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ALLELOPATHIC EFFECTS OF HACKBERRY IN A BOTTOMLAND FOREST COMMUNITY

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Abstract—Hackberry was previously found to produce toxins that inhibit the growth of associated herbaceous species in a grassland community. Nevertheless, it was hypothesized that bare areas under hackberry trees in a bottomland forest community may not be caused by allelopathy, since inhibitory compounds may be leached or metabolized faster than in a grassland community. Investigations indicated that the relatively bare areas under hackberry were not due to competition for minerals, light, or water, or to differences in texture or pH. The percent of soil moisture was always higher under hackberry trees than under bur oak trees where herbaceous species were prominent. Decaying hackberry leaves, leaf leachate, and soil collected from under hackberry trees significantly reduce seed germination and seedling growth of test species. Ferulic, caffeic, gentisic, and *p*-coumaric acids, and scopolin, and scopoletin were identified as phenolic phytotoxins produced in hackberry leaves. Thus it appears that the reduced growth of herbaceous vegetation associated with hackberry trees in both grassland and forest communities is due primarily to allelopathy, with the initial inhibition being accentuated by competition.

Key Words—allelopathy, *Celtis laevigata*, forest community, phenolic, ferulic acid, caffeic acid, gentistic acid, *p*-coumaric acid, scopolin, scopoletin, phytotoxins.

INTRODUCTION

A previous study of *Celtis laevigata* (hackberry) demonstrated that it exerted allelopathic effects against all important tall grass species in a grassland research plot containing hackberry trees (Lodhi and Rice, 1971).

Because of the possible ecological significance of the inhibitors of seed

germination and seedling growth (Lodhi and Rice, 1971), additional work was initiated with *Celtis laevigata* in a bottomland forest where these inhibitors possibly can be leached and metabolized much faster and may lose their allelopathic activity. Bare areas frequently occur under and around hackberry, although several herbaceous species may grow profusely under adjacent tree species that cause shade that is just as dense. This paper reports on experiments designed to determine whether the relatively bare areas under the hackberry in a forest community are chiefly because of competition for minerals, water, or light; or because of chemicals produced by the hackberry. The nomenclature used here follows Waterfall (1966).

LOCATION AND DESCRIPTION OF STUDY AREA

A bottomland plot was established in Oliver Wildlife Preserve located on the University of Oklahoma campus in Norman (Sec. 7, T8NR2W in Cleveland County). The bottomland plot is on a level flood plain of the South Canadian River. The soil is a sandy clay loam. The vegetation consisted of a floodplain forest dominated by *Fraxinus pennsylvanica* (green ash), *Quercus macrocarpa* (bur oak), and hackberry, with several minor tree species.

The growth of herbaceous species was observed to be considerably better under bur oak than hackberry. Light intensities were measured under several hackberry and bur oak trees. Readings were taken twice a month in June and July of 1969. Ten readings were taken with a Weston light meter under each species at each sampling time. An average range of 600–700 ft-c light intensity was obtained under both hackberry and oak trees. No differences were obtained that could explain the differences in growth of herbaceous species under test and control trees.

To describe quantitatively the zone of reduced growth associated with hackberry trees in the Oliver Preserve, 30 randomly located quadrats, 0.25 m^2 in area, were clipped under hackberry trees and 30 under bur oaks in July. Species were separated, oven-dried, and weighed. To obtain quantitative data for *Bromus japonicus* (brome grass), 30 quadrats were clipped for this species in late May and June 1971, because this species is a winter annual. Oven-dry weights of all species sampled were significantly lower under hackberry trees than under bur oaks (Table 1).

EXPERIMENTATION AND RESULTS

Physical and Chemical Analyses of Soil

Soil moisture, pH, texture, and several selected mineral analyses were

TABLE 1. RESULTS OF FIELD CLIPPING OF SPECIES ASSOCIATED WITH HACKBERRY AND BUR OAK IN OLIVER PRESERVE

Species	Mean oven dry weights in g/0.25 m²	
	Hackberry	Bur oak
Elymus virginicus	1.72±0.34	3.65±0.49[a]
Solidago gigantea	8.80±0.66	14.14±0.42[a]
Ambrosia trifida	1.75±0.23	3.76±0.55[a]
Other species	1.47	4.28
Mean total weight	13.72	24.80
Bromus japonicus (May)	1.78±0.20	3.68±0.31[a]
Bromus japonicus (June)	2.21±0.78	5.32±0.45[a]

[a] Dry weight significantly different from that under hackberry at 0.05 level.

made to see if the differences in the vegetation under the hackberry trees were due primarily to physical or chemical properties of the soil.

