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ALLELOPATHIC EFFECTS OF *Platanus occidentalis* L. IN RELATION TO THE PATTERNING OF VEGETATION

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ALLELOPATHIC EFFECTS OF *Platanus occidentalis* L.

IN RELATION TO THE PATTERNING OF VEGETATION

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CHAPTER I

INTRODUCTION

Allelopathic effects of woody species on herbaceous species have been reported for *Juglans nigra* L. and *Juglans cinerea* L. (Cook 1921, and Massey 1925), *Quercus robur* L. (Ljubic 1955), *Ailanthus altissima* (Mill.) Swingle (Mergen 1959), *Salvia leucophylla* Greene, *S. apiana* Jeps., and *Artemisia californica* Less. (Muller and Muller 1964; Muller, Muller, and Haines 1964; Muller 1965; and Muller and del Moral 1966), and *Eucalyptus globulus* Labill (del Moral and Muller 1969).

Cynodon dactylon (L.) Pers., *Lolium multiflorum* Lam., and *Poa pratensis* L. will not grow beneath *Platanus occidentalis* (sycamore) on The University of Oklahoma campus even with adequate irrigation and fertilization because of chemical inhibitors produced by the sycamore (Al-Naib, unpublished M.S. thesis, Univ. of Okla., 1968). Extracts or decaying materials of leaves, fruits, and buds were found to be inhibitory to the growth of the

above mentioned grasses and to selected species associated with sycamore in natural areas in Oklahoma. Observations in natural areas indicated also that virtually no herbaceous plants grow under sycamore trees except for isolated trees where the fallen leaves blow away.

This project was undertaken to determine whether the bare areas under the sycamore in natural areas were due chiefly to competition for light, water, or minerals or to chemical inhibitors produced by the sycamore.

CHAPTER II

LOCATION AND DESCRIPTION OF STUDY AREA

A stand of Platanus occidentalis located in Section 34, T6NR3E, near Wanette, Pottawatomie County, Oklahoma was chosen for study. The area in which the stand was located had not been grazed for a considerable period prior to the study. In this stand and others a definite zone of reduced growth of associated species in and around the stand of sycamore was observed. Very few species were found within the stand and individuals were scattered and had a sparse growth. These included Ambrosia trifida L. var. texana Scheele, Elephantopus carolinianus Willd., Elymus virginicus L., Spartina pectinata Link, and Symphoricarpos orbiculatus Moench. On the other hand, the following species were found away from the edge of the canopy and area of leaf fall and accumulation: Andropogon glomeratus (Walt.) B.S.P., Andropogon virginicus L., Ambrosia psilostachya DC., Cynodon dactylon, Elymus virginicus, Panicum anceps Michx., Panicum scribnerianum Nash, Panicum virgatum L., Setaria viridis (L.) Beauv., Symphoricarpos orbiculatus, and Tridens flavus (L.) Hitchc.

Sampling was accomplished by locating thirty 0.25 m^2 quadrats at the edge of the canopy, 30 quadrats 0.5 m outward from the first series, and 30 additional quadrats 0.5 m outward from the second series. All plants in the quadrats were clipped, separated by species, and oven-dry weights were determined. The weights of all species were found to be significantly lower in the quadrats at the edge of the canopy than in those farther away (Table 1). The area immediately below the sycamore canopy was not sampled as there was virtually no growth of herbaceous plants there.

Table 1. Field clippings of species associated with sycamore.

Species	Mean oven-dry weight in g/0.25 m^2			F_s
	QUADRATS			
	A	B	C	
Ambrosia psilostachya	10.5	13.0	16.7	45.07[a]
Andropogon glomeratus	14.3	17.5	40.5	355.38[a]
Andropogon virginicus	12.6	18.0	42.5	960.88[a]
Panicum scribnerianum	0.7	1.2	2.3	126.73[a]
Panicum virgatum	8.5	12.2	18.1	713.44[a]
Setaria viridis	2.1	4.7	8.3	263.51[a]
Tridens flavus	2.5	6.0	8.5	1164.57[a]

Quadrat A at the edge of the canopy.

Quadrat B extends 0.5 m from quadrat A.

Quadrat C extends 0.5 m from quadrat B.

[a]Significant difference among quadrats at 0.05 level or better.

CHAPTER III

EXPERIMENTATION

Competition Studies

Experiments were designed first to try to determine if the distribution of herbaceous plants near sycamore was due to competition in the usual sense. Measurements were made of soil moisture, pH, and mineral content under the trees and away from their canopies. Tests were run also to determine if shading was a critical factor.

