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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

# ALLELOPATHIC EFFECTS OF *Celtis laevigata* Willd. AS RELATED TO PATTERNING OF VEGETATION

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ALLELOPATHIC EFFECTS OF *Celtis laevigata* Willd.

AS RELATED TO PATTERNING OF VEGETATION

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# ALLELOPATHIC EFFECTS OF *Celtis laevigata* Willd. AS RELATED TO PATTERNING OF VEGETATION

## CHAPTER I

## INTRODUCTION

Kershaw (1964) and Muller (1966) reported that the chemical inhibition of one plant by another (allelopathy) was reported over a century ago by De Candolle. Evidence concerning the roles and ecological importance of allelopathy has been reviewed by many workers (Evenari, 1949; Bonner, 1950; Börner, 1960; Woods, 1960; Garb, 1961; Patrick, Toussoun and Koch, 1964; Muller, 1966; and Rice, 1967).

Apparently the first tree species found to produce chemical inhibitors of other plants was *Juglans nigra*[1] (Cook, 1921; Massey, 1925; Davis, 1928). Several tree species have subsequently been demonstrated to produce substances toxic to other plant species (Proebsting and Gilmore, 1950; Ljubic, 1955; Mergen, 1959; Jameson, 1961;

[1]Nomenclature follows Waterfall (1966) unless authority is given.

Al-Naib, unpublished Ph.D. dissertation, University of Oklahoma, 1970).

Bare areas frequently occur under and around Celtis laevigata (Hackberry), although several herbaceous species may grow profusely under adjacent tree species which cast just as dense shade. I hypothesized, therefore, that hackberry might produce chemical inhibitors of certain herbaceous species often associated with it. Appropriate investigations were undertaken to test the hypothesis.

## CHAPTER II

## LOCATION AND DESCRIPTION OF STUDY AREAS

An upland plot containing hackberry was established in the University of Oklahoma Grasslands Research Plots, 8 miles southwest of Norman, Oklahoma in McClain County (Sec. 12, T8NR4W), and a bottomland plot was established in Oliver Wildlife Preserve located on the University of Oklahoma campus in Norman (Sec. 7, T8RNR2W in Cleveland County).

The Grasslands Plots are on a gently rolling upland with moderately deep sandy loam soil over a soft red sandstone bedrock. Vegetation of the area is tallgrass prairie which has been invaded by woody species since the elimination of burning and grazing starting in 1949. Dominant species in the plot are Andropogon scoparius, A. gerardi, Panicum virgatum, and Sorghastrum nutans.

The bottomland plot is on a level floodplain of the South Canadian River. The soil is a sandy clay loam. The vegetation consists of a flood-plain 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 in the Oliver Preserve and better under Prunus mexicana (plum) than hackberry in the Grasslands Plots. Light intensities were measured under several hackberry and bur-oak trees in the Oliver Preserve, and under several hackberry and plum trees in the Grasslands Plots. Readings were taken twice a month in June and July of 1969. Ten readings were taken with a Weston Light Meter under each species in each study area at each sampling time. An average range of 600-700 ft-c light intensity was obtained under both hackberry and oak trees in the Oliver Preserve, and 2600-3300 ft-c under both hackberry and plum in the Grasslands Plots. No differences were obtained which could explain the differences in growth of herbaceous species under test and control trees.

To describe quantitatively the zones 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. Species were separated, oven dried and weighed, and weights of all species sampled were significantly lower under hackberry trees than under bur-oaks (Table 1).

To quantify these observations in the Grasslands Plots, 0.25 $m^2$ quadrats were located along lines extending outward from the tree trunks. Three quadrats were sampled

Table 1. Results of field clipping of species associated with hackberry and oak in Oliver Preserve.

| Species | Mean oven dry weights in g/0.25 $m^2$ | |
|---|---|---|
| | Hackberry | Oak |
| *Elymus virginicus* | 1.71±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 |

[a]Dry weight significantly different at .05 level from that under hackberry.

along each line with one starting 0.5 m from the trunk, one at 1.5 m and one at 2.5 m. Ten of such series of quadrats were located under hackberry trees and 10 under plum trees, the plants were clipped, separated by species, oven dried and weighed. The weights of all the species separated were significantly lower under hackberry trees than under plum trees, except in the quadrats farthest from the tree trunk (Table 2). The quadrats farthest from the tree trunks were almost in the open prairie.

