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FACTORIAL ALLELOPATHY: STRESS OF AGING, POSSIBLE MINERALIZATION, NITRIFICATION, AND INDIVIDUALISTIC PRESSURE OF DIFFERENT TREE SPECIES IN A FOREST COMMUNITY

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Abstract

Herbaceous biomass, soil moisture, and soil factors pH, P,N, Ca, K, Mg, and Mn, when compared from under sycamore, hackberry, red oak, and white oak trees were significantly variable. The differential herbaceous growth pattern did not correspond with soil moisture or variability of different soil factors under different tree species. The amounts of available ammonium and nitrate nitrogen were invesely related to the amounts of total nitrogen under all trees. Ammonium nitrogen was always found to be significantly higher than nitrate nitrogen in all cases. Sycamore, hackberry, red oak, and white oak trees exerted differential allelopathic influence on their understoroy vegetation, inhibition of nitrification, and mineralization. All species exhibited greater allelopathic impact with aging stress of the trees. Many phytotoxins were isolated from sycamore, hackberry, red oak, and white oak plant material. Phytotoxins identified were mainly phenolics, flavanoids, hydrolyzable and condensed tannins.

Introduction

A few authors have considered allelopathy at an ecosystem level and have demonstrated that many species can develop ecological niches by exerting their differential allelopathic pressure within a community. Zinke (1962) reported that the patterns of soil properties under single forest trees were due to the differences in the litter of different species. Gersper & Holowaychuk (1971) attributed the soil chemistry variability under different tree species to variable organic litter or to a combination of litter and stem flow. The variable soil chemistry, in turn, will affect the understorey vegetation under different species. Del Moral & Cates (1971), demonstrated that the differential ground-cover composition beneath the canopy of different species was due to the allelopathic factors of the canopy. Lodhi (1976) reported a low productivity of herbaceous species and relatively bare areas under various tree species in the same forest due to allelopathy. Further, the tree species were found to vary in their effects on soil properties (Lodhi. 1977). The variable litter under different species plays an important role in controlling nitrification, mineralization, and soil pH, thus developing various physio-ecological niches, which can determine the variable undergrowth vegetation (Lodhi, 1976, 1977).

Rice (1974) has discussed that the amounts of allelopathic compounds can vary under different stress conditions, and there is some evidence that the aging stress may cause quantitative shifts in phytotoxins. Morgan (1964), Koeppe et al. (1970), and Lehman & Rice (1972) found the concentration of several phytotoxins increased with age in the leaf tissue. However, none of these reports demonstrated any changes in allelopathic behaviour due to the increased amounts of phytotoxins under aging stress.

With this in mind, a study was conducted to determine if differential allelopathic activity of different tree species on understorey vegetation is related to: (1) differences in understorey herbaceous growth, (2) differences in soil mineralization and nitrification, and (3) differences in stress of aging of various tree species.

Materials and Methods

Location and description of study area. – A forest plot was established in St. Louis Country, Missouri. The vegetation was dominated by *Platanus occidenalis* (sycamore), *Celtis occidentalis* (hackberry), *Quercus borealis* (red oak,) *Q. alba* (white oak), and *Ulmus americana* (American elm). The under-canopy vegetation was mainly dominated by *Eupatorium urticaefolium, Symphoricarpos orbiculatus, Elymus canadensis, Parthenocissus quinquefolia,* and *Bromus tectorum.* Nomenclature follows Fernald (1950).

To describe quantitatively the zones of differential reduced growth associated with allelopathic trees (Lodhi, 1976), sycamore, hackberry, red oak, and white oak, ten randomly located quadrats, 0.25 m^2 in area, were clipped under each species in July. Species were separated, oven dried, and weighed.