Soil Moisture determination.--Soil moisture was determined under the sycamore canopies and away from the canopies in June, July, and August when soil moisture was most likely to be limiting. Samples were taken at two levels, 0-6 inches and 6-12 inches, at 10 sites under the canopies and 10 at least 5 m from the canopies during each sampling period. These samples were weighed immediately, oven-dried for 48 hrs. at 105°F, cooled in a desiccator, reweighed, and the percentage soil moisture was computed based on the oven-dry weight of the soil (Table 2). The percent soil moisture was greater under the canopy at both levels at all sampling periods, although the

Table 2. Comparison of soil moisture under the sycamore canopy and away from it. Each value represents average of 10 soil samples.

Level of the Soil Samples	MOISTURE %								
	June 1969		F_s	July 1969		F_s	August 1969		F_s
	under	outside		under	outside		under	outside	
0-6"	25.5	21.0[a]	5.73	15.6	15.0	0.13	21.1	18.9[a]	4.77
6-12"	24.9	16.7[a]	71.73	16.7	12.2[a]	11.27	21.7	19.3	2.54

[a]Difference from appropriate level under the canopy significant at 0.05 level or better.

difference was not always statistically significant. Most of the roots of the herbaceous plants of interest in this study occur in the top 12 in. of soil so the failure of the herbs to grow under the canopies certainly was not due to a moisture deficiency.

Determination of Soil Reaction (pH).--Ten soil samples were taken under the sycamore canopy and ten samples away from the canopy, all at the 0-12 in. level. These samples were used for determination of pH, nitrogen, phosphorus, copper, iron, and zinc. The samples were air-dried, the undecayed pieces of organic matter were picked out and discarded, and the samples were run through a 2 mm sieve. After determination of the pH, the samples were ground in a soil mill to pass a 0.5 mm sieve.

The soil reaction was determined for each soil sample by the glass electrode method of Piper (1942). There was virtually no difference in pH between the two sites (Table 3).

Total Nitrogen.--Total nitrogen was determined for each of the previously described soil samples by the macro-Kjeldahl method (Bremner, 1965). There was no statistically significant difference in total nitrogen under the canopy compared with soil away from the canopy (Table 3).

Total Phosphorus.--Total phosphorus was determined by the method of Shelton and Harper (1941). There was no

Table 3. Comparison of selected soil factors in and outside the stand of sycamore. Each value represents average of 10 soil samples.

Location of the Soil Samples	pH	Total Nitrogen %	Total Phosphorus %	Iron (ppm)	Copper (ppm)	Zinc (ppm)
Within the stand	8.2	.025	.019	164.57	1.59	6.03
Outside the stand	8.3	.026	.019	159.85	1.69	6.24
F_s [a]	2.6	.078	.500	1.240	1.31	.04

[a] No statistically significant difference at 0.05 level.

significant difference in total phosphorus under the canopy and away from it (Table 3).

Trace Elements: Cu, Fe, and Zn.--Exchangeable and easily soluble Cu, Fe, and Zn were extracted by the method suggested by Perkin-Elmer (1968), and amounts were determined by atomic absorption spectrometry using a Perkin-Elmer, Model 303, Spectrophotometer. There were no significant differences in amounts of any of these trace elements under the canopy and away from it.

Shading Effects.--Six shading devices with dimensions of 2.5 X 1.5 m and consisting of a framework with a top cover of several layers of cheesecloth were distributed randomly in the herbaceous vegetation away from the sycamore canopies. These devices were designed to reduce the light intensity to the average light intensity under the sycamore canopy at about noon on a clear day, 291 ft-c. The shade devices were placed in the field in mid-spring of 1968, and these were checked every week or two. In October 1968 at the time chosen for clipping, they were found removed and destroyed by vandals. Their exact location could not be determined, because there were no noticeable differences in growth where they were located. The experiments were repeated in 1969, and in October 1969, all the plants under the canopies were clipped, separated by species, and their oven-dry weights were

determined. At the same time thirty randomly distributed 0.25 m^2 quadrats were clipped in the same area but in full sunlight, and oven-dry weights of species were determined. The weights per unit area of all the species under the artificial shading canopies were not significantly different from weights of the same species which grew in full sunlight (Table 4).

These results indicated that the herbaceous test species grew just as well in shade equivalent to that under the sycamore canopy as in full sunlight.

The results of all experiments up to this point indicate, therefore, that the failure of the test species to grow under the canopy was not due to normal competition. Consequently, numerous tests were run to determine if allelopathy might be the cause of the failure of the test species to grow under the sycamore canopy.

Chemical Inhibition Studies

Effects of Decaying Sycamore Leaves.--Field sampling of sycamore leaves on the ground after leaf fall indicated that there were 6.16 grams air-dried weight of sycamore leaves per 454 g of soil to a depth of approximately 17 cm. In order to determine the effects of decaying sycamore leaves on the test species, ten test pots and ten control pots were set up for each species. Each test pot contained a mixture of two parts of soil

Table 4. Effects of shade on growth of herbaceous species associated with sycamore.