There was a pronounced reduction in total biomass of herbaceous species under hackberry trees as compared with bur-oak trees in the Oliver Preserve, and plum trees in the Grasslands Plots (Tables 1, 2).

Table 2. Results of field clippings of species associated with hackberry and plum in Grasslands Research Plots.

| Species | Mean oven dry weight in g/0.25 $m^2$ Quadrats[a] | | | | | |
|---|---|---|---|---|---|---|
| | A | | B | | C | |
| | Hackberry | Plums | Hackberry | Plums | Hackberry | Plums |
| Andropogon gerardi | 0.79±.19 | 1.71±.27[b] | 5.48±.64 | 7.46±.20[b] | 12.34±1.50 | 11.70±1.46 |
| Andropogon scoparius | 0.76±.20 | 1.52±.26[b] | 5.08±.49 | 7.28±.36[b] | 13.08±1.29 | 13.02±1.06 |
| Panicum virgatum | 0.92±.19 | 1.74±.22[b] | 5.74±.52 | 7.50±.24[b] | 12.53±1.36 | 12.29±1.23 |
| Sorghastrum nutans | 0.83±.17 | 1.91±.30[b] | 5.84±.57 | 7.69±.27[b] | 12.88±.93 | 12.97± .77 |
| Other Species | 0.59 | 1.02 | 1.18 | 2.20 | 2.90 | 3.98 |
| Mean total weight | 3.90 | 7.90 | 23.30 | 32.15 | 53.75 | 54.00 |

[a]Quadrat A starts 0.5 m from tree trunk.

Quadrat B starts 0.5 m from Quadrat A.

Quadrat C starts 0.5 m from Quadrat B.

[b]Dry weight significantly different at .05 level from that of the quadrats taken under hackberry.

# CHAPTER III

## EXPERIMENTATION AND RESULTS

### Physical and Chemical Analyses of Soil

Soil moisture, pH, texture and several selected mineral analyses were made to see if the differences in the vegetation under the hackberry trees were due primarily to physical and chemical properties of the soil.

Soil moisture was determined during the summer of 1969 in both research plots 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 the plum trees at each sampling time in the Grasslands Research Plots. Samples were taken similarly in the Oliver Wildlife Preserve under hackberry and bur-oak trees at each sampling period. All samples were weighed, oven dried for 48 hr at 105° 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. Percent soil moisture was always significantly higher under hackberry trees than under bur-oak trees in the Oliver Wildlife Preserve or under plum trees in the Grasslands Plots (Table 3).

Table 3. Comparision of soil moisture under hackberry trees and under control trees. Each value represents average of 10 soil samples.

| Time of soil collection | Level the soil | Oliver Wildlife Preserve | | Grasslands Research Plots | |
|---|---|---|---|---|---|
| | | Under hackberry | Under oak | Under hackberry | Under plum |
| June 1969 | 0-15 cm | 22.70±.75 | 19.80±.60[a] | 8.41±.19 | 7.66±.11[a] |
| | 15-30 cm | 20.60±.44 | 16.90±.80[a] | 9.04±.21 | 7.65±.23[a] |
| July 1969 | 0-15 cm | 22.62±.74 | 19.82±.61[a] | 8.44±.18 | 7.52±.90[a] |
| | 15-30 cm | 20.38±.44 | 16.57±.79[a] | 9.10±.22 | 7.50±.16[a] |
| August 1969 | 0-15 cm | 22.20±.73 | 19.91±.60[a] | 8.40±.22 | 7.20±.70[a] |
| | 15-30 cm | 19.90±.45 | 16.24±.77[a] | 8.90±.28 | 7.10±.18[a] |

[a]Percent moisture significantly different at .05 level from amount under appropriate control.

