THE EFFECTS OF WATER EXTRACTS FROM *JUGLANS NIGRA* L. ON THE GERMINATION OF NATIVE DECIDUOUS TREE SPECIES

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ABSTRACT OF THESIS

THE EFFECTS OF WATER EXTRACTS FROM JUGLANS NIGRA L. ON THE GERMINATION OF NATIVE DECIDUOUS TREE SPECIES

Juglans nigra L., black walnut, produces an allelopathic effect on other plant species. Seed from eight deciduous tree species were used to test the allelopathic effects of water extracts from Juglans nigra leaves and hulls on germination, timing of developmental stages, and survival of seedlings.

After dormancy was broken, the seed were planted in wooden flats containing a 50-50 mixture of washed sand and sphagnum moss and maintained under continuous fluorescent lighting. Four treatments of a given species were placed side by side in a plot, and four replica plantings were made of each species. Water extracts prepared from dry leaves, fresh leaves, and dry hulls of Juglans nigra were used to treat three flats in each plot of each species. Seed germination and seedling growth of these eight species were not affected by the extracts.

To check for short term effects, the early stages of germination of Liquidambar styraciflua L., Platanus occidentalis L., and Robinia pseudoacacia L. were investigated. Seed were treated with water extracts from fresh leaves or dry hulls of Juglans nigra and cultured in petri dishes in a controlled environment. Four dilutions were prepared of both extracts, and the seed were soaked in the extracts or placed on filter paper soaked in the extracts. Four replicas of this experiment were conducted. As germination began, the germinating seed of each species were counted. A least-squares analysis for a four factor factorial design plus control was used to analyze the data. The method of treatment, source of extracts, and dilution of extracts affected the germination rate of each species in a separate fashion. The effects on the germination rate in these species were temporary. Although many researchers have reported that black walnut is allelopathic to herbaceous plant species, pine and apple, the water extracts used in this investigation did not have long lasting effects on either germination or seedling growth of the eight deciduous tree species tested.

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INTRODUCTION

Interactions between species at the community level are complex. Competition for light, water, and minerals limits the number of individuals and species of higher plants in the community. In addition to the above limiting factors, Bonner (6), Börner (8), Garb (20), Muller (34, 35), Tukey (49), Whittaker and Feeny (53) and Woods (55) report that higher plants also limit the growth of others by releasing chemical substances.

This phenomenon, allelopathy, was demonstrated in both cultivated and wild species in communities from rain-forests to deserts (53). The allelopathic influence of species in these communities was not limited to any particular class or family (20, 55).

Black walnut, Juglans nigra L., was reported to produce allelopathic effects on other species (5, 9, 10, 11, 23, 24, 29, 43, 45, 50). The primary interest of these studies involved species of agricultural importance. The present study was devised to determine whether water extracts of black walnut exhibit an allelopathic influence on seed germination of black walnut and seven other tree species native to the deciduous forest.

LITERATURE REVIEW

Allelopathic substances are found in all plant organs and belong to the group of compounds called secondary plant substances (20, 52, 53, 55). These substances do not affect the cells in which they are produced for they are stored as glycosides, polymers, or crystals (52, 53). They may also be deposited outside the living cells in intercellular spaces, dead cells, or glandular hairs on the surface of the plant (52).

Liberation of allelopathic substances into the environment may occur by leaching of above ground parts (48, 49, 53), by volatilization from leaves (34, 40, 49, 52), excretion by roots (55), or decay of plant parts (13, 25, 52).

Al-Naib and Rice (2) and Lodhi and Rice (27) reported that decaying leaves, leaf leachates, and soils collected under sycamore, Platanus occidentalis L., and hackberry, Celtis laevigata Willd., significantly reduced germination and seedling growth of associated herbaceous species in Oklahoma. Rain wash from the leaves and leaf litter of Eucalyptus camaldulensis Dehnhardt was found to inhibit the growth of herbaceous species in California (18). Fog drip from the leaves of Eucalyptus globulus Labill also exhibited this phenomenon (17). Mergen (30) found a water soluble substance in Ailantus altissima

(Mills) Swingle which stopped the growth of pine and birch seedlings.

The California chaparral consists of several species of deep rooted shrubs which lack an understory of herbaceous plants (31, 37, 42). When the aerial portions of the shrubs are destroyed by fire, herbaceous species grow profusely in the burned over area until the shrubs regenerate themselves (31, 37, 42). Salivia species and others produce volatile germination and growth inhibitors (33, 34, 36, 37, 38, 39, 41). Water soluble substances, leached from living plant surfaces and leaf litter of Adenostoma fasciculatum Hook, and Arn., Arctostaphylos species, and other chaparral shrub species, prevent germination and growth of the herbaceous species (31, 37).

Gray and Bonner (22) reported that substances leached from dried leaves of a desert shrub, Encelia farnosa Gray, inhibited the growth of tomatoes, peppers, and corn, but did not affect the growth of barley, oats, sunflowers, and Poa seedlings growing in nutrient sand culture. An extract, prepared by soaking fresh E. farosa leaves in water for 12 hr, inhibited the growth of tomato seedlings in aqueous nutrient culture (22). Bennett and Bonner (4) demonstrated the presence of water soluble toxins in another desert shrub, Thamnosma montana Torr. and Frem. Mullerand Muller (40) and Muller (32) extracted toxic stubstances from dried leaves of the above species and Franseria dumosa Gray. Leaf samples were dried on

newspapers in a well ventilated room. The dried leaves were crushed and soaked in distilled water for 12 hr before filtering to remove the plant tissue. These extracts caused the wilting and death of tomatoes and desert herbs growing in aqueous nutrient culture (32, 40). Yardeni and Evanari (56) worked with fresh and ground dry leaves of four woody species: Myrtus communis L., Eucalyptus rostrata Cav., Laurus nobilis L., and Pinus halepensis Mill. Fresh or ground dry leaves of each species were soaked in distilled water for 12 hr and filtered to remove the leaves (56). These extracts, poured on filter paper in petri dishes containing 50 spring wheat seeds each, inhibited germination (56). Selleck (46) perpared aqueous extracts from dried whole plants of small everlasting, Antennaria microphylla Rydb., by grinding the plants and soaking them in distilled water for 48 hr. Several concentrations were prepared from this extract and used to treat 200 seed samples of spring wheat, Triticum vulgare L., smooth brome, Bromis inermis Leyss., wild mustard, Brassica kaber L., and crested wheatgrass, Agropyron cristatum L., in petri dishes on felt. Distilled water served as the control treatment. These extracts reduced the percent germination of these species.

Some species of higher plants exhibited both allelopathic and autotoxic properties. Chaparral shrubs reproduce only following a fire which removes the adult shrubs. This indicates that these shrubs are autotoxic

to their own seed (31, 37). Curtis and Cottam (13), working with Helianthus clones in Wisconsin, reported that flowering and growth of plants in the center of the clones were significantly reduced due to the presence of autotoxic decay products from the rhizomes. Johnson grass, Sorghum halepense (L.) Pers., was found by Abdul-Wahab and Rice (1) to produce water soluble compounds and decay products which inhibited germination and growth of its own seedlings and other weed species in early stages of old-field succession in Oklahoma. Wilson and Rice (54) demonstrated the production of allelopathic and autotoxic substances by Helianthus annuus L. Water extracts of various plant organs, decaying leaves, root exudates, leaf leachates, and soils collected from around H. annuus inhibited germination and seedling growth (54) as did Johnson grass (1). Bonner and Galston (7) noted a depression in the growth of young guayule plants watered with nutrient solutions leached from mature guayule plants growing in sand cultures. This autotoxic effect explains the wide spacing of this desert plant (6, 7). Keever (25), studying old-field succession in the piedmont of North Carolina, found that decaying horseweed, Leptilon canadense L., contributed to the rapid decline of horseweed and asters by inhibiting their seed germination and growth (25).