Species	Mean oven-dry weights in g/0.25 m^2		F_s [a]
	In Artificial Shade	In Full Sunlight	
Ambrosia psilostachya	17.0	17.3	0.16
Andropogon glomeratus	34.8	35.9	1.61
Andropogon virginicus	23.1	24.6	1.19
Panicum virgatum	32.4	32.0	1.39
Setaria viridis	7.1	7.5	0.58

[a]No difference at .05 level.

to one part of sand and 1 g of pulverized sycamore leaf material per 454 g of the soil and sand mixture. Each control pot contained 1 g of milled peat moss per 454 g of the mixture to keep the organic matter content the same. Fifty test seeds were planted in each pot. In the case of Ambrosia psilostachya, seedlings were transplanted from the field because the seeds germinate very poorly even after cold treatment. All plants were kept under greenhouse conditions, and after germination was completed, the plants were thinned to the five largest plants per pot. The plants were allowed to grow for an additional three weeks, at which time they were harvested and oven-dry weights were determined.

The decaying sycamore leaves inhibited seed germination and seedling growth of all test species (Table 5). The results indicate that the leaves contain inhibitory compounds which are released into the soil during the decay process causing inhibition of the test species.

Effect of Leaf Leachate.--A fine mist of cistern water was sprayed over fresh mature leafy sycamore branches, and the water which dripped from the leaves was collected on plastic sheets. This leachate was used to water pots containing a mixture of two parts of soil to one part sand and 50 seeds each of one of the test species. In the case of Ambrosia psilostachya, seedlings were again transplanted from the field. Control pots received equal

Table 5. Effects of decaying sycamore leaves on seed germination and seedling growth.

Species	Exp. No.	Mean oven-dry weight of seedlings, mg		F_s	Germination[b]
		Control	Test		
Ambrosia psilostachya	1	937.0	236.7[a]	187.87	--
	2	823.0	220.6[a]	176.50	--
Andropogon glomeratus	1	620.7	64.9[a]	35.48	37
	2	624.8	63.1[a]	36.37	21
Andropogon virginicus	1	589.8	15.8[a]	59.19	33
	2	566.4	15.9[a]	36.59	25
Cynodon dactylon	1	241.2	53.3[a]	187.79	39
	2	251.5	46.5[a]	250.14	44
Elymus virginicus	1	123.6	23.8[a]	422.03	51
	2	128.3	27.2[a]	113.45	62
Panicum virgatum	1	376.7	100.9[a]	237.70	77
	2	355.5	30.0[a]	33.12	88
Setaria viridis	1	296.7	17.5[a]	1,660.60	60
	2	390.0	18.2[a]	4,707	75
Tridens flavus	1	944.6	67.0[a]	138.37	56
	2	905.5	54.5[a]	134.90	52

[a]Dry weight significantly different from control.

[b]Expressed as percent of control.

volumes of cistern water that had not passed over sycamore leaves. Ten test pots and ten control pots were used for each species. After germination was completed, the number of seeds germinated was recorded, and the plants were thinned to the five largest ones per pot. The plants were allowed to grow for an additional three weeks after which the oven-dry weights of the entire plants were determined.

The leachate reduced the percentage germination appreciably in all species except Panicum virgatum and Elymus virginicus (Table 6). The leachate also significantly reduced the oven-dry weights of all species except Elymus virginicus and Panicum virgatum. The failure of the leachate to inhibit germination and growth of E. virginicus seems significant in view of the fact that this species was one of few which grew at least sparsely under the canopy.

Effects of Field Soil.--Soil, minus litter, was taken from under the sycamore canopy by means of a post-hole digger to prevent disturbing the vertical stratification of the soil, and placed directly in 4-inch plastic pots. Control soil was obtained in a similar way outside the canopy and away from the area of leaf fall. Ten test pots and ten control pots were set up for each species. These soil collections were made in July, 1968 when the sycamores were in full leaf and in November, 1968 after

Table 6. Effects of leaf leachate of sycamore on germination and seedling growth.

Species	Exp. No.	Mean oven-dry weights of seedlings, mg		F_s	Germination % of Control
		Control	Test		
Ambrosia psilostachya	1	937.0	358.5[a]	133.33	--
	2	823.0	321.5[a]	127.50	--
Andropogon glomeratus	1	181.8	178.2	2.20	70
	2	157.7	92.0[a]	8.74	63
Andropogon virginicus	1	283.4	201.8[a]	4.75	77
	2	313.3	215.9[a]	6.19	71
Cynodon dactylon	1	189.8	18.6[a]	92.71	61
	2	165.9	15.0[a]	86.86	53
Elymus virginicus	1	102.5	100.5	1.37	97
	2	87.4	84.3	3.19	91
Panicum virgatum	1	155.3	150.3	2.18	95
	2	124.2	121.2	1.76	98
Setaria viridis	1	296.7	187.5[a]	21.76	64
	2	329.9	232.5[a]	33.40	52
Tridens flavus	1	944.6	371.0[a]	48.54	67
	2	861.1	298.0[a]	18.03	74