For physical and chemical soil analyses, ten soil samples minus litter were collected at the 0-30 cm level under hackberry and 10 under bur-oak trees in the Oliver Wildlife Preserve, and similar collections were made under hackberry and plum trees in the Grasslands Research Plot. 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 Bouyoucos hydrometer method (Bouyoucos, 1936; Piper, 1942). After the pH and texture were determined, the samples were ground in a soil mill to pass through a 0.5 mm sieve. Total phosphorus was determined by the method of Shelton and Harper (1941), total carbon by the Walkley 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 (Table 4). Apparently, the failure of herbaceous species to grow well under hackberry was not due to any of the soil factors

Table 4. Comparision of physical and mineral properties of soils under hackberry and control trees. Each value represents the average of ten soil samples.[a]

| Test | Oliver Wildlife Preserve | | Grasslands Research Plots | |
|---|---|---|---|---|
| | Hackberry | Bur-Oak | Hackberry | Plum |
| pH | 8.29 ± .002 | 8.29 ± .002 | 6.85 ± .02 | 6.86 ± .021 |
| sand % | 62.04 ± .14 | 62.0 ± .13 | 76.20 ± .08 | 76.16 ± .07 |
| silt % | 14.55 ± .021 | 14.39 ± .021 | 14.77 ± .02 | 14.74 ± .04 |
| clay % | 23.41 ± .04 | 23.61 ± .08 | 9.03 ± .06 | 9.10 ± .03 |
| Total N% | 0.111± .005 | 0.113± .003 | 0.157± .007 | 0.165± .004 |
| Total C% | 1.134± .064 | 1.123± .051 | 0.917± .011 | 0.919± .012 |
| Total P% | 0.081± .0007 | 0.081± .0006 | 0.028± .0006 | 0.028± .0006 |
| Fe ppm | 107.1 ±4.32 | 104.2 ±3.62 | 223.3 ±10.36 | 220.7 ±11.45 |
| Zn ppm | 14.91 ± .72 | 14.06 ± .71 | 7.52 ± 1.15 | 7.50 ± 1.05 |
| Cu ppm | 18.34 ± .98 | 18.13 ± .69 | 7.21 ± .63 | 7.25 ± .77 |
| Mn ppm | 178.3 ±4.27 | 175.77 ±5.85 | 320.2 ±14.53 | 317.1 ±14.90 |

[a]No differences were statistically significant.

discussed above.

Experiments were subsequently initiated to determine if hackberry trees produce chemical inhibitors of certain herbaceous species often associated with them.

## Effects of Decaying Hackberry Leaves on Germination and Seedling Growth

Thirty seeds of each test species (except *Elymus virginicus* and *Sorghastrum nutans* where a large number of seeds was used because of poor germination) were planted in each of ten 10 cm glazed pots containing 1 g of air dried hackberry leaf powder per 454 g of a 3:2 soil and sand mixture. One gram of peat moss per 454 g of the soil-sand mixture was used in the control pots. All experiments were run in growth chambers with a 16 hr photo period at 29° C and a dark period temperature of 21° C. Germination was determined after 2 weeks, and the plants were thinned to the four largest seedlings per pot. Seedlings were allowed to grow for 2 additional weeks and then harvested and oven dried for 48 hr at 36° C. In all experiments described in this paper, hackberry trees growing in the Oliver Preserve were used in tests with *Elymus virginicus* and *Bromus japonicus* and hackberry trees growing in the Grasslands Research Plots were used in tests with *Andropogon scoparius*, *A. gerardi*, *Panicum virgatum* and *Sorghastrum nutans*.

Seed germination and seedling growth of all test species were significantly reduced by decaying leaf material indicating an allelopathic effect (Table 5).

## Effects of Leaf Leachate on Germination and Seedling Growth

A fine mist of cistern water was sprayed over freshly collected leafy hackberry branches. The leachate collected in this manner was used to water 10 pots of each test species, each pot containing 30 seeds of a test species and the 3:2 soil-sand mixture. Many seeds of Elymus virginicus and Sorghastrum nutans were planted per pot for reasons previously explained. Ten control pots of each species were treated in the same manner except they were watered with equal amounts of cistern water which was not passed over hackberry branches. Germination was determined after 2 weeks, after which the plants were thinned to the 4 largest seedlings per pot. The seedlings were allowed to grow for 2 additional weeks, harvested, oven dried for 48 hr and weighed.

The leachate reduced the percent germination of most test species and significantly reduced the oven dry weights of all test species (Table 6).