Soil moisture was determined during the summer of 1969, by taking soil samples at the 0–15-cm and 15–30-cm levels. Ten samples were taken at each level under hackberry trees and ten at each level under bur oak trees at each sampling time. All samples were weighed, oven-dried for 48 hours at 100°C, and reweighed to determine the amount of water present. Soil moisture was calculated on the basis of the oven-dry weight of the soil. The percent of soil moisture was always significantly higher under hackberry trees than under bur oak trees (Table 2).

TABLE 2. COMPARISON OF SOIL MOISTURE UNDER HACKBERRY TREES AND UNDER BUR OAK TREES

Time of soil collection	Level of the soil (cm)	Under hackberry	Under bur oak
June, 1969	0–15	22.70±0.75	19.80±0.60[a]
	15–30	20.60±0.44	16.90±0.80[a]
July, 1969	0–15	22.62±0.74	19.82±0.61[a]
	15–30	20.38±0.44	16.57±0.79[a]
August, 1969	0–15	22.20±0.73	19.91±0.60[a]
	15–30	19.90±0.45	16.24±0.77[a]

[a] Percent moisture significantly different from amount under hackberry at 0.05 level.

For physical and chemical soil analyses, 10 soil samples minus litter were collected at the 0–30-cm level under hackberry and 10 under bur oak trees. Visible pieces of organic matter were removed by hand, after which the soil was passed through a 2-mm sieve. The pH was determined by the glass electrode method of Piper (1942), and a mechanical analysis with a modified Bouyoucus hydrometer method (Bouyoucos, 1963; Piper, 1942). After the pH and texture were determined, the samples were ground in a soil mill to pass a 0.5-mm sieve. Total phosphorus was determined by the method of Shelton and Harper (1941), total carbon by the Walkey and Black method (Piper, 1942), and total nitrogen by the macro-Kjeldahl method of Bremner (1965). Iron, zinc, manganese, and copper were determined by using a Perkin-Elmer Model 303 atomic absorption spectrophotometer after extraction according to the instructions in the analytical manual supplied with the instrument (Perkin-Elmer Corporation, 1968). All calculations were based on the oven-dry weight of the soil. No significant differences were found in the pH, texture, organic carbon, or amounts of any of the mineral elements under hackberry as compared with control soil (data available upon request). These studies showed that the failure of the herbaceous species to grow well under hackberry was not due to any of the factors discussed above.

Experiments were subsequently initiated to determine if hackberry trees produce chemicals inhibitory to select herbaceous species from the bottomland forest community.

Effects of Decaying Hackberry Leaves on Germination and Seedling Growth

Thirty seeds of brome grass were planted in each of ten 10-cm glazed pots containing 1 g air-dried hackberry leaf powder per 454 g of a 3:2 soil and sand mixture, and because of poor germination, a large number of *Elymus virginicus* seeds were planted in each of 10 pots containing a similar mixture. The hackberry leaves employed were harvested and air dried in October, 1969. In the control pots, one gram of peat moss per 454 g of the soil-sand mixture was used, and 10 pots were planted as described above with each species.

After two weeks, the plants were thinned to the four largest seedlings per pot. The plants were grown for two additional weeks, then harvested and oven-dried for 48 hr at 36°C. Seedling growth of both test species was significantly reduced by decaying leaf material, and seed germination of brome grass was inhibited slightly, indicating an allelopathic effect (Table 3).

Effects of Leaf Leachate on Germination and Seedling Growth

A fine mist of cistern water was spread over freshly collected leafy hack-

TABLE 3. EFFECTS OF DECAYING HACKBERRY LEAVES ON GERMINATION AND SEEDLING GROWTH

Species and experiment no.	Mean oven-dry weight of seedling (mg)		Germination (% control)
	Control	Test	
Elymus virginicus			
1	157±7.23	100±7.76[a]	—
2	127±7.64	100±8.59[a]	—
Bromus japonicus			
1	131±9.30	101±6.70[a]	88
2	141±8.51	97±7.73[a]	94

[a] Dry weight significantly different from control at 0.05 level.

berry branches, and the leachate collected in this manner was used to water 10 pots of each test species in a 3:2 soil–sand mixture. Planting was done as described above. Ten control pots of each species were treated in the same manner, except they were watered with equal amounts of cistern water that was not passed over hackberry branches. After two weeks the plants were thinned to the four largest seedlings per pot. Seedlings were allowed to grow for two additional weeks, harvested, oven-dried for 48 hr and weighed.