[a]Weight significantly different from that of the control.

the leaves and some fruits had fallen. The two soil collections were treated as separate experiments. Ambrosia psilostachya seedlings were again transplanted from the field. Seeds of the other test species were planted in the pots, 50 seeds per pot, allowed to germinate; and after the amount of germination was recorded, the five largest plants in each pot were allowed to grow for an additional three weeks. The oven-dry weights of the entire plants were then determined. Seed germination of all species was significantly reduced by the soil from under the canopy during both sampling periods (Table 7). The soil from under the canopy significantly reduced the oven-dry weights of all test species during both sampling periods also. The soil collected under the canopy in November caused a greater degree of inhibition of both germination and seedling growth than that collected in July. This was probably due to a greater accumulation of sycamore debris on the soil surface in November with the resulting leaching of substances from the debris into the soil.

Identification of Phytotoxins.--The procedures used to isolate and identify the phytotoxins from sycamore were mainly those of Rice (1965). A 10% aqueous extract of leaves or fruits of sycamore was acidified to pH 2.5 with 1 N HCl and extracted with two half volumes of diethyl ether. The ether fraction was evaporated by an

Table 7. Effects of field soils from the sycamore stand on germination and growth of test species.

Species	Date Soil Taken	Mean oven-dry weights of seedlings, mg		F_s	Germination % of Control
		Control	Test		
Ambrosia psilostachya	July	365.2	275.0[a]	16.53	--
	November	312.1	196.1[a]	35.50	--
Andropogon glomeratus	July	453.8	38.3[a]	72.77	57
	November	543.8	32.0[a]	106.23	31
Andropogon virginicus	July	176.0	25.8[a]	43.24	34
	November	148.1	42.8[a]	84.00	29
Cynodon dactylon	July	88.7	3.4[a]	27.56	46
	November	10.0	2.2[a]	49.59	39
Elymus virginicus	July	78.4	39.0[a]	15.83	62
	November	90.2	25.6[a]	19.33	61
Panicum virgatum	July	140.2	16.6[a]	38.24	77
	November	126.8	18.0[a]	48.51	76
Setaria viridis	July	281.5	113.2[a]	32.22	39
	November	253.0	40.0[a]	92.94	28
Tridens flavus	July	192.4	83.0[a]	12.65	60
	November	75.6	6.3[a]	14.47	54

[a]Weight significantly different from that of the control.

air current and the residue was taken up in 3 ml of 95% ethanol. The ether fraction was chromatographed on Whatman No. 3 MM paper with n-butanol-acetic acid-water (63:10:27,v/v), BAW. The chromatograms were inspected with short (2537 A°) and long (3360 A°) ultraviolet light. Three distinctive bands were present on the chromatograms of the leaf extracts and seven bands on the chromatograms of fruit extract. The bands from the leaf extract were cut from the paper and eluted with 90% ethanol. The eluates were reduced to dryness in vacuo, taken up in 3 ml of ethanol, and chromatographed on Whatman No. 3 MM paper in four different solvent systems: BAW; 6% aqueous acetic acid, 6% AA; isopropanol-ammonia-water (200:10:20,v/v), IAW; and isopropanol-butanol-water (140:20:40,v/v), IBW. The R_f's in various solvent systems, colors in UV light with or without exposure to NH_3, and tests with various reagents (Rice, 1965) suggested that the fluorescent bands of the leaves were Phytotoxin 1, chlorogenic acid (3-caffeoylquinic acid); Phytotoxin 6, scopoletin (6-methoxy-7-hydroxy coumarin) and Phytotoxin 5, scopolin (the 7 β-D-glucoside of scopoletin) (Table 8).

The absorption spectrum of the suspected chlorogenic acid in 90% ethanol was determined with a Beckman Model DB-G Spectrophotometer before and immediately after the addition of 2 drops of 2 N NaOH to the cuvette.

Table 8. Chromatography of phytotoxins from sycamore.

