevailing conditions underwent no important change in 3 years insofar as their quality may be judged from the Durham test.[10]

STABILITY OF THE COLOR-GIVING SUBSTANCES IN THE GROUND ROOT

In connection with the tests of the same individual plants in several successive years, it was possible to make some observations on the stability of the color-giving substances present in the ground root. The surplus material from these tests was stored in the ground condition in cork-stoppered jars in subdued light and portions of it tested with the Durham test from time to time. The samples from the 1933 tests were examined after 6-, 18-, and 24-month intervals, and those from the 1934 tests after a 12-month interval. In all cases the results were the same as in the initial test in 1933—namely, two plants gave a trace of color, four a light blue, one a blue, and one a dark blue. These results indicate that the total amount of color-giving substances in a sample of ground roots kept under the conditions described remains unchanged for at least 2 years. Biological tests or rotenone determinations are necessary to secure accurate information concerning the extent to which conditions of storage affect the quality of the root.

CULTURAL EXPERIMENTS

In midsummer of 1934 Little's experimental plot near Milano, Tex., with additional land totaling about 5 acres, was included in the project

[10] Data secured since this paper was submitted indicate that in some localities considerable changes in this respect occur in individual plants from one year to another and suggest that seasonal variations in toxicity may be considerable.

of the Department under the cooperative agreement with the Texas Agricultural Experiment Station.[11] On this acreage *Tephrosia virginiana* is being established as rapidly as good propagating material can be secured (pl. 5, *A*). The major portion of the field will be devoted to experiments on cultural practices, harvesting, yields, production costs, etc., while other portions are being used for miscellaneous investigations pertaining to the relation of various factors to toxicity, studies on heredity and selection, and for trial plantings of other species of *Tephrosia*.

The cultural experiments will be conducted mainly on the Milano plot, but, as has already been mentioned, plantings are also being made in other localities, and exchange of planting stock between these and Milano are expected to furnish information on the significance of various factors concerning growth and toxicity. At the Plant Introduction Garden of the Bureau of Plant Industry at Glenn Dale, Md., seedlings of the western type of *Tephrosia virginiana*, grown from seed from toxic Texas plants, have been planted as well as seedlings grown from seed of nontoxic plants of the eastern type of the species from southern Virginia. Crown divisions of *T. latidens* from western Florida were also planted there in the fall of 1935. Facilities for similar experiments have been provided by the Georgia Coastal Plain Experiment Station at Tifton, Ga., and the Sandhill substation of the South Carolina Agricultural Experiment Station, Pontiac, S. C., the former being in one of the secondary districts of toxic plants and the latter in a district where the wild plants are abundant but nontoxic.