The oven-dried weight was reduced significantly in each species by the leachate, and the germination of brome grass seeds was reduced slightly in one experiment (Table 4).

TABLE 4. EFFECT OF LEAF LEACHATE ON GERMINATION AND SEEDLING GROWTH

Species and experiment no.	Mean oven-dry weight of seedlings (mg)		Germination (% control)
	Control	Test	
Elymus virginicus			
1	158±7.94	103±7.00[a]	—
2	131±7.53	100±8.95[a]	—
Bromus japonicus			
1	126±9.32	101±6.36[a]	93
2	141±8.09	98±7.50[a]	99

[a] Dry weight significantly different from control at 0.05 level.

TABLE 5. EFFECT OF FIELD SOIL FROM UNDER HACKBERRY TREES ON GERMINATION AND SEEDLING GROWTH

Species and date soil taken	Mean oven-dry weight of seedlings (mg)		Germination (% control)
	Control	Test	
Elymus virginicus			
July, 1969	128±6.32	127±6.78	—
Jan., 1970	151±7.76	99±7.56[a]	—
Bromus japonicus			
July, 1969	144±8.67	141±8.32	106
Jan., 1970	149±8.56	102±7.56[a]	54
June, 1971	158±6.39	121±8.20[a]	61

[a] Dry weight significantly different from control at 0.05 level.

Effects of Field Soils on Germination and Seedling Growth

To determine if the phytotoxins of hackberry are stable in the soil under field conditions, soil collections were made in July, 1969, January, 1970, and June, 1971 under hackberry (test) and oak trees (control) in the Oliver Preserve. Collections were made with a sharp-nose shovel, and the soil was transferred directly into the pots in order to disturb the profile as little as possible. Seeds of test species were placed in pots, as explained before. Ten test pots and ten control pots were planted with each species. After two weeks the plants were thinned to the four largest seedlings per pot. These were allowed to grow for two additional weeks, harvested, oven-dried for 48 hr and weighed.

The July, 1969 soil did not significantly affect germination or seedling growth (Table 5). The January, 1970 soil, however, reduced germination of brome grass appreciably and significantly inhibited seedling growth of both test species (Table 5). Apparently the toxic compounds are more active in soil in late fall and winter after the accumulation of hackberry leaves and other plant parts, as reported by Lodhi and Rice (1971). The inhibitors were possibly either leached from the soil by the early summer rains of 1969, or were oxidized because of exceptionally hot weather in late July of 1969. To check these possibilities, a soil collection was made in June, 1971 and was treated in the same manner as the previous collections but with only one test species, brome grass. Interestingly, seed germination was lowered appreciably and seedling growth was significantly reduced (Table 5). Therefore, it appears that the phytotoxins of hackberry are stable in the soil under field conditions unless some exceptional weather conditions occur.

Identification of Phytotoxins from Hackberry Extracts

The two procedures used to isolate the compounds from hackberry leaves were those of Rice (1965) and Guenzi and McCalla (1966). The identifications were based on the methods of Rice (1965).

Ten percent aqueous extracts of hackberry leaves were acidified to pH 2.5 using 2 N HCl, and extracted with two half volumes of diethyl ether. Ether and water fractions were evaporated to dryness and were taken up in 5 ml 95% ethanol and 10 ml distilled water, respectively. These fractions were chromatographed in two dimensions on Whatman 3 MM paper with *n*-butanol–acetic acid–water (63:10:27 v/v/v), BAW, followed by 6% aqueous acetic acid, (6% AA). The chromatograms were inspected with short (2537 Å) and long (3360 Å) ultraviolet light. Compounds were marked under UV light and subsequently eluted with 95% ethanol. The eluates were reduced to dryness in vacuo, taken up in 3 ml 95% ethanol, and chromatographed in one dimension on Whatman No. 1 paper in three different solvent systems: BAW, 6% AA, and isopropanol–butanol–water (140:20:60 v/v/v) IBW. The R_fs in various solvent systems, colors in UV light, colors in various reagents (Rice, 1965), and maximum absorption peaks in 95% ethanol before and immediately after the addition of 2 drops of 2 N NaOH per cuvette, indicated the presence of scopolin and scopoletin in the extracts (Tables 6 and 7).