Some research indicates that black walnut produces a toxic material in its roots. Cook (11) noted that tomatoes wilted within the root spread of black walnut

trees. Massey (29) planted tomatoes within the root spread of a black walnut tree along one side of a garden. The tomatoes within the root spread began to die in two months; potatoes also were affected while beans, beets, and corn were not. Root contact between the dead tomatoes and the walnut tree was observed. Tomatoes grown in soil or aqueous nutrient solution containing pieces of walnut root were stunted in growth and wilted (29). Alfalfa was replaced by timothy within the root spread of other black walnut trees (29). Smith (47) indicated that black walnut could improve pastures on poor soils by excluding weeds and favoring useful grass species. When alfalfa and tomato seed were placed on strips of black walnut, apple, and sumac root as well as paper towels, Brown (10) observed inhibition of seed germination and seedling growth on the strips of walnut root. Perry (43), Schneiderhan (45) and von Althen (50) reported the death and inhibition of apple trees and pine trees growing near black walnut trees.

Brooks (9) surveyed 300 black walnut trees at least 12 inches in diameter at breast height in West Virginia, Virginia, Ohio, and Michigan to determine the effects of standing black walnut trees and their products on woody and herbaceous vegetation. He presented three tables listing the tree, shrub and vine, and herb species growing under each of 300 walnut trees. He did not list the number or age of the individuals of each species, but he did note the percent of the walnut trees under which each species

grew. Although a majority of the trees surveyed were in open fields and pastures, he did not consider the effects of grazing. Brooks found seven of the eight species used in the present study growing under 0.7 to 10.3% of the 300 walnut trees in his investigation. However, Brooks concluded, "This study strongly supports the assumption that active antagonisms exist between black walnut and many other plants."

Bode (5), working with two year old black walnut seedlings and tomatoes, indicated that a toxic substance was washed from the leaves of black walnut to the soil. He planted tomato seedlings with black walnut seedlings in pots inside or outside the greenhouse. The rain drip from the black walnut seedlings inhibited the growth of the tomato seedlings. The weight, internodal elongation, and root growth were each reduced by spraying tomato plants with a 1 to 2 mg/l solution of synthetic juglone. The dry black walnut abscised catkins and leaves also inhibited tomato growth when mixed in the soil around the plants. The juglone and tannin content of the rain drip was measured during the summer and was found to decrease from early summer to late fall with a slight increase before leaf fall (5).

Many investigators indicate that the toxic substance in black walnut is juglone, 5 hydroxy-1,4-napthoquinone (5, 16, 23, 24, 29). Davis (16) extracted juglone from fresh hulls and roots of black walnut.

Alfalfa and tomato plants that he injected with the juglone died. Synthetic juglone was equally toxic (16). Perry (44) found that respiration of excised tomato and bean leaves is reduced 50% by a 10^{-4} M juglone solution; however, he notes that intact tomatoes are affected by black walnut while beans are not. Gries (23, 24) reported that juglone was nearly equal to copper in Bordeaux mixture as a fungicide in standard laboratory tests, but when used as a seed protectant, juglone was deleterious to germinating seeds. Daglish (14, 15) determined that juglone in Juglans regia L. occurs as a glycoside of 1,4,5-trihydroxynapthalene. Free juglone and glucose are released by hydrolysis and oxidation. According to Gries (24), drying black walnut hulls causes the disappearance of free juglone in the hull because juglone is inactivated by exposure to oxygen. Lee and Campbell (26) reported the relative juglone content of fresh leaves, hulls, and roots of black walnut in the month of September as 1.23, 6.71, and 7.73 mg/g of dry tissue respectively. The relative concentration of juglone in the leaves and hulls varied between different black walnut trees and with the season (26).

Westfall et al. (51) and Auyong et al. (3) studied the effects of a petroleum ether extract from black walnut hulls on several animal species. Crushed unripe walnuts are used as a means of immobilizing fish to take them from small streams and impoundments (51). Depression of

several fish species was induced by 7 to 15 mg/l of the water soluble fraction of the dried ether extract (52). A purified juglone extract produced sedation of fish at 0.15 mg/l of water (3). Marking (28) reported a 96 hr LC50 value of 27 to 88 ppb for several species of fish exposed to juglone solutions. The effectiveness of the solution decreased with age indicating inactivation of the material (28).

MATERIALS AND METHODS

Seed of eight deciduous tree species were used to test the allelopathic effects of water extracts of *Juglans nigra* L. leaves and hulls on germination, timing of developmental stages, and survival of seedlings. Seed was collected in October, 1971, from individual trees of eight species native to the deciduous forest (Table 1). Individual seed trees were used to reduce the effects of genetic variability. Voucher specimens of these individual trees are on file with accession numbers 03625 to 03640 in the Morehead State University Herbarium. Nomenclature is according to Gleason (21).

Many tree species native to the temperate region exhibit embryo or seed coat dormancy. Seed of the following species, used in the present investigation, exhibit embryo dormancy:

SCIENTIFIC NAME	COMMON NAME
Acer negundo L.	Box elder
Acer saccharum Marsh.	Sugar maple
Juglans nigra L.	Black walnut
Liquidambar styraciflua L.	Sweet gum
Platanus occidentalis L.	Sycamore
Quercus velutina Lam.	Black oak

Robinia pseudoacacia L., black locust, seed has seed coat dormancy. *Quercus alba* L., white oak, seed is physio-

logically active as soon as it matures. Here after common names will be used in this paper.

The U. S. Forest Service has recorded the methods of breaking tree seed dormancy in the Woody-Plant Seed Manual (19). Embryo dormancy is broken by stratifying the seed in moist sand and storing at 0 to 5 C for a time interval specific for each species (Table 1). Seed coat dormancy is broken by scarification of the seed coat. Black locust seed was scarified by soaking the seed for 30 min in concentrated sulfuric acid. The scarified seed was then rinsed in running water for 15 min to remove excess acid.

After dormancy was broken, seed of each species was planted in wooden flats containing a 50-50 mixture of washed sand and sphagnum. This medium bears a low number of soil borne disease causual organisms (19). The number of seed planted in each test varied with the species (Table 1). Sample size used was that recommended in the Woody-Plant Seed Manual (19). Four treatments of a given species were placed side by side in a plot and four replica plantings were made of each species except for three replicas of white oak. These germination tests were conducted in the laboratory under continuous fluorescent lighting.

Black walnut leaves were collected in October, 1971, from young black walnut trees and dried at room temperature. The leaves were crushed and stored at room

Table 1. Seed experiments conducted in the laboratory.

Species	Date Collected	Days Stratified	No. Seed per Flat	Date Planted
Box elder	10/23/71	97	200	3/23/72
Black locust	10/29/71	0	200	5/18/72
Black oak	10/22/71	97	50	3/23/72
Black walnut	10/16/71	157	25	5/19/72
Sugar maple	10/30/71	68	200	3/4/72
Sweet gum	10/22/71	153	300	5/18/72
Sycamore	10/30/71	154	200	5/19/72
White oak	10/15/71	0	50	3/4/72

Table 2. Dates water extracts were applied to experimental plots in the laboratory.

Species	Treatments		
	Dry leaves or hulls		Fresh leaves
	First	Second	
Box elder	3/30/72	4/11/72	6/18/72
Black locust	6/15/72	6/21/72	6/24/72
Black oak	4/13/72	4/26/72	6/24/72
Black walnut	6/15/72	6/21/72	6/24/72
Sugar Maple	3/17/72	3/30/72	6/18/72
Sweet gum	5/31/72	6/15/72	6/24/72
Sycamore	5/31/72	6/13/72	6/24/72
White oak	4/13/72	4/26/72	6/24/72

temperature until used. Hulls were removed from the nuts of black walnut in October, 1971, dried and stored at room temperature until used.