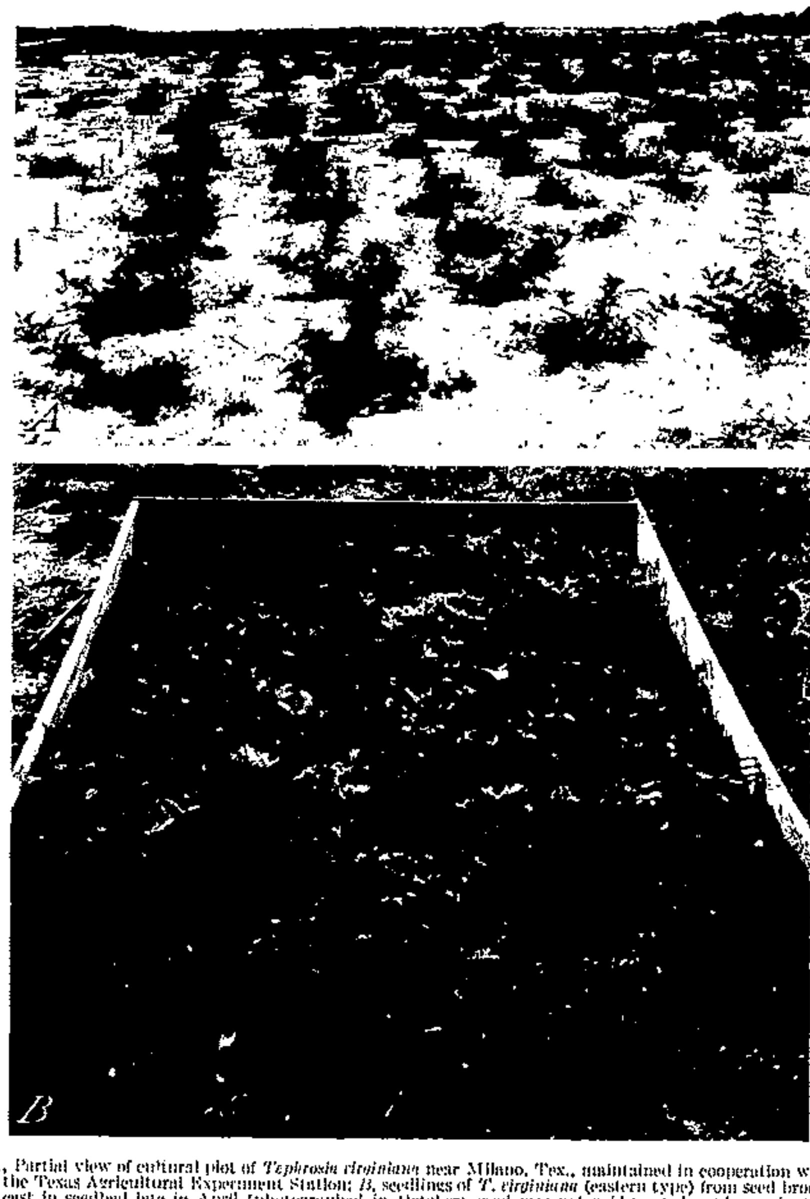
The wide distribution of *T. latidens* in the Choctawhatchee National Forest in western Florida has been referred to (p. 14). Inasmuch as this species appears to be uniformly toxic throughout this district and the soil is also very uniform, cultural experiments were undertaken through the cooperation of the Forest Service and with the assistance of District Ranger H. A. Snyder in the forest near Valparaiso, Fla. In November 1935 and March 1936 plants of *T. virginiana* from Texas and Virginia and plants of *T. latidens* obtained locally were planted. Through these several exchanges of planting stock, it is hoped that some of the broader problems of the project may be worked out.

PROPAGATION

According to present information *T. virginiana* may be propagated from seed and by crown division. Both methods possess some advantage, but which one should be recommended will depend largely on the circumstances, and a detailed study of the various factors involved is being made. Little (*6*), from his experiences with the propagation of *T. virginiana*, points out that (1) the easiest means of propagation is by seed; (2) seed is frequently very scarce due to the ravages of insects that destroy it or the bloom; (3) the germination of the seed is improved by treating it with sulphuric acid; (4) the crown may be divided into several portions with roots attached and these planted; (5) the roots alone are worthless for propagating; (6) propagation from seed is cheaper, but propagation by crown division produces larger plants in a given time; and (7) the plant grows much more rapidly under cultivation than it does in the wild state.

The damage done to the flowers and seed of *Tephrosia virginiana* by various insects, which appears to be general throughout its range, is

[11] See footnote 2, p. 1.