Following Guenzi and McCalla (1966), 10 g plant material were ground to pass a 10-mesh screen and hydrolyzed with 150 ml 2 N NaOH in an autoclave for 45 minutes. The extract was filtered and acidified to pH 2.0 with HCl and extracted with two half volumes of diethyl ether. The ether extract was shaken with two half-volumes of 5% $NaHCO_3$ and the ether portion was discarded. The alkaline portion was acidified again to pH 2.0 and reextracted with two half-volumes of ether. The ether fraction was evaporated to dryness and the residue was taken up in 5 ml 95% ethanol. Acid hydrolysis was carried out on a similar amount of ground material by refluxing with 150 ml 2 N HCl for 30 minutes. Ether extractions were carried out as previously described.

Ferulic, caffeic, and *p*-coumaric acids were identified from alkaline hydrolysis (Tables 6 and 7). Only one compound, gentisic acid, was identified from acid hydrolysis.

The biological activity of all the compounds identified was determined. Ethanolic eluates of all the compounds identified and of a similar sized area from a blank chromatogram were evaporated to dryness and were taken up in 2 ml of phosphate buffer, pH 5.65. These buffer solutions were added to petri plates containing 50 seeds each of *Amaranthus palmeri* or brome grass on filter paper. The amounts of the inhibitors applied were not known. The

TABLE 6. CHROMATOGRAPHY OF PHYTOTOXINS FROM *Celtis laevigata*

Compound	R_fs on Whatman No. 1[a]			UV fluorescence[c]		Reagent colors[b,c]		
	BAW	6%	IBW	long	short	Sulfan. acid	$FeCl_3$–$K_3Fe(CN)_6$	*p*-nit.
Scopolin	0.53	0.80	0.52	b bl	b bl	none	none	none
Suspected scopolin	0.53	0.79	0.53	b bl	b bl	none	none	none
Scopoletin	0.80	0.46	0.83	b bl	b bl	f br rose	bl	bl black
Suspected scopoletin	0.81	0.46	0.83	b bl	b bl	f br rose	bl	bl black
Ferulic acid	0.88	0.40	0.77	b bl	b bl	f tan	bl	f br black
Suspected ferulic acid	0.87	0.39	0.76	b bl	b bl	f tan	bl	f br black
p-Coumaric acid	0.90	0.46	0.70	0.86	pur abs	pur abs	or red	bl	br black
Suspected *p*-coumaric acid	0.89	0.47	0.71	0.85	pur abs	pur abs	or red	bl	br black
Caffeic acid	0.80	0.32	0.66	0.71	bl	bl	none	bl	f br black
Suspected caffeic acid	0.81	0.32	0.66	0.71	bl	bl	none	bl	f br black
Gentisic acid	0.85		0.65	0.65	bl	bl	f tan	bl	f br black
Suspected gentisic acid	0.85		0.64	0.65	bl	bl	f tan	bl	f br black

[a] See text for solvent systems.
[b] Diazotized sulfanilic acid, ferric chloride–potassium ferricyanide, and diazotized *p*-nitraniline.
[c] b = bright; bl = blue; br = brown; f = faint; abs = absorption; or = orange; pur = purple.

TABLE 7. MAXIMUM ABSORPTION SPECTRA (IN 95% ETHANOL) OF INHIBITORS FROM HACKBERRY LEAVES AND EFFECT OF INHIBITORS ON GERMINATION

Compound	Maximum absorption (nm)	Maximum absorption with NaOH	Germination (% control)	
			Amaranthus palmeri	Brome grass
Scopolin	326	345		
Suspected scopolin	325	346	33	27
Scopoletin	344	392		
Suspected scopoletin	344	390	26	31
Ferulic acid	285	343		
Suspected ferulic acid	282	340	33	48
p-Coumaric acid	283	330		
Suspected *p*-coumaric acid	285	332	49	39
Caffeic acid	288	265		
Suspected caffeic acid	286	264	24	27
Gentisic acid	330	295		
Suspected gentisic acid	328	293	47	37

eluate from the blank paper was used as the control. Germination was determined after 5 days, and the results expressed as a percent of control germination (Table 7).