Compound	R_f's on Whatman 3MM[a]				Fluorescence[b]		Reagent colors[b,c]			
	BAW	6% AA	IAW	IBW	Long & Short UV $-NH_3$	$+NH_3$	P-Nit.	Sulfan Acid	$FeCl_3$ $K_3Fe(CN)_6$	Hoepfner Reaction
Phytotoxin 1[d,e,f]	.64	.58,.72	.01	.35	l.bl.	yel.gr.	brn.	tan	blue	red
Chlorogenic acid	.64	.58,.73	.01	.36	l.bl.	yel.gr.	brn.	tan	blue	red
Phytotoxin 2[f]	.78	.16,.31	.03	--	l.bl.	yel.gr.	brn.	tan	blue	red
Isochlorogenic acid	.79	.16,.30	.02	--	l.bl.	yel.gr.	brn.	tan	blue	red
Phytotoxin 3[f]	.54	.72,.81	.06	--	l.bl.	yel.gr.	brn.	tan	blue	red
Neochlorogenic acid	.55	.72,.82	.06	--	l.bl.	yel.gr.	brn.	tan	blue	red
Phytotoxin 4[f]	.61	.61,.72	.05	--	l.bl.	yel.gr.	brn.	tan	blue	red
Band 510	.60	.61,.74	.05	--	l.bl.	yel.gr.	brn.	tan	blue	red
Phytotoxin 5[d,e,f]	.53	.81	.29	.51	bl.	br.bl.	--	--	--	--
Scopolin	.53	.80	.30	.52	bl.	br.bl.	--	--	--	--

Table 8 (Cont.)

Compound	R_f's on Whatman 3MM[a]				Fluorescence[b]		Reagent colors[b,c]			
	BAW	6% AA	IAW	IBW	Long & Short UV $-NH_3$	Long & Short UV $+NH_3$	P-Nit.	Sulfan Acid	$FeCl_3$ $K_3Fe(CN)_6$	Hoepfner Reaction
Phytotoxin 6[d,f]	.77	.44	.45	.80	bl.	br.bl.	bl.bk.	rose	dp.bl.	--
Scopoletin	.78	.44	.46	.81	bl.	br.bl.	bl.bk.	rose	dp.bl.	--
Phytotoxin 7[f]	.91	.58	.10	--	w.bl.	w.bl.	purp-tan	dk.red bn.	f.bl.	--
o-coumaric acis	.90	.58	.10	--	w.bl.	w.bl.	purp-tan	dk.red	f.bl.	--

[a] Refer to text for solvent system. R_f's are average of many runs.

[b] l, light; bl, blue; yel, yellow; gr, green; brn, brown; br, bright; purp, purple; dk, dark; f, faint; w, white; bk, black; dp, deep.

[c] Diazotized p-nitraniline (Bray et al. 1950), diazotized sulfanilic acid (Bray et al. 1950), ferric chloride--potassium ferricyanide (Smith 1960, p. 324), and Hoepfner reaction (Hoepfner 1932).

[d] Present in extract of leaves.

[e] Present in leaf leachate.

[f] Present in extract of fruits.

The spectrum of known chlorogenic acid was similarly determined. The absorption maximum for both suspected chlorogenic and known chlorogenic acid in 90% ethanol was 330 mμ, and both the suspected and known chlorogenic acid had an absorption maximum of 370 mμ in 90% ethanol plus NaOH. The suspected chlorogenic acid was cochromatographed in two dimensions on paper with known chlorogenic acid using BAW in the first dimension and 6% AA in the second, and the spots coincided.

The biological activity of suspected chlorogenic acid was determined and compared with known chlorogenic acid (2 mg/ml concentration) using an Amaranthus retroflexus seed germination bioassay (Wilson and Rice, 1968). Petri plates containing 100 seeds on filter paper received 2.5 ml of aqueous suspected or known chlorogenic acid, and control plates received 2.5 ml of distilled water. The germination results expressed as percentage of the control germination were: suspected chlorogenic acid 38; known chlorogenic acid 25. The concentration of the suspected chlorogenic acid was not known, so this bioassay does not give comparative values.

Absorption spectra of the suspected and known scopolin and scopoletin in absolute methanol were determined with a Beckman Model DB-G Spectrophotometer. The absorption maximum for both suspected scopolin and known scopolin was 326 mμ, and both had an absorption maximum

The spectrum of known chlorogenic acid was similarly determined. The absorption maximum for both suspected chlorogenic and known chlorogenic acid in 90% ethanol was 330 mμ, and both the suspected and known chlorogenic acid had an absorption maximum of 370 mμ in 90% ethanol plus NaOH. The suspected chlorogenic acid was cochromatographed in two dimensions on paper with known chlorogenic acid using BAW in the first dimension and 6% AA in the second, and the spots coincided.

The biological activity of suspected chlorogenic acid was determined and compared with known chlorogenic acid (2 mg/ml concentration) using an Amaranthus retroflexus seed germination bioassay (Wilson and Rice, 1968). Petri plates containing 100 seeds on filter paper received 2.5 ml of aqueous suspected or known chlorogenic acid, and control plates received 2.5 ml of distilled water. The germination results expressed as percentage of the control germination were: suspected chlorogenic acid 38; known chlorogenic acid 25. The concentration of the suspected chlorogenic acid was not known, so this bioassay does not give comparative values.