Water extracts were prepared using the methods described by Cox (12). These methods include the following:

1. 15 g of crushed dry leaves were soaked in 300 ml distilled water
2. 30 g of crushed dry leaves were soaked in 300 ml distilled water
3. 30 g of dry hulls were soaked in 300 ml distilled water

After soaking for 16 hr, the above were each filtered through several layers of cheese cloth.

As germination of the eight species began, one flat in each plot was treated with one of the above three water extracts. The extracts were poured on the soil surface. The untreated flat in each plot served as a control. These treatments were each repeated in 6 to 15 days (Table 2).

In June, 1971, germination, rates of development and survival did not vary from control to treatment for any of the four replicas of the eight species. These negative effects prompted me to try extracts of fresh plant material. One hundred grams of fresh, chopped black walnut leaves were placed in 1000 ml of boiling distilled water, left for 16 hr until the extract reached room

temperature and then filtered through several layers of cheese cloth (12). This extract was poured on the soil of each experimental flat in all the plots of each species. The control flats remained untreated.

The following events were used to determine the antibiotic effects of walnut extracts on the eight tree species being investigated:

1. number of seeds germinating
2. delay in the timing of developmental stages
3. number of seedlings surviving

The above end points were the same or slightly different among treatments. Although no effects were noted in these lengthy experiments, water extracts from black walnut may have an early short term effect on germination. An experiment was then designed to test for short term effects.

In the above series of experiments in the laboratory, the first evidence of germination was the pushing of the epicotyl above soil level. To check all stages from the first emergence of the embryo from the seed coat, seed was placed on filter paper in petri dishes and cultured in a growth chamber.

Physiologically active black locust, sweet gum and sycamore seed were selected for these experiments in July, 1972. Seventy-two seed samples of each of these three species were prepared.

Water extracts were prepared from fresh black walnut leaves and dry hulls. One hundred grams of fresh,

chopped black walnut leaves were placed in 1000 ml of boiling distilled water and soaked 16 hr until the water reached room temperature (12). One hundred grams of dry black walnut hulls were placed in 1000 ml of distilled water and soaked for 16 hr (12). Both of the above preparations were filtered through several layers of cheese cloth. Four dilutions of these extracts were prepared as follows:

ml original extract	+ ml of distilled water
12.5	87.5
25.0	75.0
50.0	50.0
100.0	0.0

Four 100 seed samples of each species were treated as listed below:

A. Seed samples soaked 16 hr in the following and placed on filter paper soaked with 4.5 ml of distilled water:

1. distilled water
2. 87.5% dilution of leaf extract
3. 75.0% dilution of leaf extract
4. 50.0% dilution of leaf extract
5. 0.0% dilution of leaf extract
6. 87.5% dilution of hull extract
7. 75.0% dilution of hull extract
8. 50.0% dilution of hull extract
9. 0.0% dilution of hull extract

B. Seed samples placed on filter paper soaked with 4.5 ml of the following;

1. distilled water
2. 87.5% dilution of leaf extract
3. 75.0% dilution of leaf extract
4. 50.0% dilution of leaf extract
5. 0.0% dilution of leaf extract
6. 87.5% dilution of hull extract
7. 75.0% dilution of hull extract
8. 50.0% dilution of hull extract
9. 0.0% dilution of hull extract

Four replicas of the above 18 treatments, a total of 72 plates, were prepared for each species.

The petri dishes were incubated in a Sherer growth chamber under an 8 hr day with a temperature of 26 ± 2 C and a 16 hr night with a temperature of 16 ± 2 C. Relative humidity was maintained between 50 and 70%.

Each petri dish was observed daily. Germination, as evidenced by the radicle emerging from the seed, began on day three. Each day the germinating seed of each species were counted and removed to avoid errors in counting.

Mr. David Muse, the university statistician, suggested that the data collected from this series of experiments be analyzed using a least-squares analysis for a four factor factorial design plus control on each day of the experiment.

RESULTS

Laboratory Experiments

Water extracts of black walnut leaves or hulls were applied to seed of eight deciduous tree species native to the deciduous forest to determine whether water soluble compounds from black walnut affect germination, timing of developmental stages, and survival of seedlings.

After the embryo or seed coat dormancy was broken, the seed were planted in the laboratory under fluorescent lights in wooden flats containing a 50-50 mixture of sand and sphagnum moss (Table 1). As the seedlings of each species began to emerge through the soil, the experimental flats were treated with water extracts from dry hulls or leaves of black walnut. Untreated flats served as controls. These treatments were repeated in 6 to 15 days (Table 2). Since the treated seedlings did not differ from the controls, I decided to try extracts from fresh leaves of black walnut. This extract was applied to the soil in each experimental flat (Table 2). There were no effects which could be attributed to the water soluble substances from black walnut.

The end points of these experiments on each species are recorded in the following tables:

SPECIES	TABLE
Box elder	3
Black locust	4
Black oak	5
Black walnut	6
Sugar maple	7
Sweet gum	8
Sycamore	9
White oak	10

Table 3. Number of box elder seedlings with a given number of leaf pairs surviving 9/20/72.

Treatment (200 seed per flat)	Number Germinated	Number of leaf pairs per seedling										
		1	2	3	4	5	6	7	8	9	10	11
Control	128	0	59	42	9	6	1	1	1	1	0	0
LLLE[a]	135	1	92	40	0	1	0	1	0	0	0	0
HLLE[b]	133	2	61	37	12	7	2	1	1	0	0	0
HE[c]	136	0	79	28	3	0	1	0	0	1	0	0
Control	116	0	41	18	9	6	3	1	0	1	0	0
LLLE	116	0	35	21	12	9	1	2	2	1	0	0
HLLE	136	1	76	20	3	0	1	3	1	1	0	0
HE	117	2	50	18	10	12	0	1	0	0	0	0
Control	135	0	63	34	9	12	5	0	0	0	0	0
LLLE	130	0	65	39	17	6	2	0	1	0	0	0
HLLE	129	0	57	47	11	5	1	1	0	0	0	0
HE	139	0	67	38	6	4	3	0	0	0	0	0
Control	106	3	18	70	2	1	1	0	1	1	1	1
LLLE	132	1	64	32	6	10	1	1	1	0	2	0
HLLE	137	0	25	23	10	3	2	0	1	2	1	1
HE	132	5	45	19	12	4	4	2	1	2	1	1

a. LLLE Low level leaf extract
b. HLLE High level extract
c. HE Hull extract

Table 4. Number of black locust seedlings with a given number of leaves surviving 9/13/72.

Treatment (200 seed per flat)	Number Germi-nated	Number of leaves per seedling														
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Control	57	2	1	1	5	8	7	3	6	4	0	0	0	0	0	0
LLLE[a]	31	3	2	1	4	5	4	3	2	2	0	3	0	0	0	0
HLLE[b]	38	5	0	2	1	1	1	5	3	1	0	1	0	0	0	0
HE[c]	64	1	1	2	5	2	1	0	0	1	0	1	0	0	0	0
Control	32	3	0	4	4	5	3	2	2	5	1	1	2	0	0	0
LLLE	41	2	2	3	4	6	2	3	4	2	1	4	2	1	0	0
HLLE	30	0	0	2	6	2	3	1	3	3	0	2	0	0	0	1
HE	21	7	1	1	1	0	0	0	0	0	1	0	1	1	0	0
Control	91	2	1	1	2	4	2	6	5	10	10	3	6	5	2	1
LLLE	61	4	0	0	3	5	3	5	4	1	3	4	5	1	0	3
HLLE	106	2	1	0	1	1	6	3	8	7	3	3	2	3	1	0
HE	62	5	1	3	2	7	1	4	7	9	4	3	2	0	0	1
Control	158	1	1	1	6	2	8	11	18	18	10	9	4	0	0	0
LLLE	104	3	2	1	4	8	9	12	9	8	4	7	0	0	1	1
HLLE	165	0	0	4	9	9	9	11	11	6	9	7	8	5	1	0
HE	169	1	0	1	5	6	4	7	18	12	10	5	3	2	0	1

a. LLLE Low level leaf extract
b. HLLE High level leaf extract
c. HE Hull extract

Table 5. Number of black oak seedlings surviving 9/20/72.