A, Partial view of cultural plot of *Tephrosia virginiana* near Milano, Tex., maintained in cooperation with the Texas Agricultural Experiment Station; *B*, seedlings of *T. virginiana* (eastern type) from seed broadcast in seedbed late in April (photographed in October), seed was not acid-treated, and germination extended over 10 weeks.

of much importance in connection with the commercial cultivation of the plant. Yip (*8*) has made a study of the damage done by insects to the seed of *T. virginiana*. It is reported that in Texas the flowers are attacked by blister beetles, and no doubt this is also the case in other localities where the plants fail to produce flowers. Weather conditions may also have an important effect on the flowering. In Texas the loss of flowers appears to be greatest in wet seasons. In the case of *T. latidens* in 1935 in the Choctawhatchee National Forest the flowers were either destroyed or their development prevented by some agency to such an extent that only two or three plants with pods could be found during a day's search. A large commercial planting, if such were advisable at this time, would have to be developed slowly because of lack of seed. If the plant is to be grown commercially from seed some practical means of controlling such insects in fields of the cultivated plant may have to be found.

Treatment of the seed to improve germination has so far been confined to the sulphuric acid method described by Little (*6*) and found advantageous in that it hastens the germination and increases the number of seedlings obtained. The seed is subjected to the action of concentrated sulphuric acid for about 15 minutes, the acid is drained off, and the seed is repeatedly washed with water and then dried. With this treatment a germination of from 70 to 90 percent will be obtained with seed of good quality in several weeks. However, much of the seed collected includes a considerable proportion that is not viable from one cause or another, hence the percentage of germination would in most cases be somewhat lower than the figures given. Other methods of treatment may be equally or more effective in improving germination. The use of scarifying devices, such as are successfully used for some other hard-coated seeds, was tried on a small amount of seed and found to have possibilities.

Seedlings may be grown in pots, in seedbeds (pl. 5, *B*), or directly in the field. Field sowing appears to be quite satisfactory so far as the growth of the seedlings is concerned, but it is subject to the usual hazards which may result in an incomplete stand. This method involves less expense than growing the seedlings in pots or seedbeds with subsequent transplanting and may be found practical on a large scale, if the seed are sown thickly enough to allow for losses from various causes.

Propagation by crown division as described by Little (*6*) produces larger plants in a given time but is more expensive than propagation by seed directly in the field, and calls for a large quantity of initial propagating material. Recent experiments have shown that propagation by crown cuttings may be feasible. This is actually a form of crown division but makes it possible to secure a great many more new plants from one individual. The crown wood is cut into small pieces, which make excellent young plants with a good root system in a comparatively short time. It is not necessary that any roots be attached. In one instance a piece of crown wood 2 inches in length planted in a pot in the greenhouse in May, produced by October a fine plant with 15 inches of top growth and four principal roots from 8 to 10 inches in length with many branches. It is estimated that a 3-year-old plant would furnish material for probably several dozen such cuttings. Experiments are under way to determine whether such cuttings may be placed directly in the field with equally good results. This method is particularly considered in connection with *Tephrosia latidens*, the widely branched crown of which would perhaps lend itself well to such purpose. An observation made in the Choctawhatchee Forest further suggests that such a method may have practical possibilities. A tract of several acres typical of the forest as a whole on which pine seedlings were later planted had been thoroughly worked over with heavy implements including disks that chopped most of the small vegetation, including *T. latidens*, which was present in considerable abundance, into small fragments. Later it was observed that numerous small plants of the latter were growing all over the tract. These had the appearance of seedlings but had developed from pieces of crown wood. It may be feasible to chop the branching crowns into small pieces and scatter these in rows or broadcast them in suitably prepared ground. It is quite likely that the crown of *T. virginiana* can also be utilized in that way, though it is probably less well adapted for the purpose. Special attention is being given to the possibilities of this method as a means of expediting the propagation of that species.

SUMMARY

The discovery by Little and Clark that the roots of the devil's shoestring, *Tephrosia virginiana*, a native legume, possess insecticidal properties and contain rotenone in some cases suggested a study of the commercial possibilities of this and other native species of *Tephrosia*. Their cultivation as a commercial source of an insecticide in the South and Southeast, where they are widely distributed, would provide a new crop for those regions and at the same time be a forward step in the search for insecticides harmless to man and animals. Cultural experiments were therefore undertaken at Milano, Tex., in cooperation with the Texas Agricultural Experiment Station, with minor experimental plantings at the Bureau of Plant Industry's Arlington Experiment Farm, Arlington, Va., and Plant Introduction Garden at Glenn Dale, Md., at the Georgia Coastal Plain Experiment Station, Tifton, Ga., the Sandhill substation of the South Carolina Agricultural Experiment Station at Pontiac, S. C., and in the Choctawhatchee National Forest near Valparaiso, Fla.