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

| Species | Expt. No. | Mean oven dry weight of seedlings, mg | | Germination |
|---|---|---|---|---|
| | | Control | Test | % of Control |
| Andropogon gerardi | 1 | 162± 8.30 | 116±7.11[a] | 76 |
| | 2 | 165± 7.51 | 98±7.92[a] | 68 |
| Andropogon scoparius | 1 | 167± 8.02 | 102±8.22[a] | 72 |
| | 2 | 125± 8.83 | 87±6.53[a] | 64 |
| Panicum virgatum | 1 | 203± 5.45 | 131±8.70[a] | 64 |
| | 2 | 178± 7.70 | 129±5[a] | 68 |
| Sorghastrum nutans | 1 | 195±10.11 | 144±7.25[a] | -- |
| | 2 | 199± 6.92 | 128±9.43[a] | -- |
| 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 at .05 level from control.

Table 6. Effect of leaf leachate on germination and seedling growth.

| Species | Expt. No. | Mean oven dry weight of seedlings, mg | | Germination |
|---|---|---|---|---|
| | | Control | Test | % of Control |
| Andropogon gerardi | 1 | 201± 7.53 | 160±6.64[a] | 21 |
| | 2 | 222± 7.60 | 141±9.92[a] | 83 |
| Andropogon scoparius | 1 | 136± 9.33 | 85±5.71[a] | 77 |
| | 2 | 110± 7.47 | 83±4.45[a] | 69 |
| Panicum virgatum | 1 | 200±10.10 | 164±9.23[a] | 69 |
| | 2 | 201± 6.00 | 129±8.50[a] | 73 |
| Sorghastrum nutans | 1 | 182± 9.38 | 145±7.64[a] | -- |
| | 2 | 199± 6.55 | 126±9.50[a] | -- |
| 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 at .05 level from control.

## Effect of Field Soils on Germination and Seedling Growth

To determine the biological activity and stability of toxic compounds in the soil, soil collections were made in July 1969 and January 1970, under hackberry (test) and oak trees (control) in the Oliver Preserve and under hackberry (test) and plum (control) in the Grasslands Plots. 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 appropriate pots, 30 seeds per pot and with 10 pots of each species. Germination was determined after 2 weeks, and the plants were thinned to the 4 largest seedlings per pot. These were allowed to grow for 2 additional weeks, harvested, oven dried for 48 hr and weighed.

The July 1969 soil did not significantly reduce germination or seedling growth (Table 7). The January 1970 soil did, however, significantly reduce germination and seedling growth of all test species (Table 7). Apparently the toxic compounds are more active in soil in late fall and winter after the accumulation of hackberry leaves and other plant parts on the soil surface. The inhibitors had apparently either been leached from the soil by the early summer rains of 1969 or were possibly oxidized due to the exceptionally hot weather in late

Table 7. Effect of field soil from under hackberry trees on germination and seedling growth

| Species | Date Soil Taken | Mean oven dry weight of seedlings, mg | | Germination |
|---|---|---|---|---|
| | | Control | Test | % of Control |
| Andropogon gerardi | July 1969 | 177±7.20 | 172± 7.12 | 92 |
| | Jan. 1970 | 197±7.39 | 122± 9.31[a] | 49 |
| Andropogon scoparius | July 1969 | 111±7.18 | 101± 6.38 | 84 |
| | Jan. 1970 | 117±7.73 | 91± 4.40[a] | 51 |
| Panicum virgatum | July 1969 | 192±8.20 | 182±11.60 | 86 |
| | Jan. 1970 | 193±7.19 | 141± 5.36[a] | 47 |
| Sorghastrum nutans | July 1969 | 144±6.56 | 132± 5.58 | -- |
| | Jan. 1970 | 187±6.73 | 121± 9.83[a] | -- |
| 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 |

[a]Dry weight significantly different at .05 level from control.

July of 1969.

## 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 2N HCl, and extracted with 2 half volumes of diethyl ether. Ether and water fractions were evaporated to dryness and were taken up in 3 ml of 95% ethanol and 10 ml of 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 (2537Å) and long (3360Å) 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 of 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). The Rf's 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 2N NaOH per cuvette, indicated the presence of scopolin and scopoletin in the extracts (Table 8).