Treatment (50 seed per flat)	Number Germinated	Number of Survivors
Control	41	40
LLLE[a]	41	38
HLLE[b]	44	40
HE[c]	43	41
Control	42	40
LLLE	43	41
HLLE	44	43
HE	38	27
Control	38	34
LLLE	39	39
HLLE	43	43
HE	39	38
Control	40	38
LLLE	44	41
HLLE	43	43
HE	42	41

a. LLLE Low level leaf extract
b. HLLE High level leaf extract
c. HE Hull extract

Table 6. Number of black walnut seedlings with a given number of leaves surviving 9/13/72.

Treatment (25 seed per flat)	Number Germinated	Number of leaves per seedling											
		5	6	7	8	9	10	11	12	13	14	15	16
Control	14	1	1	2	2	3	1	1	2	1	0	0	0
LLLE[a]	11	1	1	0	3	3	1	1	0	0	0	0	0
HLLE[b]	11	2	2	1	0	1	5	0	0	0	0	0	0
HE[c]	11	0	2	0	3	2	2	1	1	0	0	0	0
Control	14	0	0	1*	2*	1*	4	3	0	0	0	0	0
LLLE	11	0	0	1	1	2	0	1	2	1	0	1	0
HLLE	11	0	0	2	2	4	3	0	0	0	0	0	0
HE	11	0	0	3	1	1	1	1	2	2	0	0	0
Control	9	0	0	0	1	2	2	1	1	0	0	0	0
LLLE	8	0	0	1	2	1	2	1	1	0	0	0	0
HLLE	10	0	1	3	1	2	1	1	0	0	0	0	1
HE	10	0	2	0	2	1	2	1	1	0	0	0	0
Control	15	1	0	2	3	4	3	1	0	0	0	0	0
LLLE	9	0	0	3	0	1	1	0	1	1	0	0	0
HLLE	4	0	1	0	1	0	1	0	1	0	0	0	0
HE	11	0	0	4	2	1	3	1	0	0	0	0	0

a. LLLE Low level leaf extract
b. HLLE High level leaf extract
c. HE Hull extract
* One plant with four shoots

Table 7. Number of sugar maple seedlings with a given number of leaf pairs surviving 9/5/72.

Treatment (200 seed per flat)	Number Germinated	Number of leaf pairs per seedling		
		1	2	3
Control	48	40	0	0
LLLE[a]	42	42	0	0
HLLE[b]	35	35	0	0
HE[c]	35	32	0	0
Control	35	30	0	0
LLLE	38	32	0	0
HLLE	48	48	0	0
HE	39	37	0	0
Control	57	52	0	0
LLLE	37	28	2	1
HLLE	49	47	2	0
HE	26	26	0	0
Control	54	47	1	0
LLLE	40	39	1	0
HLLE	39	32	2	0
HE	46	38	0	0

a. LLLE Low level leaf extract
b. HLLE High level leaf extract
c. HE Hull extract

Table 8. Number of sweet gum seedlings with a given number of leaves surviving 9/20/72.

Treatment (300 seed per flat)	Number Germinated	Number of leaves per seedling				
		1	2	3	4	5
Control	204	8	33	33	19	22
LLLE[a]	169	3	25	56	21	16
HLLE[b]	179	6	32	47	14	31
HE[c]	179	10	30	56	23	21
Control	176	8	30	75	17	20
LLLE	190	0	16	52	34	21
HLLE	196	7	27	50	26	29
HE	145	0	10	30	28	11
Control	174	12	41	19	32	9
LLLE	137	2	17	25	11	18
HLLE	152	0	10	11	11	0
HE	182	1	4	29	10	6
Control	182	10	24	19	37	4
LLLE	198	5	27	48	25	12
HLLE	154	9	10	23	24	17
HE	168	1	14	14	27	4

a. LLLE Low level leaf extract
b. HLLE High level leaf extract
c. HE Hull extract

Table 9. Number of sycamore seedlings with a given number of leaves surviving 9/5/72.

Treatment (200 seed per flat)	Number Germinated	Number of leaves per seedling						
		2	3	4	5	6	7	8
Control	75	0	4	5	5	4	0	1
LLLE[a]	85	0	0	6	27	18	4	0
HLLE[b]	59	0	0	5	4	7	1	0
HE[c]	54	0	0	1	2	0	0	0
Control	81	0	3	23	21	2	1	0
LLLE	87	2	3	12	9	0	0	0
HLLE	75	0	3	11	14	15	17	4
HE	75	0	2	14	20	2	0	0
Control	83	0	7	24	18	1	0	0
LLLE	62	0	2	6	9	2	0	0
HLLE	57	0	4	1	11	0	0	0
HE	75	1	5	13	8	3	0	0
Control	75	0	6	8	7	1	0	0
LLLE	72	0	2	3	1	0	0	0
HLLE	82	0	3	16	10	7	0	0
HE	85	0	3	15	7	8	0	0

a. LLLE Low level leaf extract
b. HLLE High level leaf extract
c. HE Hull extract.

Table 10. Number of white oak seedlings surviving 9/20/72.

Treatment (50 seed per flat)	Number Germinated	Number of Survivors
Control	42	37
LLLE[a]	44	39
HLLE[b]	45	40
HE[c]	38	36
Control	47	44
LLLE	49	47
HLLE	33	32
HE	41	36
Control	25	23
LLLE	45	40
HLLE	46	41
HE	41	32

a. LLLE Low level leaf extract
b. HLLE High level leaf extract
c. HE Hull extract

Growth Chamber Experiment

The first evidence of germination in the laboratory experiments was the emergence of the epicotyl above the soil level. To observe the early stages of germination, physiologically active black locust, sweet gum, and sycamore seed were placed on filter paper in petri dishes and cultured in a growth chamber.

Water extracts of fresh leaves or dry hulls of black walnut were prepared. Distilled water was added to each of these extracts to prepare 87.5, 75.0, 50.0 and 0.0 percent dilutions. Four 100 seed samples of each species were soaked in each of the above eight dilutions or distilled water for 16 hr then placed on filter paper soaked with 4.5 ml distilled water. Four 100 seed samples were also placed in petri dishes on filter paper soaked with 4.5 ml of the above eight dilutions or distilled water.

Germination, as evidenced by emergence of the radicle, began on day three. Each day the germinating seed of each species were counted and removed from each petri dish.

The percent seed germination of each species is recorded according to the method of treatment in the following tables:

SPECIES	TREATMENT	TABLE
Black locust	Soaked seed	11
Black locust	Soaked filter paper	12
Sweet gum	Soaked seed	13
Sweet gum	Soaked filter paper	14
Sycamore	Soaked seed	15
Sycamore	Soaked filter paper	16

Table 11. Daily germination of four replicas of 100 seed samples of black locust soaked in water or four dilutions of water extracts of black walnut leaves or hulls.

		Percent Dilution							
		Leaf Extract				Hull Extract			
Date	Water	87.5	75.0	50.0	0.0	87.5	75.0	50.0	0.0
7/10	18	14	22	24	21	30	27	22	31
	22	37	29	5	21	21	32	19	25
	18	27	29	21	14	27	26	22	23
	22	29	20	17	14	17	17	15	20
Total	80	107	100	67	70	95	102	78	99
7/11	2	8	6	7	2	2	1	12	1
	1	0	1	19	0	12	4	6	10
	4	8	1	1	5	3	9	0	1
	0	8	17	0	5	0	0	1	0
Total	7	24	25	27	12	17	23	19	12
7/12	5	6	3	5	5	2	3	1	0
	3	5	5	6	0	6	3	4	2
	4	4	6	2	0	0	4	6	4
	0	1	4	0	2	3	6	9	4
Total	12	16	18	13	7	11	16	20	10
7/13	11	3	3	0	3	0	2	3	4
	12	0	4	12	6	2	0	2	1
	3	2	4	0	3	4	2	1	1
	6	0	0	2	2	4	1	2	4
Total	32	5	11	14	14	10	5	8	10

Table 12. Daily germination of four replicas of 100 seed samples of black locust on filter paper soaked with water or four dilutions of water extracts of black walnut leaves or hulls.