To determine if the differential herbaceous growth under different tree species was due to the factors not influenced by allelopathy, several physical and chemical analyses were done. Soil moisture was determined during the months of June and July by taking samples at each of two depths (0-15 cm and 15-30 cm). Ten samples at each level were taken under sycamore, hackberry, red oak, and white oak trees. All samples were weighed, oven dried for 48 hr at 100°C, and percent soil moisture calculated on the basis of oven-dry weight of the soil. For chemical analyses, ten soil samples minus litter were collected at the 0-30 cm level under dsycamore, hackberry, red oak, and white oak trees. The soil pH was determined by the method of Piper (1942). Total nitrogen was determined by the macrokjeldahl method of Bremner (1965), and total phosphours was determined by the method of Jackson (1958). Calcium, potassium, magnesium, and manganese were determined by using a Perkin-Elmer Model 303 atomic absorption spectrophotometer, after extraction according to the instruction in the analytical manual supplied with the instrument.

Effects of aging of allelopathic trees on test species. – To determine the effects of aging on allelopathic behavior of sycamore, hackberry, red oak, and white oak trees, germinating brome grass (*Bromus tectorum*) seeds were exposed to extracts from trees of different ages. Brome grass was used as a test species due to its association with the trees and also due to its rapid germination rate. Two age periods, young and old, were selected, represented by May and late September samples, respectively. The plant material collected in May was oven dried for 48 hr at 50°C and stored in air-tight containers in the refrigerator until September. The plant material collected in September was treated in the same manner before futher experimentation.

Ten percent aqueous extracts of May and September leaves of sycamore, hackberry, red oak and white oak were prepared with dried leaves. The leaf extract of each test species was prepared by immersing leaves in hot distilled water (50°C) for 5 min, grinding in a Waring blender for 5 min, and removing the particulate matter by centrifugation.

All extracts were made to 100 ml with distilled water, and pH was adjusted to 5.6. The water potential of all original extracts (stock solution) was determined by thermocouple psychrometry to be -0.5 bar or less. Twenty-five brome grass seeds were germinated in each Petri plate on filter paper saturated with 10 ml of one of the following solutions: (1) control: 90% phosphate buffer pH 5.6, 10% water; (2) test: 90% phosphate buffer pH 5.6, 10% extract solution of May or September plant material of each tree species; or (3) test 2: 90% phosphate buffer pH 5.6, 5% extract solution of May or September plant material of each tree species, 5% water. Seeds were allowed to germinate in the dark at room temperature (25°C) for five days. Germinated seeds were thinned to 10 seeds with longest radicles per Petri plate and were allowed to grow for two additional days. All Petri plates were rinsed with distilled water and resaturated with 5 ml of appropriate solutions of control, test 1, or test 2, before radicles were allowed to grow for additional days. The radicle growth of all tests and control was recorded.

To determine the aging effect of various allelopathic trees on brome grass plants were grown in a sand-vermiculite mixture for two weeks in Hoagland's nutrient solution (Hoagland & Arnon, 1950). Seedlings were transferred to plastic vials containing one of of the following solutions: control: 95% Hoagland's solution, 5% H_2O; test: 95% Hoagland's solution, 5% extract solution of May or September plant material of all individual species. Seedlings were allowed to grow for ten additional days, harvested, oven dired, and weighed.

Identification of phytotoxins. – The procedures used to isolate the compounds were the same as used previously by Guenzi & McCalla (1966), Feeny & Bostock (1968), Lodhi & Rice (1971), and Lodhi (1975). Ten percent aqueous extracts of fresh or air-dried sycamore, hackberry, red oak, and white oak leaves (fruits and leaves in the case of

sycamore) were acidificed to pH 2.0 using HCl, and extracted with 3 half volumes of diethyl ether. Ether and water fractions were evaporated to dryness and were taken up in 5 ml of 95% ethanol and 10-12 ml of distilled water, respectively. These fractions were chromatographed in two dimensions on Whatman No. 1 paper with N-butanol-acetic acid-water (63:10:27v/v/v), BAW, followed by 6% aqueous acetic acid, 6% A.A. The chromatograms were ispected 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 several different solvent systems: BAW, 6% A.A; isopropanol-butanol-water (140:20:60: v/v/v), IBW; isopropanol-ammonia-water (200:10:20v/v/v), IAW. The chromatograms were dipped in various reagents (Rice, 1965) and maximum absorption peaks in 95% ethanol were determined with a Beckman DBG spectrophotometer before and after the addition of 2 drops of 2 N NaOH to the cuvette.