Following Guenzi and McCalla (1966), 10 g of plant material were ground to pass a 10 mesh screen, and hydrolyzed with 150 ml of 2N NaOH in an autoclave for 45 minutes. The extract was filtered and acidified to pH 2.0 with 1N HCl, and extracted with two half volumes of diethyl-ether. The ether extract was shaken with 2 half volumes of 5% $NaHCO_3$ and the ether portion was discarded. The alkaline portion was acidified again to pH 2.0 and re-extracted with 2 half volumes of ether. The ether fraction was evaporated to dryness and the residue was taken up in 3 ml of 95% ethanol. Acid hydrolysis was carried out on a similar amount of ground material by refluxing with 150 ml of 2N HCl. Ether extractions were carried out as previously described.

The procedures used to identify the compounds resulting from acid and alkaline hydrolysis were chiefly those of Rice (1965) as described above. Ferulic, caffeic and p-coumaric acids were identified from alkaline hydrolysis (Table 8). 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

Table 8. Chromatography of phytotoxins from Celtis laevigata.

| Compound | Rf's on Whatman No. 1[a] | | | Fluorescence[c] | | Reagent colors[b,c] | | | Maximum Absorption | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BAW | 6% | IBW | long U.V. | short U.V. | Sulfan. acid | $FeCl_3$ $K_3Fe(CN)_6$ | p-nit. | without NaOH | with NaOH |
| Scopolin | .53 | .80 | .52 | b bl | b bl | none | none | none | 326 | 345 |
| Suspected Scopolin | .53 | .79 | .53 | b bl | b bl | none | none | none | 325 | 346 |
| Scopoletin | .80 | .46 | .83 | b bl | b bl | f br rose | bl | bl black | 344 | 392 |
| Suspected Scopoletin | .81 | .46 | .83 | b bl | b bl | f br rose | bl | bl black | 344 | 390 |
| Ferulic Acid | .88 | .40 | .77 | b bl | b bl | f tan | bl | f br black | 285 | 343 |
| Suspected Ferulic Acid | .87 | .39 | .76 | b bl | b bl | f tan | bl | f br black | 282 | 340 |
| p-Coumaric Acid | .90 | .46,.70 | .86 | pur abs | pur abs | or red | bl | br black | 283 | 330 |
| Suspected p-Coumaric Acid | .89 | .47,.71 | .85 | pur abs | pur abs | or red | bl | br black | 285 | 332 |

Table 8. Continued.

| Compound | Rf's on Whatman No. 1[a] | | | Fluorescence[c] | | Reagent colors[b,c] | | | Maximum Absorption | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BAW | 6% | IBW | long U.V. | short U.V. | Sulfan. acid | $FeCl_3$ $K_3Fe(CN)_6$ | p-nit. | without NaOH | with NaOH |
| Caffeic Acid | .80 | .32,.66 | .71 | bl | bl | none | bl | f br black | 288 | 265 |
| Suspected Caffeic Acid | .81 | .32,.66 | .71 | bl | bl | none | bl | f br black | 286 | 264 |
| Gentisic Acid | .85 | .65 | .65 | bl | bl | f tan | bl | f br black | 330 | 295 |
| Suspected Gentisic Acid | .85 | .64 | .65 | bl | bl | f tan | bl | f br black | 328 | 293 |

[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.

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 on filter paper. The eluate from the blank paper was used as the control. Germination was determined after 5 days, and the results epxressed as percent of control germination were scopoletin, 26; scopolin, 31; ferulic acid, 35; caffeic acid, 28; p-coumaric acid, 47; and gentisic acid, 43. Thus, all the compounds identified were inhibitory to germination.

# CHAPTER IV

## DISCUSSION

The reduced growth of test species under hackberry trees was apparently not due primarily to physical factors, or to 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 in control areas under bur-oak trees in the Oliver Preserve and under plum trees in the Grasslands Plots. Soil moisture was always significantly higher under hackberry trees than under the control trees.