		Percent Dilution							
		Leaf Extracts				Hull Extracts			
Date	Water	87.5	75.0	50.0	0.0	87.5	75.0	50.0	0.0
7/10	16	15	16	29	23	14	20	15	6
	17	14	13	18	13	12	11	12	5
	20	9	17	18	13	11	19	15	8
	19	3	21	13	10	11	12	10	7
Total	72	41	67	78	59	48	62	52	26
7/11	5	9	6	0	6	13	1	6	4
	11	1	11	4	9	9	14	8	10
	0	9	3	5	8	3	2	1	10
	7	24	4	9	6	8	2	6	10
Total	23	43	24	18	29	33	19	21	34
7/12	8	4	4	10	6	2	5	6	8
	7	8	5	4	5	4	1	1	8
	1	6	2	5	3	8	6	2	9
	2	3	3	7	7	3	12	7	6
Total	18	21	14	26	21	17	24	16	31
7/13	2	9	5	0	1	1	4	4	6
	5	1	4	1	1	9	5	13	1
	2	4	4	0	2	3	1	7	6
	6	1	2	4	7	3	10	2	5
Total	15	15	15	5	11	16	20	26	18

Table 13. Daily germination of four replicas of 100 seed samples of sweet gum soaked in water or four dilutions of water extracts of black walnut leaves or hulls.

		Percent Dilution							
		Leaf Extracts				Hull Extracts			
Date	Water	87.5	75.0	50.0	0.0	87.5	75.0	50.0	0.0
10/9	6	6	2	1	0	5	5	2	7
	2	3	0	5	6	4	3	13	9
	4	4	1	12	3	2	14	10	1
	0	0	3	1	1	3	4	5	13
Total	12	13	6	19	10	14	26	30	30
10/10	23	17	25	16	20	12	18	20	28
	24	20	24	24	24	23	30	26	32
	24	32	20	21	28	20	4	26	11
	5	0	8	20	12	19	8	14	36
Total	76	69	77	81	84	74	60	86	107
10/11	10	14	18	8	17	16	16	13	21
	19	14	22	9	28	7	6	14	8
	13	15	18	8	17	11	12	14	12
	32	0	12	23	29	23	26	16	25
Total	74	43	70	48	91	57	60	57	66
10/12	4	8	10	21	7	8	9	1	6
	11	8	5	8	16	15	12	2	7
	17	7	18	4	9	12	9	13	10
	16	2	16	10	6	19	12	18	7
Total	48	25	49	43	38	54	42	34	30

Table 14. Daily germination of four replicas of 100 seed samples of sweet gum on filter paper soaked with water or four dilutions of water extracts of walnut leaves or hulls.

		Percent Dilution							
		Leaf Extracts				Hull Extracts			
Date	Water	87.5	75.0	50.0	0.0	87.5	75.0	50.0	0.0
10/9	38	34	32	18	36	34	34	37	15
	28	22	16	20	22	42	31	30	5
	32	37	48	25	14	46	42	40	26
	0	0	25	26	18	23	33	22	5
Total	98	93	121	89	90	145	140	129	51
10/10	24	24	15	15	8	17	8	17	10
	11	16	23	28	9	23	13	35	24
	10	10	3	16	13	14	19	19	19
	3	1	11	9	19	12	21	17	26
Total	48	51	52	68	49	66	61	88	79
10/11	5	6	10	5	6	13	4	1	9
	13	11	26	19	22	14	8	8	25
	8	5	4	10	5	6	5	9	15
	10	10	30	10	9	21	6	6	19
Total	36	32	70	44	42	54	23	24	68
10/12	2	9	5	4	7	3	8	3	12
	5	4	7	10	10	7	20	4	5
	9	6	8	12	5	11	2	12	10
	12	6	11	7	6	8	4	8	7
Total	28	25	31	33	28	29	34	27	34

Table 15. Daily germination of four replicas of 100 seed samples of sycamore soaked in water or four dilutions of water extracts of black walnut leaves or hulls.

		Percent Dilution							
		Leaf Extract				Hull Extract			
Date	Water	87.5	75.0	50.0	0.0	87.5	75.0	50.0	0.0
10/9	21	15	13	2	0	27	7	12	23
	7	3	3	0	0	10	21	9	14
	20	4	6	0	0	17	9	25	16
	26	9	2	0	0	15	13	19	4
Total	74	31	24	2	0	69	50	65	57
10/10	4	11	8	2	0	5	11	6	6
	16	7	15	0	0	8	7	10	14
	7	7	8	0	1	8	4	2	1
	7	15	15	0	0	9	6	9	4
Total	34	40	46	2	1	30	28	27	25
10/11	7	2	6	0	0	0	10	7	0
	6	14	5	0	1	4	3	7	7
	3	3	6	0	0	0	0	0	0
	4	0	10	0	0	3	3	3	1
Total	20	19	27	0	1	7	16	17	8
10/12	0	2	0	4	0	2	3	1	5
	1	2	5	2	0	1	0	2	1
	1	0	6	0	0	0	0	1	0
	1	0	5	1	0	0	0	0	0
Total	3	4	16	7	0	3	3	4	6

Table 16. Daily germination of four replicas of 100 seed samples of sycamore on filter paper soaked with water or four dilutions of water extracts from walnut leaves or hulls.

Date	Water	Percent Dilution							
		Leaf Extract				Hull Extract			
		87.5	75.0	50.0	0.0	87.5	75.0	50.0	0.0
10/9	46	20	32	28	23	35	38	33	22
	29	33	25	31	17	10	23	26	0
	32	42	34	23	19	42	31	27	24
	28	21	24	32	22	34	43	36	22
Total	135	116	115	114	81	121	135	122	68
10/10	3	2	2	3	4	0	3	5	5
	1	3	2	9	7	6	1	4	0
	3	4	1	2	3	0	0	1	7
	7	4	2	3	0	3	2	0	5
Total	14	13	7	17	14	9	6	10	17
10/11	0	1	1	1	5	1	0	1	5
	2	3	2	3	1	6	0	2	0
	2	2	1	0	1	1	0	4	0
	0	1	0	3	3	2	0	0	4
Total	4	7	4	7	10	10	0	7	9
10/12	1	0	0	0	2	0	0	1	0
	0	0	0	2	1	0	0	0	5
	0	0	0	0	0	0	0	1	1
	1	0	0	0	0	1	2	0	3
Total	2	0	0	2	3	1	2	2	9

ANALYSIS AND DISCUSSION

Various allelopathic compounds tested at different concentrations inhibit germination and seedling growth at lower concentrations than those that result in death (4, 20). Inhibitors found in dry climate soils decrease the germination of herbaceous species during the rainy season (35). Although some seed may germinate, the seedling growth is reduced (35). As the soil becomes drier and these plants die, a bare zone developes around the plant source of the inhibitor (35, 42).

Water extracts of black walnut leaves and hulls applied to the soil around germinating seed of eight deciduous tree species in our laboratory did not affect the germination, timing of developmental stages or seedling survival. Since many researchers have reported that black walnut is allelopathic to several plant species (5, 9, 11, 43, 45, 50), the effects of water extracts of black walnut were tested on the early stages of germination of black locust, sweet gum, and sycamore seed in petri dishes in a growth chamber.