Following Guenzi & McCalla (1966), 10 g air-dried leaves of hackberry and white oak, separately, were ground to pass a 10 mesh screen and hydrolyzed with 250 ml of 2N NaOH in an autoclave for 45 min. The extract was filtered and acidified to pH 2.0 with HCl, and extracted with 3 half volumes of diethyl ether. The ether extract was shaken with 2 half volumes of 5% $NaHCO_3$ and the ether fraction was discarded. The alkaline portion was acidified again to pH 2.0 and reextracted with 2 half volumes of ether. The ether fraction was evaporated to dryness and the residue taken up in 5 ml of 95% ethanol. The toxins present were identified by the procedure described above.

Table 1. Results of field clipping of herbaceous species associated with different tree species. Mean oven dried wt. g/0.25 m^2.

Herbaceous species	Sycamore	Hackberry	Red Oak	White Oak
Eupatorium	1.25	1.09	1.37	1.05
Symphoricarpos	2.27	2.03	1.31[b]	1.06[b,c]
Parthenocissus	0.04[a,b,c]	1.70	0.27[a,b]	0.76[a]
Elymus	1.20	0.74[b,c]	1.71	0.75[a,b]
Bromus	0.06[a,b,c]	0.56[a]	0.51[b]	0.77
Other Species	1.33	1.33	1.46	1.38
Total biomass	6.15	7.45	6.63	5.77[b,c]

[a]Dry weight significantly different at .05 level from the next higher value in the same row.
[b]Dry weight significantly different at .05 level from the second higher value in the same row.
[c]Dry weight significantly different at .05 level from the third higher value in the same row.

Condensed tannis were isolated according to Feeny & Bostock (1968) only from red oak. The extracting producere used was the same as explained above. The condensed tannins were more concentrated in water extract and streaked in second dimension when developed in 6% A.A. Streaks of condensed tannis from several paper chromatograms were eluted with 30% ethanol for 24 hr. The ethanolic solution of condensed tannis was evaporated to reduce the volume and boiled for 5 min with 2:1 ratio of concentrated HCl and n-butanol. A cherry-red color developed in the solution, which was more intense in the butanol layer. The butanol portion was spotted on chromatography paper and developed in one dimension with water-acetic acid-hydrochloric acid (10:30:30v/v/v), WAHC, and maximum absorption peak of the butanol layer was determined with a Beckman DBG spctrophotometer.

Results

The differential effects of allelopathy of previously known phytotoxic tree species were well pronounced in the field conditions (Table 1). The dry weights of various herbaceous species were found to be significantly different when compared under sycamore, hackberry, red oak, and white oak. Further, the total herbaceous biomass under different tree species was also significantly variable (Table 1). Percent soil moisture showed a smiliar trend only at the 0-15 cm level, where moisture was significantly different in many cases when compared under different tree species (Table 2). On the other hand, no

Table 2. Precent soil moisture under different tree species.

Time of soil collection	Level of the soil	Sycamore	Hackberry	Red Oak	White Oak
June	0-15 cm	35.47±1.50	30.91±1.58[a,b,c]	37.13±1.91	36.91±1.04
	15-30 cm	31.35±1.02	28.56±0.47	28.04±0.83	29.88±0.74
July	0-15 cm	39.36±1.60	33.07±1.57[a]	31.98±2.82[b]	30.73±1.51[c]
	15-30 cm	29.07±0.63	28.19±0.11	29.49±1.01	28.39±0.08

[a]Percent soil moisture significantly different at .05 level from the next higher value in the same row.
[b]Percent soil moisture significantly different at .05 level from the second higher value in the same row.
[c]Percent soil moisture significantly different at .05 level from the third higher value in the same row.