Absorption spectra of the suspected and known scopolin and scopoletin in absolute methanol were determined with a Beckman Model DB-G Spectrophotometer. The absorption maximum for both suspected scopolin and known scopolin was 326 mμ, and both had an absorption maximum

of 346 mμ in absolute methanol with 2 drops of 2 N NaOH added to the cuvette. The absorption maximum for the suspected and known scopoletin in absolute methanol was 344 mμ, and both had an absorption maximum of 392 mμ in absolute methanol with 2 drops of 2 N NaOH added to the cuvette.

The two suspected compounds were cochromatographed in two dimensions with known scopolin and scopoletin using BAW in the first dimension and 6% AA in the second. The suspected scopolin and known scopolin spots coincided as did the suspected scopoletin and known scopoletin spots. It is clear, therefore, that the chief toxins in the leaf extract are chlorogenic acid, scopoletin, and scopolin.

The biological activity of suspected scopolin and scopoletin and known scopolin and scopoletin (2 mg/ml concentration) were tested against A. retroflexus germination. The germination percentages as percent of the control were: known scopoletin, 15; and suspected scopoletin, 29; known scopolin, 19; and suspected scopolin, 32.

The seven bands from the fruit extract were eluted and subjected to the same sorts of tests used with toxins from the leaf extract. Colors in UV light, R_f's in various solvents, and tests with various reagents indicated that the inhibitors in the fruit extract were: Phytotoxin 1, chlorogenic acid; Phytotoxin 2, isochlorogenic

acid (a mixture chiefly of three dicaffeoylquinic acids); Phytotoxin 3, neochlorogenic acid (5-caffeoylquinic acid); Phytotoxin 4, band 510 (4-caffeoylquinic acid); Phytotoxin 5, scopolin; Phytotoxin 6, scopoletin; and Phytotoxin 7, o-coumaric acid (2-hydroxycinnamic acid) (Table 8). The absorption spectra of the suspected and known compounds were determined before and immediately after the addition of 2 drops of 2N NaOH to the cuvette. The absorption maximum of both suspected isochlorogenic acid and known isochlorogenic acid in 90% ethanol was 329 mμ, and both had an absorption maximum of 377 mμ in 90% ethanol plus NaOH. The absorption maximum of both suspected neochlorogenic acid and known neochlorogenic acid in 90% ethanol was 328 mμ, and both had an absorption maximum of 350 mμ in 90% ethanol plus NaOH. The absorption maximum of the suspected and known band 510 in 90% ethanol was 320 mμ, and both had an absorption maximum of 372 mμ in 90% ethanol plus NaOH. The maximum absorption of the suspected and known o-coumaric acid was 280 mμ in 70% ethanol, and both had an absorption maximum of 282 mμ in 70% ethanol plus NaOH. The absorption maxima of the suspected and known chlorogenic acid, scopoletin, and scopolin coincided with the results obtained from the leaf extracts. The toxins in the fruit extracts, therefore, are chlorogenic acid, isochlorogenic acid, band 510, neochlorogenic acid, o-coumaric

acid, scopoletin, and scopolin.

The biological activity of the compounds not encountered in the leaf extract was determined as described for the leaf toxins. The germination results expressed as percentage of the control were: suspected isochlorogenic acid, 62; the known isochlorogenic acid, 51; suspected neochlorogenic acid, 37; the known neochlorogenic acid, 41; suspected band 510, 31; the known band 510, 33; suspected o-coumaric acid, 32; and the known o-coumaric acid, 24.

Phytotoxins from Sycamore Leaf Leachate.--Two liters of leaf leachate were evaporated to dryness in vacuo, the residue was taken up in 10 ml of absolute methanol, 4 ml of the solution were streaked on Whatman No. 3 MM paper and developed in BAW. Two distinctive bands were observed, and these were eluted and subjected to the same sorts of tests used with toxins from leaf and fruit extracts. Colors in UV, R_f's in various solvents, tests with various reagents and absorption spectra indicated that the inhibitors in the leaf leachate were chlorogenic acid and scopolin (Table 8).

CHAPTER IV

DISCUSSION

In natural areas, the usual explanation given for the virtual absence of herbaceous plants under many species of trees is competition (personal discussions with many ecologists). Evidence from this investigation indicates that this is not a satisfactory explanation in the case of sycamore. The mineral content and pH of the soil were not significantly different under the canopy than away from it. Soil moisture was consistently higher under the canopy than away. The shading experiment indicated that the herbaceous test species grew as well in a shade, equivalent to that under the sycamores, as in full sunlight. Therefore, the failure of the test species to grow under the canopy was definitely not due primarily to competition.