The data from the growth chamber experiment were analyzed using a least-squares analysis for a four factor factorial design plus control on each day of the experiment. The four factors in the experimental design are the species, the four dilutions of the extracts and the

water controls, the hull or leaf extracts, and soaked seed or soaked filter paper. Comparisons of the germination rate due to each of these factors and interactions involving each of these factors are presented in tables as follows:

1. species - a comparison of the germination rate among the three species.
2. dilution - a comparison of the germination rate in the four dilutions and the water controls.
3. hull-leaf - a comparison of the germination rate of seed treated with hull or leaf extracts.
4. soak-paper - a comparison of the germination rate of soaked seed or seed on soaked filter paper.
5. dilution, hull-leaf - a second order interaction involving the effect of both the source and dilution of the extracts on germination rate.
6. dilution, soak-paper - a second order interaction involving the effect of both source of the extracts and method of treatment on germination rate.
7. dilution, hull-leaf, soak-paper - a third order interaction involving the effect of dilution, source of the extracts, and method of treatment on germination rate.
8. species, dilution - a second order interaction involving the effect of both species and dilution on germination rate.
9. species, soak-paper - a second order interaction

involving the effect of species and method of treatment on germination.

10. species, dilution, hull-leaf - a third order interaction involving the effect of the species, dilutions, and source of the extracts on germination rate.
11. species, dilution, soak-paper - a third order interaction involving the effect of the species, dilution, and method of treatment on germination rate.
12. species, dilution, hull-leaf, soak-paper - a fourth order interaction involving the effect of the species, dilution, source of the extracts and method of treatment on germination rate.

Analysis of Day Three Germination

Table 17 summarizes the analysis on day three of the experiment. The significant fourth order interaction at the 1% level involving species, dilution, source of extract and the method of treatment indicates that interactions of each of these factors affect germination rate. These interactions conceal the effects of the individual factors. The data for each of the factors involved in the significant fourth order interaction are analyzed to determine their individual effect on germination. The species factor offers a reasonable basis for segmenting the full experiment (Tables 18, 19, and 20).

The analyses for black locust (Table 18) and

Table 17. Analysis of variance for seed of all species germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	66781.50	1	66781.50	1330.53**
Species	508.86	2	254.43	5.07**
Dilution	1468.97	4	367.24	7.31**
Hull-Leaf	471.88	1	471.88	9.40**
Soak-Paper	7450.08	1	7450.08	148.43**
Dilution Hull-Leaf	88.43	3	29.48	.59
Dilution Soak-Paper	216.53	4	54.13	1.07
Dilution Hull-Leaf Soak-Paper	3519.34	4	879.84	17.52**
Species Dilution	2600.02	8	325.00	6.48**
Species Hull-Leaf	430.01	2	215.01	4.28*
Species Soak-Paper	11295.45	2	5647.72	112.52**
Species Dilution Hull-Leaf	166.49	6	27.75	.55
Species Dilution Soak-Paper	1498.74	8	186.22	3.71**
Species Dilution Hull-Leaf Soak-Paper	4366.62	8	545.83	10.87**
Error	8131	162	50.19	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 18. Analysis of variance for black locust seed germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	23580.68	1	23580.68	915.26**
Dilution	2498.94	4	624.74	24.24**
Hull-Leaf	11.39	1	11.39	.44
Soak-Paper	1341.39	1	1341.39	52.06**
Dilution Hull-Leaf	5.80	3	1.93	.07
Dilution Soak-Paper	811.34	4	202.84	7.87**
Dilution Soak-Paper Hull-Leaf	351.44	4	87.86	3.41*
Error	1391.25	54	25.76	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 19. Analysis of variance for sweet gum seed germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	17298.00	1	17298.00	293.37**
Dilution	472.25	4	118.06	2.00
Hull-Leaf	240.25	1	240.25	4.07*
Soak-Paper	9900.25	1	9900.25	167.91**
Dilution Hull-Leaf	215.50	3	71.83	1.21
Dilution Soak-Paper	607.03	4	151.76	2.57**
Dilution Soak-Paper Hull-Leaf	432.75	4	108.19	1.83
Error	3814.00	54	58.96	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 20. Analysis of variance for sycamore seed germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	26411.68	1	26411.68	487.48**
Dilution	1097.82	4	274.45	5.06**
Hull-Leaf	650.25	1	650.25	12.00**
Soak-Paper	7503.89	1	7503.98	138.49**
Dilution Hull-Leaf	33.62	3	11.21	.21
Dilution Soak-Paper	287.90	4	71.98	1.32
Dilution Soak-Paper Hull-Leaf	1888.44	4	472.11	8.71**
Error	2925.75	54	54.18	

** Significant difference at the 1 percent level

sweet gum (Table 19) each indicate significant interactions at the 1% level involving dilution and method of treatment. A significant third order interaction involving dilution, method of treatment and source of extract occurs in sycamore (Table 20). These significant interactions make it necessary to further segment the analysis. The method of seed treatment is used as a basis for further segmenting the full experiment.

Table 21 summarizes the analysis for germination rate of soaked seed of all species, and Table 22 summarizes the analysis for germination rate of seed on soaked filter paper. An interaction involving the species and source of extract, significant at the 1% level for soaked seed, indicates that germination of each species varies according to the source of the extracts (Table 21). The effect of the extracts on each individual species could not be determined because of this interaction. An interaction between species and dilution factors, significant at the 1% level, indicates that the germination of each species on soaked paper varied with the dilution (Table 22). Therefore, each species is analyzed according to the method of seed treatment.

Neither dilution nor source of the extract significantly affected germination of soaked black locust seed (Table 23). Source of the extracts and the dilutions did significantly affect the germination rate of black locust seed on soaked paper (Table 24). The germination

Table 21. Analysis of variance for soaked seed of all species germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	16378.70	1	16378.70	582.51**
Species	5865.41	2	2932.70	104.30**
Dilution	166.88	4	41.72	1.48
Hull-Leaf	737.04	1	737.04	26.21**
Dilution Hull-Leaf	158.54	3	52.85	1.87
Species Dilution	612.07	8	76.51	2.72*
Species Hull-Leaf	433.58	2	216.79	7.71**
Species Dilution Hull-Leaf	92.08	6	15.35	.54
Error	2277.50	81	28.12	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 22. Analysis of variance for seed of all species germinated on soaked paper on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	56398.37	1	56398.37	780.43**
Species	4240.80	2	2120.40	29.34**
Dilution	1744.42	4	436.10	6.03**
Hull-Leaf	12.76	1	12.76	.18
Dilution Hull-Leaf	401.25	3	133.75	1.85
Species Dilution	6543.58	8	817.95	11.39**
Species Hull-Leaf	263.27	2	131.64	1.82
Species Dilution Hull-Leaf	564.54	6	94.09	1.30
Error	5853.50	81	72.26	

** Significant difference at the 1 percent level

Table 23. Analysis of variance for soaked black locust seed germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	17689.00	1	17689.00	513.55**
Dilution	310.25	4	77.56	2.25
Hull-Leaf	28.12	1	28.12	.82
Dilution Hull-Leaf	110.63	3	36.88	1.07
Error	930.00	27	34.44	

** Significant difference at the 1 percent level

Table 24. Analysis of variance for black locust seed germinated on soaked paper on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	7084.03	1	7084.03	414.90**
Dilution	297.85	4	74.46	4.36**
Hull-Leaf	101.53	1	101.53	5.95*
Dilution Hull-Leaf	128.32	3	42.77	2.50
Error	461.25	27	17.07	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

rate is greater on filter paper soaked with leaf extract than with hull extract (Table 12). Table 12 shows that the 0.0% dilution of hull extract reduces germination.