Field soils from under hackberry trees were collected and extracted according to Wang *et al.* (1967), and then were treated for isolation and identifications following the procedure of Rice (1965). The papers were examined under UV light and the visible spots were eluted with 95% ethanol. These eluates were then tested for biological activity by the *A. palmeri* germination bioassay and were found to be very toxic to this species. However, I was not able to identify any of these toxins and suspected that they consisted of phenolics bound to other compounds. Therefore, a hydrolytic method modified from Guenzi and McCalla (1966) was used to extract these compounds from the soil. Ferulic, caffeic, and *p*-coumaric acids were identified from this method. All compounds identified from the January soil collection were present in concentrations of 800–1100 μg/g of soil.

DISCUSSION

The reduced growth of test species under hackberry trees was apparently not primarily because of physical factors, deficient soil moisture, or mineral deficiencies. Light intensity, pH, soil texture, organic carbon, and amounts

of mineral elements measured were not significantly different under hackberry than under bur oak trees. Soil moisture was always significantly higher under hackberry trees than under control trees. Decaying hackberry leaves, leaf leachate, and soil from under hackberry trees were all found to inhibit seed germination and seedling growth of herbaceous species that grow considerably better when away from hackberry trees than when under them. Apparently the allelopathic effects of hackberry trees in a forest community are just as effective as in a grassland community (Lodhi and Rice, 1971). Thus, the original hypothesis that hackberry may not be allelopathic in a forest community because of a rapid removal of toxins was not supported.

The phytotoxins identified from hackberry leaves were scopolin, scopoletin, and ferulic, caffeic, *p*-coumaric, and gentisic acids. Scopolin and scopoletin were found in aqueous extracts of leaves, whereas all others were found only after acid or alkaline hydrolysis. Lodhi (1975) reported that ferulic, caffeic, and *p*-coumaric acids were identified from the soil under hackberry trees only after alkaline hydrolysis. Guenzi and McCalla (1966) found ferulic and *p*-coumaric acids in the residues of corn, oats, sorghum, and wheat and that *p*-coumaric acid can be released in amounts sufficient to inhibit plant growth. Wang *et al.* (1967) sampled soil from several croplands and found *p*-coumaric acid and ferulic acid plus several other phenolic acids.

The concentration of phenolic acids quantified in many soils was found to suppress the growth of several young crop plants when applied to plants growing in nutrient culture solution. Rasmussen and Rice (1971) isolated ferulic and *p*-coumaric acids from *Sporobolus pyramidatus* and found allelopathic effects on associated species resulting in either reduced growth or elimination from the stand. Langdale and Giddens (1967) reported that small quantities of ferulic and *p*-coumaric acids are effective in inhibiting IAA activity in *Avena* coleoptiles. Zenk and Muller (1963) found that *p*-coumaric and ferulic acids increase IAA decarboxylation, resulting in reduced growth. Olmsted and Rice (1970) found that *p*-coumaric acid was significantly inhibitory to the growth of 12-day-old seedlings of *Amaranthus retroflexus*. Del Moral and Muller (1970) found that *p*-coumaric, ferulic, and caffeic acids from *Eucalyptus camaldulensis* were toxic to the germination of test seeds. Hennequin and Juste (1967) found that caffeic, ferulic, and *p*-coumaric acids have phytotoxic effects on seed germination and seedling growth. Rice (1965) found that *Ambrosia psilostachya* produced a glucose ester of caffeic acid that is inhibitory to nitrogen-fixing and nitrifying bacteria, and Rice (1968, 1971) reported that *A. psilostachya* and its leaf leachate caused a significant reduction of nodulation in three legume species. Neill and Rice (1971) reported that the root exudate, leaf leachate, and decaying leaves of *A. psilostachya* inhibited many of the early invaders of abandoned fields.

Al-Naib and Rice (1971) found that *Platanus occidentalis* inhibited seed

germination and seedling growth of many associated species, and scopolin, scopoletin, and other phenolic compounds were isolated from *P. occidentalis* leaves and mature fruits. Einhellig *et al.* (1970) found that growth of tobacco, sunflower, and pigweed was inhibited by a 5×10^{-4} M scopoletin concentration. Net photosynthesis in tobacco plants treated with a 10^{-3} M concentration of scopoletin was depressed to 34% of that of the controls. Lodhi and Nickell (1973) found that osmotically inactive water extracts of hackberry significantly reduced the shoot growth and the rate of photosynthesis, but significantly increased the rate of dark respiration of *Andropogon gerardi*, *A. scoparius*, and brome grass (species associated with hackberry). Lodhi (1975), Rice (1971), Al-Naib and Rice (1971), and Wilson and Rice (1968) suggested that the additive effect of a combination of inhibitors may be more detrimental than each compound separately.