Table 3. Comparison of chemical properties of soils under tree species. Each value represnts the average of ten soil samples. Amounts of minerals are expressed as μg/g ± S.E.

Test	Sycamore	Kackberry	Red Oak	White Oak
pH	6.70 ± .23	6.80 ± .08	5.10 ± .07[a,b]	4.90 ± .03[b,c]
Ca	148.28 ± 4.32	125.18 ± 2.93	73.59 ± 5.72[a,b]	54.81 ± 6.31[b,c]
K	137.51 ± 1.80[b]	152.58 ± 2.02	134.89 ± 7.63[c]	143.51 ± 6.23
Mg	296.04 ± 11.33	290.83 ± 12.09	208.17 ± 16.39[a,b]	183.99 ± 29.73[b,c]
Mn	272.94 ± 8.36	297.50 ± 9.71	301.43 ± 13.75	282.91 ± 8.23
N	1690.90 ± 48.63	1620.62 ± 62.39	1480.60 ± 55.00[b,c]	1520.00 ± 71.36[a,b]
P	790.63 ± 27.08[c]	931.01 ± 28.61	834.00 ± 31.33	803.68 ± 19.07[b]

[a]Amounts significantly different at .05 level from the next higher value.
[b]Amounts significantly different at .05 level from the second higher value.
[c]Amounts significantly different at .05 level from the third higher value.

significant differences were found at the 15-30 cm level (Table 2). In general, percent soil moisture was lowest under hackberry trees. On the other hand, total herbaceous biomass under hackberry trees was the highest. Thus, the differences in soil moiture could not explain the differential growth of herbaceous vegetation under sycamore, hackberry, red oak, and white oak trees.

Several soil mineral analyses were found to be significantly variable under different tree species. The amounts of Ca, K, Mg, Mn, N, P, and pH were significantly variable

Table 4. Available soil nitrogen under different tree species. Amounts expressed as μg/g of soil[a].

Soil from	1977			1978		
Underneath	NH_4^+-N[b]	NO_3^--N[b]	total	NH_4^+-N[b]	NO_3^--N[b]	total
Sycamore	6.82	2.42	9.24	7.50	2.10	9.60
Hackberry	6.27	2.44	8.71	7.15	3.17	10.32
Red Oak	16.05	2.19	18.24[c]	14.28	2.83	17.11[c]
White Oak	7.49	1.94	9.43	8.93	1.80	10.73
Pooled Mean	9.15	2.25		8.81	2.53	

[a]Data modified from Lodhi (1977, 1978b).
[b]Amounts of NH_4^+-H significantly different than the amounts of NO_3^--N.
[c]Amounts of NH_4^+-N significantly different than all other values in the same column at 0.5 level.

under different species with the exception of Mn (Table 3). Sycamore and hackberry soils were found to be richer in minerals as compared to red oak and white oak soils. Similarly the soil pH under sycamore and hackberry was slightly acidic as compared to very acidic under red oak and white oak trees. The high and low total biomass trend did not correspond with the trend of soil chemical properties (Tables 1,3). Due to the growing evidence of the role of allelopathy in nitrification and also the importance of available nitrogen an compared to total nitrogen, the NH_4^+-N and NO_3^--N amounts were compared under different tree species (Table 4). The lowest and the highest amounts of total nitrogen under red oak and sycamore, respectively, showed an inverse relationship with the total available nitrogen (Tables 3,4). Further, NH_4^+-N was always significantly higher than NO_3^--N in all cases. Apparently the differential understorey vegetation under major tree species, based on all field and laboratory analyses, was not chiefly due to any of the factors discussed above.

The differential understory growth pattern was chiefly due to allelopathy with its variable activity dependent on different species and age (Table 5). Germination and radicle growth of brome grass seeds were significantly reduced in all test series when

Table 5. Aging factor and effects of osmotically inactive leaf extracts of different tree species on brome grass seed germination and its radicle growth. Germination is expressed as percent of control.