Germination of sweet gum seed soaked in hull extracts is greater than seed soaked in leaf extracts (Table 13). The difference is significant at the 5% level (Table 25). Neither dilution nor source of the extract significantly affect germination of sweet gum seed on soaked paper (Table 26). On day three, the germination of sweet gum seed exhibits no evidence of inhibition due to water soluble compounds from black walnut.

Dilution and source of the extracts significantly affect germination of soaked sycamore seed (Table 27). The leaf extract inhibits germination (Table 15), and the dilution factor is significant at the 5% level. The germination rate in the leaf extracts is reduced as the percent dilution decreases (Table 15). The analysis for sycamore seed germination on soaked paper also indicates a significant difference in germination rate in the dilutions of extracts (Table 28). Germination is reduced at the higher concentrations of leaf and hull extracts (Table 16).

Analysis of Day Four Germination

Seed germination on day four is also analyzed. Significant interactions involving species and method of treatment indicate that germination rate of each species varies with the treatment method (Table 29). Therefore,

Table 25. Analysis of variance for soaked sweet gum seed germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	711.11	1	711.11	51.13**
Dilution	44.14	4	11.03	.79
Hull-Leaf	84.50	1	84.50	6.07*
Dilution Hull Leaf	30.56	3	10.19	.73
Error	375.50	27	13.91	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 26. Analysis of variance for sweet gum seed germinated on soaked paper on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	25387.11	1	25387.11	199.35**
Dilution	1035.14	4	258.78	2.03
Hull-Leaf	162.00	1	162.00	1.27
Dilution Hull-Leaf	933.75	3	311.25	2.44
Error	3438.50	27	127.35	

** Significant difference at the 1 percent level

Table 27. Analysis of variance for soaked sycamore seed germinated on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	3844.00	1	3844.00	106.78**
Dilution	426.75	4	106.69	2.96*
Hull-Leaf	1058.00	1	1058.00	29.39**
Dilution Hull-Leaf	109.25	3	36.47	1.01
Error	972.00	27	36.00	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 28. Analysis of variance for sycamore seed germinated on soaked paper on day three.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	28168.03	1	28168.03	389.27**
Dilution	958.97	4	239.74	3.31*
Hull-Leaf	12.50	1	12.50	.17
Dilution Hull-Leaf	19.92	3	6.64	.09
Error	1953.75	27	72.36	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 29. Analysis of variance for seed of all species germinated on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	18834.67	1	18834.67	553.13**
Species	7617.79	2	3808.89	111.86**
Dilution	56.89	4	14.22	.42
Hull-Leaf	24.80	1	24.80	.73
Soak-Paper	190.01	1	190.01	5.58*
Dilution Hull-Leaf	196.85	3	65.62	1.93
Dilution Soak-Paper	43.79	4	10.95	.32
Dilution Hull-Leaf Soak-Paper	55.98	4	13.99	.41
Species Dilution	426.59	8	53.32	1.56
Species Hull-Leaf	121.03	3	40.34	1.18
Species Soak-Paper	537.32	2	268.66	7.88**
Species Dilution Hull-Leaf	38.76	6	6.46	.19
Species Dilution Soak-Paper	377.63	8	47.20	1.39
Species Dilution Hull-Leaf Soak-Paper	133.46	8	16.68	.49
Error	5516.25	162	34.05	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

the method of treatment is analyzed for each species.

The germination rate of black locust (Table 30) and sweet gum (Table 31) differed significantly with the treatment method. Black locust seed germination is significantly less for soaked seed than seed on soaked paper (compare Tables 11 and 12). In contrast, soaked sweet gum seed germination was greater than seed germination on soaked filter paper.

Table 32 summarizes the analysis for sycamore seed germination on day four. Because the dilution and method of treatment interaction is significant at the 1% level, the method of treatment is used to segment the analysis of sycamore seed germination further. Table 33 indicates a significant interaction involving dilution and source of the extracts for soaked sycamore seed. Therefore, source of the extracts is used in a one way analysis of variance to determine the effect of the dilution factor. Table 35 indicates a significant difference in the germination rate in the dilutions of leaf extract; however, Table 36 indicates no significant difference in the germination rate of sycamore seed soaked in hull extracts. The analysis of sycamore seed germination on soaked paper indicates no significant difference in germination rate (Table 34).

Analysis of Day Five Germination

The analysis of the complete experiment on day five is found in Table 37. The significant interaction

Table 30. Analysis of variance for black locust seed germinated on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	150.22	1	150.22	5.75**
Dilution	79.59	4	19.90	.76
Hull-Leaf	17.02	1	17.02	.65
Soak-Paper	118.26	1	118.26	4.53*
Dilution Hull-Leaf	20.17	3	6.72	.26
Dilution Soak-Paper	102.56	4	25.54	.98
Dilution Hull-Leaf Soak-Paper	11.94	4	2.98	.11
Error	1409.25	54	26.10	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 31. Analysis of variance for sweet gum seed germinated on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	22613.56	1	22613.56	338.17**
Dilution	320.32	4	80.08	1.20
Hull-Leaf	126.56	1	126.56	1.89
Soak-Paper	361.00	1	361.00	5.40*
Dilution Soak-Paper	110.99	4	27.74	.41
Dilution Hull-Leaf Soak-Paper	65.62	4	16.41	.24
Error	3611.00	54	66.87	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 32. Analysis of variance for sycamore seed germinated on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	1605.56	1	1605.56	174.80**
Dilution	83.57	4	20.89	2.27
Hull-Leaf	2.25	1	2.25	.24
Soak-Paper	248.06	1	248.06	27.01**
Dilution Hull-Leaf	98.38	3	32.79	3.57**
Dilution Soak-Paper	207.88	4	51.97	5.66**
Dilution Hull-Leaf Soak-Paper	111.88	4	27.97	3.04*
Error	496.00	54	9.18	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 33. Analysis of variance for soaked sycamore seed germinated on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	1508.03	1	1508.03	118.28**
Dilution	267.60	4	66.90	5.25**
Hull-Leaf	13.78	1	13.78	1.08
Dilution Hull-Leaf	189.34	4	47.34	3.71*
Error	344.25	27	12.75	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 34. Analysis of variance for sycamore seed germinated on soaked paper on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	318.03	1	318.03	56.58**
Dilution	23.85	4	5.96	1.06
Hull-Leaf	2.53	1	2.53	.45
Dilution Hull-Leaf	6.84	3	2.28	.41
Error	151.75	27	5.62	

** Significant difference at the 1 percent level

Table 35. One way analysis of variance for sycamore seed soaked in leaf extract germinated on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Within	435.19	3	151.06	18.74**
Between	96.75	12	8.06	

** Significant difference at the 1 percent level

Table 36. One way analysis of variance for sycamore seed soaked in hull extract germinated on day four.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Within	3.25	3	1.08	.08
Between	166.50	12	13.88	

Table 37. Analysis of variance for seed of all species germinated on day five.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	9640.04	1	9640.04	498.18**
Species	4892.33	2	2446.17	126.41**
Dilution	126.76	4	31.69	1.64
Hull-Leaf	2.76	1	2.76	.14
Soak-Paper	141.80	1	141.80	7.32**
Dilution Hull-Leaf	97.85	3	32.62	1.68
Dilution Soak-Paper	101.06	4	24.26	1.30
Dilution Soak-Paper Hull-Leaf	153.52	4	38.38	1.98
Species Dilution	196.92	8	24.61	1.27
Species Hull-Leaf	13.54	2	6.77	.34
Species Soak-Paper	442.62	2	221.31	11.44**
Species Dilution Hull-Leaf	237.17	6	39.53	2.04
Species Dilution Soak-Paper	115.01	8	14.38	.74
Species Dilution Soak-Paper Hull-Leaf	220.54	8	27.57	1.42
Error	3134.75	162	19.35	

** Significant difference at the 1 percent level

involving the species and the method of treatment indicates that the germination rate of each species varies with the treatment method. The data are then analyzed at the level of the species factor (Tables 38, 39, 40).