In 1934 composite root samples of *T. virginiana* were collected in many localities from Maryland to Oklahoma and Texas and from a few localities outside of that general region. Acetone extracts of these samples were tested by the Durham color test to indicate roughly the amount of rotenone and related substances present, and their toxicity to flies was determined by the Bureau of Entomology and Plant Quarantine. The results indicated that: (1) Plants from all but a few restricted districts are lacking in insecticidal value; (2) toxic plants are found almost exclusively in the region extending south and west of central Georgia, in northeast Florida, and in northeast Texas; and (3) the Durham color test, which is easily made, gives a reasonably reliable indication of the plant's toxicity to flies and is, therefore, an excellent means of determining the value of plants rapidly in the field.

More elaborate field studies in 1935 confirmed the conclusion that the species is toxic only in a few relatively small districts in Texas, Florida, and Georgia, the location and size of which are described.

Twelve of the seventeen species of *Tephrosia* occurring in the South and East were studied with regard to their taxonomy and toxicity. Judged by their insecticidal properties and their favorable growth habits, *T. virginiana* and *T. latidens* are the only species of immediate interest, but several others should be further investigated. *T. latidens* is abundant in the Choctawhatchee National Forest in the western part of Florida where its toxicity is quite uniform. This fact and its growth habits, which are advantageous in propagation, suggest that it may have commercial possibilities.

The soil and vegetative environments of toxic and nontoxic plants of the two species mentioned were studied in many localities with a view to finding some correlation between soil types and the toxicity of the plants. These studies were inconclusive, but the observations made and their possible significance are discussed. Some chemical analyses of the root ash and also spectographic analyses of the ground roots were made. The limited data obtained do not indicate a definite relation between the inorganic constituents and rotenone content of the roots.

No relation could be observed between any botanical characters and the toxicity of individual plants of *T. virginiana*. The eastern and western types of this species, though quite distinct, are both subject to wide variations in toxicity.

Data have been obtained suggesting that the capacity of several of the species to produce rotenone and related toxic substances is a genetic character, and that a variety or strain of greater insecticidal value than any thus far observed may be developed by selection and breeding. There are indications, however, that such a character is subject to modification by environment.

Miscellaneous studies of *Tephrosia virginiana* furnished the following information: (1) More than 200 plants growing on 1 square acre in Texas varied greatly in toxicity as determined by the Durham test, individuals growing in close proximity to one another showing, in several instances, a wide difference in this respect; (2) the herbage is apparently always nontoxic, the seed is sometimes toxic, and when the roots are toxic they appear to be uniformly so, without regard to size, and the toxic substances are present in the wood of the roots rather than in the bark; (3) toxic plants apparently remain so in successive years if environments remain unchanged; and (4) the color-giving substances in the ground root properly stored remain unchanged for some time.

For the principal cultural studies the original plot at Milano established by Little has been enlarged and plantings are being increased to about 5 acres. Here methods of propagation, cultivation, and harvesting are being determined as rapidly as possible and data obtained on yields, production costs, and the general economic aspects of the project. Exchanges of planting stock are being made between this plot and the smaller ones established elsewhere in cooperation with the several agencies mentioned to note the effects of environment on growth and toxicity and in connection with efforts to improve the toxicity of the plant in degree and uniformity by selection and breeding. To study the possibilities of *T. latidens* as a cultivated crop, a cultural plot has been established in the Choctawhatchee National Forest in western Florida.

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