Decaying sycamore leaves significantly reduced seed germination and seedling growth of all test species. Moreover, sycamore leaf leachate significantly reduced the percentage of seed germination and seedling growth of all test species except Elymus virginicus and Panicum virgatum. The failure of the leachate to inhibit

germination and growth of E. virginicus seems significant in view of the fact that this species was one of very few which grew at least sparsely under the canopy. The results of these experiments are in agreement with the findings of Morgan and Tukey (1964) that organic compounds are leached from leaves and are often allelopathic to the associated plants (Grümmer, 1961). Wilson and Rice (1968) reported that leaf leachate of Helianthus annuus reduced the percentage germination and oven-dry weights of many associated species, and identified scopolin and a derivative of ∝-naphthol as the inhibitory compounds in the leachate.

Field soil collected under the sycamore canopy in July and November significantly reduced the percentage of seed germination and amount of seedling growth of all test species. These experiments showed that the phytotoxins are apparently stable in the soil under field conditions for appreciable time periods. The colloidal materials of the soil may play a role in accumulating these phytotoxins to a toxic level (Wilson and Rice, 1968).

Results of all experiments concerning allelopathic effects of sycamore indicate that the production of chemical inhibitors by that species is the basic cause of the failure of most associated herbaceous species to grow under the sycamore trees. Once the herbaceous species

are slowed in germination and development, even moderate competitive effects would serve as a feed-back mechanism, thus accentuating the retardation in growth.

Chemical analyses of the phytotoxins indicated the presence of chlorogenic acid, scopolin, and scopoletin in the leaf extract, while in the fruit extract the same toxic compounds were produced along with isochlorogenic acid, neochlorogenic acid, band 510, and o-coumaric acid. Only two of the inhibitory compounds identified in leaf extracts were found in appreciable quantities in leachates of living leaves. However, all inhibitors identified in the extracts of leaves and fruits are at least slightly water soluble and could easily leach out of dead leaves and fruits because the differentially permeable membranes are no longer present. In fact, the fruit extracts used in identification of inhibitors were aqueous extracts of dried fruits. Moreover, all compounds could be released from decaying leaf and fruit material.

The biochemical effects of the identified compounds on metabolism and growth were not investigated. However, chlorogenic acid is known to be a strong inhibitor of several enzyme systems (Sondheimer, 1962). Sondheimer and Griffin (1960) found that indoleacetic acid oxidase is inhibited by chlorogenic acid, isochlorogenic acid, neochlorogenic acid, band 510, and

dihydrochlorogenic acid. Olmsted (unpublished M.S. thesis, Univ. of Okla., 1967) found that chlorogenic acid is toxic to 12 day old seedlings of Amaranthus retroflexus in a concentration as low as 0.83×10^{-7} M, and to Bromus japonicus seedlings at 0.83×10^{-3} M. Scopoletin inhibits the oxidation of indoleacetic acid and it has been suggested that it acts as a competitive inhibitor (Andreae, 1952, and Andreae and Andreae, 1953). Sequeira (1964) noted the inhibition of indoleacetic acid oxidase which was correlated with an increase in scopoletin in diseased tobacco tissue, but his results indicated a non-competitive inhibition. Pollock, Goodwin, and Greene (1954) found that scopoletin inhibited the growth of roots of Phleum pratense L. and Avena sativa L. Einhellig (unpublished Ph.D. Diss., Univ. of Okla., 1969) found that a 5×10^{-4} M scopoletin solution was inhibitory to tobacco, sunflower, and pigweed. He found that net photosynthesis in tobacco plants treated with a 10^{-3} M concentration of scopoletin was depressed to as low as 34% of that of the controls. He concluded, therefore, that the reduction in net photosynthesis contributed to plant inhibition.

The many inhibitors produced by sycamore might have synergistic effects which could cause even greater plant inhibition of ecological significance, than either one could separately.

CHAPTER V

SUMMARY

Field observations in native stand of sycamore indicated a definite zone of reduced growth of associated species under the sycamore canopy and in areas of accumulation of fallen sycamore leaves. Experiments were designed to determine if the distribution of herbaceous plants near the sycamore trees was due to competition in the usual sense. The results of these studies showed that the mineral content and pH of the soil were not significantly different under the canopy than away from it. Soil moisture was consistently higher under the canopy than away. Experiments using artificial shading devices indicated that the herbaceous test species grew as well in a shade equivalent to that under sycamores as in full sunlight. Therefore, the failure of the test species to grow under the canopy was apparently not due primarily to competition.

Chemical inhibition studies showed that decaying sycamore leaves significantly reduced seed germination and seedling growth of all test species. Sycamore leaf leachate significantly reduced the percentage

germination and seedling growth of most test species. Field soil collected under the sycamore canopy significantly reduced the percentage of seed germination and the amount of seedling growth of all test species.

Results of all experiments indicate that the production of chemical inhibitors by sycamore is the basic cause of the failure of most herbaceous species to grow under the sycamore trees. Competition could actually serve as a feedback mechanism to accentuate the growth retarding effects of the inhibitors.