Source	Sample time	Control	Test 1[a]		Test 2[a]	
		Radicle length mm.	Germination	Radicle length mm.	Germination	Radicle length mm.
Sycamore	May	32.0	76.0	23.0	63.0	26.8
	Sept.	32.0	43.0	18.9[c]	78.0	22.6[c]
Hackberry	May	32.0	66.0	21.2	42.0	21.3
	Sept.	32.0	56.0	20.6	49.0	18.8
Red Oak	May	32.0	73.0	23.0	55.0	22.0
	Sept.	32.0	67.0	13.5[b]	38.0	16.4[b]
White Oak	May	32.0	31.0	22.0	66.0	24.8
	Sept.	32.0	53.0	16.8[c]	52.0	22.7

[a]Radicle length of all tests significantly different from control at .05 level.

[b]Radicle length of September sample significantly different from May sample for the same tree species at .05 level or better.

[c]Radicle length of September sample significantly different from May sample for the same tree species at .1 level.

compared with controls (Table 5). The reduction in radicle growth of brome grass due to the September leaf extract was significantly higher than the May extract in most smaples (Table 5). The red oak leaf extract seems to be most potent in its phytotoxicity with the aging factor (Table 5). The age-related accentuated allelopathic behavior of all tree species was even more pronounced against the seedling growth (Table 6). The seedling growth of

Table 6. Aging factor and effects of osmotically inactive leaf extracts of different tree species on brome grass seedlings.

Source	Mean oven dry weight of seedlings, mg. Control	Test[a] May	Test[a] Sept.
Syacamore	154 ±5.4	127 ± 6.8	103 ±9.6[b]
Hackberry	154 ±5.4	131 ± 4.7	114 ±6.8[c]
Red Oak	154 ±5.4	126 ±10.0	98 ±7.4[b]
White Oak	154 ±5.4	119 ± 8.3	105 ±6.2[c]

[a]Dry weight significantly different from control in all cases at .05 level.
[b]Dry weight significantly different from control in all cases at .05 level.
[c]Dry weight significantly different from May samples at .1 level.

brome grass was significantly reduced by sycamore, hackberry, red oak and white oak leaf extracts when compared with controls. Further, the extracts from the September material of all species caused signicantly higher reduction in seedling growth as compared to the growth reduction due to the extracts of the young leaves collected in May (Table 6).

Inhibitors identified from sycamore, hackberry, red oak, and white oak trees were the same as reported previously by Lodhi (1976). Toxins identified from sycamore leaf and fruit aqueous extracts were scopolin, chlorogenic acid, isochlorogenic acid, neochlorogenic acid, caffeic acid, and Band-510 (Table 7). From hackberry leaf aqueous extracts the phytotoxins identified were scopolin and scopoletin; after alkaline hydrolysis of hackberry leaves, additional toxins identified were caffeic acid, ferulic acid, and p-coumaric acid (Table 7). Inhibitors identified from aqueous extracts of red oak leaves wer were scopolin, scopoletin, chlorogenic acid, caffeic acid, ferulic acid, gallic acid, ellagic acid, digallic acid, quercetin, cyanidin and traces of B-resorcylic acid (Table7). Inhibitors from aqueous extracts of white oak leaves were scopolin, quercetin and traces of caffeic acid, and from alkaline hydrolysate of white oak leaves the toxins were gallic acid, ellagic acid, ferulic acid, homovanillic acid, p-OH benzoic acid, salicyl alcohol and caffeic acid. (Table 7). Details of Chromatopathy are not included in the table.