Thus, it appears that allelopathy expressed by hackberry in a grassland community (Lodhi, 1975; Lodhi and Rice, 1971) or in a bottomland forest community may be important ecologically in helping determine the patterning of herbaceous vegetation.

REFERENCES

Al-Naib, F.A.G., and Rice, E.L. 1971. Allelopathic effects of *Platanus occidentalis*. *Bull. Torrey Bot. Club* 98:75–82.

Bouyoucos, G. 1963. Directions for making mechanical analysis of soils by the hydrometer method. *Soil Sci.* 42:225–229.

Bremner, J.M. 1965. Total nitrogen, pp. 1149–1178, *in* C.A. Black (ed.), Methods of Soil Analysis, Part 2. American Society of Agronomy, Inc., Madison, Wisconsin.

del Moral, R., and Muller, C.H. 1970. The allelopathic effects of *Eucalyptus camaldulensis*. *Am. Midl. Nat.* 83:254–282.

Einhellig, F.A., Rice, E.L., Risser, P.G., and Wender, S.H. 1970. Effects of scopoletin on growth, CO_2 exchange rates, and concentration of scopoletin, scopolin, and chlorogenic acids in tobacco, sunflower, and pigweed. *Bull. Torrey Bot. Club* 97:22–33.

Guenzi, W.D., and McCalla, T.M. 1966. Phenolic acids in oats, wheat, sorghum and corn residues and their phytotoxicity. *Agron J.* 58:303–304.

Hennequin, J.R., and Juste, C. 1967. Présence d'acides phénols libres dans le sol. Étude de influence sur la germination et la croissance des végétaux. *Ann. Agron.* (Paris), 18:545–569.

Langdale, G.W., and Giddens, J.E. 1967. Phytotoxic phenolics compounds in sericea lespendeza residues. *Agron. J.* 59:581–584.

Lodhi, M.A.K. 1975. Soil-plant phytotoxicity and its possible significance in patterning of herbaceous vegetation in a bottomland forest. *Am. J. Bot.* In press.

Lodhi, M.A.K., and Nickell, G.L. 1973. Effects of leaf extracts of *Celtis laevigata* on growth, water content, and carbon dioxide exchange rates of three grass species. *Bull. Torrey Bot. Club* 100:159–165.

Lodhi, M.A.K., and Rice, E.L. 1971. Allelopathic effects of *Celtis laevigata*. *Bull. Torrey Bot. Club* 94:83–89.

NEILL, R.L., and RICE, E.L. 1971. Possible role of *Ambrosia psilostachya* on patterning and succession in old-fields. *Am. Midl. Natur.* 86:344–357.

OLMSTED, C.E., and RICE, E.L. 1970. Relative effects on known plant inhibitors on species from first two stages of old-field succession. *Southwest. Nat.* 15:165–173.

PERKIN-ELMER CORPORATION 1968. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer Corporation, Norwalk, Connecticut.

PIPER, C.S. 1942. Soil and Plant Analysis. The University of Adelaide, Adelaide, Australia. 368 pp.

RASMUSSEN, J.A., and RICE, E.L. 1971. Allelopathic effects of *Sporobolus pyramidatus* on vegetational patterning. *Am. Midl. Nat.* 86:309–326.

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.

RICE, E.L. 1968. Inhibition of nodulation of inoculated legumes by pioneer plant species from abandoned fields. *Bull. Torrey Bot. Club* 95:346–358.

RICE, E.L. 1971. Inhibition of nodulation of inoculated legumes by leaf leachates from pioneer plant species from abandoned fields. *Am. J. Bot.* 58:368–371.

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

WANG, T.S.C., YANG, T.-K., and CHUANG, T.-T. 1967. Soil phenolics as plant growth inhibitors. *Soil Sci.* 103:239–249.

WATERFALL, U.T. 1966. Keys to the flora of Oklahoma. Third edition. Published privately by author. Stillwater, Oklahoma.

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

ZENK, M.H., and MULLER, G. 1963. In-vivo destruction of exogenously applied indolyl-3-acetic acid as influenced by naturally occurring phenolic acids. *Nature* 200:761–763.