The chief phytotoxins identified were chlorogenic acid, scopolin, and scopoletin in the leaf extract; and the same compounds plus isochlorogenic acid, neochlorogenic acid, band 510, and o-coumaric acid in the fruit extracts. Only two of these inhibitory compounds were identified in the leaf leachate, chlorogenic acid and scopolin.

The many inhibitors produced by sycamore might have synergistic effects which could cause greater plant inhibition than either one could separately.

LITERATURE CITED

Andreae, W. A. 1952. Effects of scopoletin on indoleacetic acid metabolism. Nature 170:83-84.

Andreae, W. A., and S. R. Andreae. 1953. Studies on indoleacetic acid metabolism. I. The effect of methyl umbelliferone, maleic hydrazide, and 2,4-D on indoleacetic oxidation. Can. J. Bot. 31:426-437.

Bray, H. G., W. V. Thorp, and K. White. 1950. The fate of certain organic acids and amides in rabbit 10. The application of paper chromatography to metabolic studies of hydroxybenzoic acid and amides. Biochem. J. 46:271-275.

Bremner, J. M. 1965. Total Nitrogen. In Methods of soil analysis, part 2, p. 1149-1176. Black, C. A. ed. Amer. Soc. Agron., Madison, Wisc.

Cook, M. T. 1921. Wilting caused by walnut trees. Phytopathology 11:346.

Del Moral, R. and C. H. Muller. 1969. Fog drip: a mechanism of toxin transport from Eucalyptus globulus. Bull. Torrey Bot. Club. 96:467-475.

Grümmer, G. 1961. The role of toxic substances in the interrelationships between higher plants. In Mechanisms in biological competition. Symp. Soc. Exp. Biol. 15:219-228. Academic Press, N.Y.

Hoepfner, W. 1932. Zwei neue Reaktionen für Kaffesaüre und Chlorogensaüre. Chem. Zeit. 56:991.

Ljubic, F. P. 1955. The interaction of root systems of different tree species growing in mixture. Agrobiologija. 1955:112-116. In Russian. Reviewed in Forestry Abstracts 17:37.

Massey, A. B. 1925. Antagonism of the walnuts (Juglans nigra, and J. cinerea) in certain plant associations. Phytopathology 15:773-784.

Mergen, F. 1959. A toxic principle in the leaves of Ailanthus. Bot. Gaz. 121:32-36.

Morgan, J. V. and H. B. Tukey, Jr. 1964. Characterization of leachate from plant foliage. Plant Physiol. 39:590-593.

Muller, C. H. 1965. Inhibitory terpenes volatilized from Salvia shrubs. Bull. Torrey Bot. Club 92: 38-45.

_____, and R. del Moral. 1966. Soil toxicity induced by terpenes from Salvia leucophylla. Bull. Torrey Bot. Club 93:130-137.

_____, W. H. Muller, and B. L. Haines. 1964. Volatile growth inhibitors produced by shrubs. Science 143:471-473.

Muller, W. H., and C. H. Muller. 1964. Volatile growth inhibitors produced by Salvia species. Bull. Torrey Bot. Club 91:327-330.

Perkin-Elmer. 1968. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer, Norwalk, Conn.

Piper, C. S. 1942. Soil and Plant Analysis. The Univ. of Adelaide, Adelaide, Australia.

Pollock, B. M., R. H. Goodwin, and S. Greene. 1954. Studies on roots. II. Effects of coumarin, scopoletin and other substances on growth. Amer. J. Bot. 41:521-529.

Rice, E. L. 1965. Inhibition of nitrogen-fixing and nitrifying bacteria by seed plants. II. Characterization and identification of inhibitors. Physiol. Plant. 18:255-268.

Sequeira, L. 1964. Inhibition of Indoleacetic acid oxidase in tobacco plants infected by Pseudomonas solanacearum. Phytopathology 54:1078-1083.

Shelton, W. R. and H. J. Harper. 1941. A rapid method for the determination of total phosphorus in soil and plant material. Iowa State Coll. J. Sci. 15:408-413.

Smith, I., Ed. 1960. Chromatographic and electrophoretic techniques. Vol. 1. Chromatography. Interscience Publishers, Inc., New York.

Sondheimer, E. 1962. Chlorogenic acids and related compounds. In Plant Phenolics and their Industrial Significance (V. C. Runeckles, ed.), p. 15-37. Symp. Plant Phenolics Group of North America. Imperial Tobacco Co. of Canada, Montreal, Quebec.

_____, and Griffin, D. H. 1960. Activation and inhibition of indoleacetic acid oxidase activity from peas. Science 131:672.

Wilson, R. E., and E. L. Rice. 1968. Allelopathy as expressed by Helianthus annuus and its role in old-field succession. Bull. Torrey Bot. Club 95:432-448.