Discussion

Lodhi (1976) reported that sycamore, hackberry, red oak, and white oak trees reduced the rate of herbaceous biomass production when compared with control plots. However, nothing was reported where differential allelopathic effects of forest trees were analyzed in the same forest community. In this study the reduced growth of various herbaceous species and total biomass were found to be significantly variable under all four above-mentioned allelopathic species. Maximum growth was under hackbery followed by red oak and sycamore, with minimum growth under white oak trees. This growth pattern did not correspond with the soil moisture under different reees. Percent soil moisture was significantly lower under hackberry than under the other tree species, whereas the maximum growth was under hackberry trees. Similar patterns have been documented

Table 7. The phenolic acids identified from test species and their maximum absorbtion spectra in 95% ethanol (with or without NaOH).

Phytotoxins	Sycamore	Hackbeery	Red Oak	White Oak	Max. absorption mu -NaOH	+NaOH
Caffeic acid	+	+[a]	+	+[a]	280	265
Ferulic acid	–	+[a]	+	+[a]	284	421
Chlorogenic acid	+	+[T]	+	–	301	370
Gallic acid	–	–	+	+[a]	273	S250
Digallic acid	–	–	+	–	282	245
Ellagic acid	–	–	+	+[a]	255	S280
Neo-chlorogenic acid	+	–	–	–	329	349
Isochlorogenic acid	+	–	–	–	330	377
B-resorcylic acid	–	–	+[T]	–	295,256	300,270
Homovanillic acid	–	–	–	+	282	290,242
p-OH Benzoic acid	–	–	–	+[a]	250	280
Salicyl aclohol	–	–	–	+[a]	277	–
Band 510	+	–	–	–	320	371
p-Coumaric acid	–	+[a]	–	–	282	331
Scopoletin	+	+	+	–	341	391
Scopolin	+	+	+	+	325	345
Quercetin	–	–	+	+	375,257	257
Cyanidin	–	–	+	–	460,558[b]	-

(+) = present, (-) = absent, T = trace, a = alkaline hydrolysate, S = shoulder, b = maximum absorption in n-butanol and compared with the maximum absorption published by Feeny & Bostock (1968)

in many reports dealing with allelopathy (Al-. aib & Rice, 1971; Lodhi & Rice, 1971). Similarly, many soil factors—pH, amounts of Ca, K, Mg, Mn, P, and total N—were significantly variable under sycamore, hackberry, red oak, and white oak trees. In general, sycamore soils represented many significantly higher values, whereas red oak and white oak soils showed many significantly lower values. The amounts of total nitrogen were significantly higher under sycamore and hackberry trees when compared with the oaks. There is growing evidence that the total nitrogen may not represent the amounts of available nitrogen. Further, the available nitrogen in the form of NO_3^--N and NH_4^+-N is highly pH dependent. Alexander (1961) reported that nitrification usually decreases below pH 6.0 and becomes almost negligible at pH 5.0. In the present study a wide range of pH could cause an appreciable shift in the amounts of available nitrogen. The amounts of available nitrogen showed an inverse relationship with the total nitrogen under different trees. Red and white oak soils low in total nitrogen were high in available nitorgen, and in all soils NH_4^+-N was always significantly higher than NO_3^--N under each tree species. Obviously, the larger amounts of NH_4^+-N in all cases could not be due to the pH fator alone. Also, the remarkably large amounts of NH_4^+-N in red oak soils did not correspond to the lowest pH. Therefore, the inhibition of nitrification is not due to pH but chiefly due to the intact vegetation. Lodhi (1978b) reported that the soils associated with sycamore, hackberry, red oak, and white oak trees were very low in *Nitrosomonas* and *Nitrobacter* and ther low numbers showed a direct relationship with the low amounts of NO_3^--N and an inverse relationship with the signicantly large amounts of NH_4^+-N. Rice & Pancholy (1972, 1973, 1974) reported significant increases in NH_4^+-N, and significant decreases in NO_3^--N and nitrifying bacteria as succession progressed. They inferred, therefore, that the climax vegetation inhibits nitrification. Lodhi (1979) reported the inhibition of nitrifying bacteria and nitrification in variously aged spoil-soils, and compared to the unmined climax area.