On the other hand, decaying hackberry leaves, leachate from hackberry leaves, and soil under hackberry trees were all found to inhibit seed germination and seedling growth of herbaceous species which grow well away from hackberry trees but not under them. The relatively bare areas under hackberry are therefore apparently due primarily to allelopathic effects of the hackberry. Normal competitive mechanisms no doubt accentuate the retarding effect of the chemical inhibitors.

The chief phytotoxins identified were scopolin, scopoletin, ferulic acid, caffeic acid, p-coumaric acid and gentisic acid. Scopolin and scopoletin were found in aqueous extracts of leaves, whereas all others were found only after acid or alkaline hydrolysis. There is little doubt that most, if not all, of the bound phenolics would be released readily by decomposers in the soil. Thus, they undoubtedly represent realistic inhibitors (Guenzi and McCalla, 1966). No attempts were made to isolate and identify the inhibitors from the soil under hackberry trees. Wang, Yang and Chuang (1967) sampled soil from several areas and found p-coumaric acid and ferulic acids plus several other phenolic acids. The concentration of phenolic acids found in many soils was found to suppress the growth of young wheat, corn and soybean plants when applied to plants growing in nutrient culture solution. They stated that phenolic acids would, at least in part, be present in adsorbed and bonded forms in soils, and that effects of these upon plant growth would probably not be the same in soils as it would be in the nutrient solutions. The level of phenolic acids in soil can increase tremendously under certain circumstances according to Wang, Cheng and Tung (1967).

Zenk and Muller (1963) reported that p-coumaric and ferulic acids increase IAA decarboxylation, resulting in reduced growth, and that p-coumaric acid has an

extremely stimulating effect on this reaction. Henderson and Nitsch (1962) found a drastic inhibiting influence of p-coumaric acid on IAA induced growth also. Wang, Yang, and Chuang (1967) reported that Knoesel found that adding phenolic acids to soil caused a shift in the microbiological balance. Rice (1964, 1965) found that many plants important in old-field succession are very inhibitory to selected test strains of nitrogen-fixing and nitrifying bacteria and most inhibitors identified were phenolics. Thus, it is quite likely that phenolic acids affect higher plant growth through their influence upon soil microorganisms in addition to direct effects on the plants.

Pollack, Goodwin and Greene (1954) found that scopoletin inhibits root growth of Phleum pratense L. and Avena sativa and also seems to inhibit the whole growth process. They found that the addition of BAL (2,3,dimercaptopropanol, a sulfhydryl enzyme protector) relieved inhibition caused by unsaturated lactones such as coumarin but did not relieve the inhibition of root growth by scopoletin. Einhellig et al. (1970) found that growth of tobacco, sunflower and pigweed was inhibited by a $5 \times 10^{-4}$ M scopoletin concentration. Net photosynthesis in tobacco plant treated with a $10^{-3}$ M concentration of scopoletin was depressed to as low as 34% of that of the controls.

Thus, it appears that allelopathy may be very

important ecologically in helping determine the patterning of vegetation, in addition to its apparently important role in plant succession (Abdul-Wahab and Rice, 1967; Wilson and Rice, 1968; Parenti and Rice, 1969).

# CHAPTER V

## SUMMARY

Bare areas frequently occur under hackberry trees, although several herbaceous species may grow relatively well under adjacent tree species which cast just as dense shade. No significant differences were found in pH, texture, organic carbon or amounts of any of the mineral elements sampled under hackberry as compared with control soils under bur-oak trees in the Oliver Wildlife Preserve and under plum trees in the University of Oklahoma Grasslands Plots. Percent soil moisture was always significantly higher under hackberry trees than under bur-oak trees in the Oliver Preserve and under plum trees in the Grasslands Plots. Apparently, the failure of herbaceous species to grow well under hackberry was not due primarily to physical factors or to deficiencies in minerals, water, or light.

Decaying leaves of hackberry, leaf leachate and soil collected from under hackberry trees in January 1970 significantly reduced seed germination and seedling growth of test species.

Scopolin, scopoletin, ferulic acid, caffeic acid, p-coumaric acid and gentisic acid were identified as the chief phytotoxins produced in hackberry leaves. Thus, it appears that the patterning of herbaceous vegetation associated with hackberry trees is due primarily to allelopathy with the initial inhibition being accentuated by competition.

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