The method of treatment significantly affects the germination rate of black locust seed (Table 38). The germination rate on soaked paper is greater than germination of soaked seed (compare Tables 11 and 12). The only factor that affects germination rate of sweet gum is method of treatment (Table 39). Soaked sweet gum seed germination rate is greater than seed germination on soaked filter paper (compare Tables 12 and 13). The method of treatment is the only factor that affects germination of black locust and sweet gum seed.

See Table 40 for an analysis of sycamore seed germination. The interaction involving dilution and method of treatment, significant at the 5% level, indicates that the germination rate varies with these factors. The effects of these individual factors can not be determined because of this interaction. An analysis of the method of sycamore seed treatment occurs in Tables 41 and 42. These tables indicate that the germination rate of sycamore seed is not affected by either dilution or source of extracts.

Analysis of Day Six Germination

Analysis of germination on day six indicates that the only difference in the germination rate is due to the species (Table 43).

Table 38. Analysis of variance for black locust seed germinated on day five.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	1343.35	1	1343.35	211.88**
Dilution	6.34	4	1.58	.25
Hull-Leaf	1.26	1	1.26	.20
Soak-Paper	66.02	1	66.02	10.41**
Dilution Hull-Leaf	18.92	3	6.31	1.00
Dilution Soak-Paper	36.01	4	9.00	1.42
Dilution Soak-Paper Hull-Leaf	30.19	4	7.55	1.19
Error	342.25	54	6.34	

** Significant difference at the 1 percent level

Table 39. Analysis of variance for sweet gum seed germinated on day five.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	12773.35	1	12773.35	282.72**
Dilution	335.59	4	83.40	1.86
Hull-Leaf	15.02	1	15.02	.33
Soak-Paper	467.64	1	467.64	10.35**
Dilution Hull-Leaf	276.67	3	92.22	2.04
Dilution Soak-Paper	82.26	4	21.56	.48
Dilution Soak-Paper Hull-Leaf	304.69	4	76.17	1.68
Error	2439.75	54	45.18	

** Significant difference at the 1 percent level

Table 40. Analysis of variance for sycamore seed germinated on day five.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	415.68	1	415.68	63.63**
Dilution	19.01	4	4.75	.73
Hull-Leaf	.0156	1	.0156	.002
Soak-Paper	50.76	1	50.76	7.77**
Dilution Hull-Leaf	39.42	3	13.14	2.01
Dilution Soak-Paper	93.81	4	23.45	3.51*
Dilution Soak-Paper Hull-Leaf	39.19	4	9.80	1.50
Error	352.75	54	6.53	

* Significant difference at the 5 percent level
** Significant difference at the 1 percent level

Table 41. Analysis of variance for soaked sycamore seed germinated on day five.

Source	Sum of Squares	Degrees of Freedom	Mean Squares	F
Mean	367.36	1	367.36	35.46**
Dilution	94.51	4	23.63	2.28
Hull-Leaf	.03	1	.03	.003
Dilution Hull-Leaf	75.34	3	25.11	2.42
Error	279.75	27	10.36	

** Significant difference at the 1 percent level

Table 42. Analysis of variance for sycamore seed germinated on soaked paper on day five.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	93.44	1	93.44	34.56**
Dilution	18.30	4	4.58	1.70
Hull-Leaf	.12	1	.12	.05
Dilution Hull-Leaf	3.13	3	1.04	.38
Error	73	27	2.70	

** Significant difference at the 1 percent level

Table 43. Analysis of variance for seed of all species germinated on day six.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F
Mean	4169.45	1	4169.45	386.14**
Species	2308.51	2	1154.25	196.90**
Dilution	41.95	4	10.49	.97
Hull-Leaf	5.67	1	5.67	.52
Soak-Paper	39.42	1	39.42	3.65
Dilution Hull-Leaf	7.95	3	2.65	.24
Dilution Soak-Paper	47.90	4	11.97	1.11
Dilution Hull-Leaf Soak-Paper	77.89	4	19.47	1.80
Species Dilution	49.03	8	6.13	.57
Species Hull-Leaf	4.91	2	2.45	.23
Species Dilution Hull-Leaf	68.78	6	11.46	1.06
Species Dilution Soak-Paper	60.26	8	7.53	.70
Species Dilution Soak-Paper Hull-Leaf	56.79	8	7.10	.66
Error	1749.25	162	10.80	

** Significant difference at the 1 percent level

The germination rate of each species varies with the method of treatment, source of the extracts, or dilution of the extracts. By day six the only source of variation in germination rates is the species.

Black locust is not affected by soaking in the extracts on day three, but the germination rate of seed on soaked paper is reduced by the 0.0% dilution of hull extract. After day three the germination rate is affected only by the method of treatment. Soaked black locust seed germinates at a slower rate than seed on soaked paper. By day six the experimental factors no longer significantly affect germination.

Water extracts from black walnut do not inhibit the germination rate of sweet gum seed. On day three, the germination of sweet gum seed is stimulated by soaking in hull extracts; germination rate of seed on soaked paper is not changed significantly. After day three the germination rate is greater for soaked seed than seed on soaked filter paper. By day six the germination rate of sweet gum seed is not affected by the extracts or method of treatment.

Sycamore seed is the most sensitive of the three species to the extracts. On day three the germination rate of seed soaked in the extracts is reduced by the leaf extracts. As the percent dilution decreases the inhibition of germination rate increases. Sycamore seed germination on soaked filter paper is reduced by the 0.0% dilutions

of leaf and hull extracts. On day four the germination rate of soaked seed is reduced in the 50.0 and 0.0% dilutions of leaf extract. On day five the germination rate of soaked sycamore seed is greater than germination of seed on soaked paper. Although the number of soaked sycamore seed germinating in the 50.0 and 0.0% dilutions of leaf extract is low for the entire experiment, the germination rate on day six was not statistically significant.

Water extracts from black walnut leaves and hulls have a short term affect on the germination rate of these three species when applied to the seed. When the extracts are applied to the soil around germinating seed of eight deciduous tree species in this investigation, the effects of the extracts were not observed.

SUMMARY

This study was initiated to investigate the allelopathic effects of water extracts from black walnut, Juglans nigra, on seed germination, seedling growth and survival of eight native deciduous tree species. Water extracts of Juglans nigra leaves and hulls did not alter germination and seedling survival of the seed of the eight deciduous tree species planted in a mixture of sand and sphagnum in the laboratory under continuous fluorescent lighting. If the extracts did have an effect on the rate of seed germination in the soil, the effects were so temporary that they were not observable by the time emergence occurred. To test for possible short term effects, germination tests were conducted in petri dishes to observe early stages of germination. Germination rates of black locust, sweet gum, and sycamore seed varied with the species, method of treatment, source of extracts, and dilution of extracts. The germination rate of black locust seed on soaked filter paper was reduced by the 0.0% dilution of hull extract; soaked seed was not affected. After day three the germination rate of black locust seed was greater on soaked filter paper than soaked seed. The germination rate of black locust seed was not significantly changed by the extracts on day six. Sweet gum seed germination was stimulated by soaking in hull extracts on

day three. After day three the germination rate of sweet gum seed was greater for soaked seed than seed on soaked paper. By day six the germination rate was not significant. Sycamore seed was the most sensitive to the extracts. On day three the germination rate of soaked sycamore seed decreased as the percent dilution decreased, and the 0.0% dilutions of hull and leaf extract reduced the germination rate on soaked paper. On day four the germination rate is reduced by the 50.0 and 0.0% dilutions of leaf extract. Germination rate of soaked sycamore seed on day five was greater than seed on soaked filter paper. The germination rate of sycamore seed was not significantly changed on day six. Water extracts from black walnut affect the germination rate of these three species, but the effect is short term and not severe enough to affect seed germination in the soil.

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