The inhibitors of nitrification are basically polyphenols which are released from the vegetation (Basaraba, 1964; Rice, 1965; Rice & Pancholy, 1972, 1973, 1974; Lodhi, & Killingbeck, 1980). Sycamore, hackberry, red oak, and white oak produced many phenolic compounds including condensed and hydrolyzable tannis. Lodhi (1978a) isolated many of the phenolics and tannins from the soils associated with sycamore, hackberry, red oak, and white oak and they were always in large enough quantities to inhibit nitrification (Rice & Pancholy, 1973, 1974).

The low amount of cations under the oaks could also be due to differential allelopathic inhibition of nitrification under various tree species. The positively charged ammonium ion could compete with other cations to attach onto negatively charged soil colloidal micelles. Such a situation will not only prevent the leaching of NH_4^+-N below the rooting zone, but may increase the leaching of cations (K^+, Ca^{++}, Mg^{++}) from the top soil. Further, the nitrate ions being negatively charged are repelled from the soil particles, thus leaching with the cations from the top soils or washed away in surface runoff. Bor-

mann et al. (1968) found with clear cutting, concentration of NO_3^--N, Ca^{++}, Mg^{++}, Na^+, and K^+ in runoff water rose several fold.

The inhibition of germination, radicle growth and seedling growth due to allelopathy has been documented (Neill & Rice, 1971; Rasmussen & Rice, 1971; Lodhi, 1975, 1976, 1978a). However, no reports were found where the variation in phytotoxicity was discussed due to the age stress. Osmotically inactive leaf extracts of sycamore, hackberry, red oak, and white oak from September and May samples significantly inhibited the seed germination and radicle growth of brome grass. The inhibition due to September samples was significantly higher than the inhibition caused by May samples, in most cases. Further, the same inhibitory effects by September extracts as compared to May extracts were more pronounced against the brome grass seedlings. There is some evidence that young, actively growing leaves resist leaching and old leaves approaching sensecence are very susceptible to leaching. Such a stiuation will provide a large nutrient pool for rapid metabolic activites during tissue development. The metabolic activities in turn will produce large amounts of secondary waste products (toxins) which will continue to increase with increasing physiological age.

Muller (1966), Whittaker & Feeny (1971), and Rice (1974) strongly suggested that phytotoxins are the secondary waste substances of various metabolic activities. Morgan (1964), Koeppe et al. (1970), and Lehman & Rice (1972) reported that several phytotoxins increased in quantities with increasing age in sunflower. Feeny & Bostock (1968) reported a variation in such phytotoxins in *Quercus robur* L. throughout its growing season. Several compounds appeared only after the initial growth period and continued to increase until late September. Condensed tannins showed a great variation of 0.5 percent in April to 5.0 percent of leaf dry weight in September. In this study, tree species initially may have had relatively smaller quantities of such phytotoxins, which gradually increased with age through September. Red oak leaves, in addition to many other phytotoxins, also contained condensed tannins. Even though no quantitative analyses were done, a very small amount of leaf material, relatively, produced large quantities of condensed tannins which visibly formed a pellet in a test tube.

The toxins isolated from different tree species are known to inhibit the growth of associated species in several ways: inhibition of nitrifiers (Rice, 1965; Rice & Pancholy, 1972, 1973, 1974; Lodhi, 1977, 1978b, 1979; Lodhi & Killingbeck, 1980), interference with enzyme systems (Gortner & Kent, 1958, Sequeira, 1964), inhibition of photosynthesis (Einhellig et al., 1970; Lodhi & Nickell, 1973). Obviously, such metabolic waste products would continue to accumulate in soils, due to annual decay cycling (Lodhi, 1978a). The continuous presence of such phytotoxins would exert its pressure on the associated organisms in the surrounding vicinity. Further, species producing different phytotoxins in various quantities during seasonal aging in the same ecosystem will modify

phytotoxins in various quantities during seasonal aging in the same ecosystem will modify the influence of phytotoxins and may develop many physioecological niches.

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