

ALLELOPATHIC EFFECTS OF JUGLONE ON GERMINATION AND GROWTH OF SEVERAL HERBACEOUS AND WOODY SPECIES¹

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Abstract—Laboratory experiments were conducted to determine juglone sensitivity of 16 species (*Trifolium incarnatum*, *Coronilla varia*, *Vicia villosa*, *Lespedeza stipulacea*, *L. cuneata*, *Acer ginnala*, *Caragana arborescens*, *Elaeagnus angustifolia*, *E. umbellata*, *Lonicera maackii*, *Quercus alba*, *Fraxinus americana*, *Liriodendron tulipifera*, *Alnus glutinosa*, *Pinus strobus*, and *P. sylvestris*) being considered for mixed plantings with *Juglans nigra* (black walnut). All species were sensitive to juglone, but seed germination and radicle elongation were less affected than shoot elongation and dry weight accumulation. Seed germination and radicle elongation were affected by juglone in 6 and 11 species, respectively, mainly by the higher concentrations (10^{-3} M and 10^{-4} M). Shoot elongation and dry weight accumulation of all species were affected by juglone; many species were sensitive to concentrations as low as 10^{-6} M. Seedlings of all species were severely wilted and eventually killed by 10^{-3} M juglone, and most were chlorotic and severely retarded by 10^{-4} M juglone. Seedlings inhibited by 10^{-6} M and 10^{-5} M juglone did not show any visible signs of injury. Based on the effects on seedling shoot elongation and dry weight accumulation, the five species found to be most sensitive to juglone were: *Lonicera maackii*, *Lespedeza cuneata*, *Trifolium incarnatum*, *Alnus glutinosa*, and *Elaeagnus umbellata*.

Key Words—Seed germination, radicle elongation, shoot elongation, dry weight accumulation, juglone, *Juglans nigra*, *Trifolium incarnatum*, *Coronilla varia*, *Vicia villosa*, *Lespedeza stipulacea*, *L. cuneata*, *Acer ginnala*, *Caragana arborescens*, *Elaeagnus angustifolia*, *E. umbellata*, *Lonicera maackii*, *Quercus alba*, *Fraxinus americana*, *Liriodendron tulipifera*, *Alnus glutinosa*, *Pinus strobus*, *P. sylvestris*.

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INTRODUCTION

Black walnut (*Juglans nigra*) is the most notorious of allelopathic trees. According to Gries (1943), the first written record of walnut toxicity can be traced back to the first century AD when Pliny and Elder wrote: "the shadow of walnut trees is poison to all plants within its compass." Walnut has been reported to be toxic to a wide variety of organisms, including herbaceous and woody plants (Brooks, 1951; Rietveld, 1979).

The principal chemical responsible for walnut allelopathy is juglone (5-hydroxy-1,4-naphthoquinone) (Davis, 1928). In living plant tissues, a colorless, nontoxic reduced form called hydrojuglone is abundant, especially in leaves, fruit hulls, inner bark, and roots (Gries, 1943; Lee and Campbell, 1969). When exposed to the air or some oxidizing substance from the roots of other plants, hydrojuglone immediately is oxidized to its toxic form, juglone. Rain washes juglone from living leaves and carries it to the soil. It is also released to the soil from decaying leaves and fruits. Little is known about the mode of action of juglone in affected plants; however, Perry (1967) found that juglone restricted respiration of leaf discs of tomato and bean by inhibiting oxygen uptake.

Juglone has been isolated from many plants in the walnut family (Juglandaceae) including black walnut, butternut (*J. cinerea* L.), Persian walnut (*J. regia* L.), Siebold walnut (*J. ailantifolia* Carr.), Manchurian walnut (*J. mandshurica* Maxim.), shagbark hickory [*Carya ovata* (Mill.) K. Koch], mockernut hickory (*C. tomentosa* Nutt.), Caucasian walnut [*Pterocarya fraxinifolia* (Lam.) Spach], and pecan [*C. illinoensis* (Wangenh.) K. Koch] (Thomson, 1971; Graves et al., 1979).

Recent research has shown that mixed plantings of certain nitrogen-fixing species with black walnut can substantially boost the growth and possibly the quality of the walnut trees (Funk et al., 1979a; Van Sambeek and Rietveld, 1981). Other species are being considered as possible cocrops with walnut to diversify vegetation and increase overall yields of products and amenities. However, only a few plant species have been tested for their sensitivity to juglone (Funk et al., 1979b). The laboratory experiments reported here were conducted to assess the allelopathic potential of black walnut juglone to several herbaceous and woody species being considered for cocrops, nurse crops, and cover crops in intensively cultured black walnut plantations.

METHODS AND MATERIALS

The study consisted of two parts: (1) seed germination and radicle elongation in response to several juglone concentrations on two media, and

(2) shoot elongation and dry weight accumulation in hydroponic cultures containing the same juglone concentrations.

The following species were tested:

Herbs—crimson clover (*Trifolium incarnatum* L.), crown vetch (*Coronilla varia* L.), hairy vetch (*Vicia villosa* Roth.), Korean lespedeza (*Lespedeza stipulacea* Maxim.), and sericea lespedeza [*Lespedeza cuneata* (Dumont) G. Don].

Shrubs—ginnala maple (*Acer ginnala* Maxim.), Siberian peashrub (*Caragana arborescens* Lam.), Russian olive (*Elaeagnus angustifolia* L.), autumn olive (*Elaeagnus umbellata* Thunb.), and amur honeysuckle (*Lonicera maackii* Maxim.).

Trees—white oak (*Quercus alba* L.), white ash (*Fraxinus americana* L.), yellow poplar (*Liriodendron tulipifera* L.), European black alder [*Alnus glutinosa* (L.) Gaertn.], eastern white pine (*Pinus strobus* L.), and Scotch pine (*Pinus sylvestris* L.).

Certain species were excluded from some tests because of problems in obtaining seed, stratification, or germination. The pines were omitted from the growth tests because they had been tested previously (Funk et al., 1979b). Seed were collected and stored until needed. Pregermination treatments used were those specified for each species in *Seeds of Woody Plants in the United States* (USDA Forest Service, 1974). Test conditions and germination criteria used were those specified for each species in *Rules for Testing Seeds* (Association of Official Seed Analysts, 1970) and *International Rules for Seed Testing* (International Seed Testing Association, 1976). Seed of all species were germinated with seed coat and cotyledons intact, except for white oak where the basal one-third of each acorn was removed and then germinated standing on end.

Germination and radicle elongation tests were run on two media—blotter paper and soil. The soil used was the "A" horizon from a forested site demonstrated to be excellent for walnut growth. The soil was screened, sterilized with methyl bromide, oven dried, ground, and stored in sealed containers until it was used. The relation between soil matric potential and soil water content was determined so that soil moisture could be maintained at field capacity (0.3 atmosphere) by maintaining total weight at a determined value.

An aqueous 10^{-3} M stock solution of juglone (Sigma Chemical Co.) was prepared by constantly stirring at 40° C for 24 hr; 10^{-4} M, 10^{-5} M, and 10^{-6} M concentrations were prepared by serial dilutions from the stock solution. Tests were run in a Stults laboratory germinator. For each of the 16 species, four trays of 100 seeds each were germinated in each of the four juglone concentrations plus one control (distilled water).

When germination was occurring at a rapid rate, 10 randomly selected germinants were set aside on each tray for measurement of radical elongation.

Radicle length was measured initially and again after radicles in the controls had elongated from 10 to 15 mm, usually from 2 to 6 days.

In the second part of the experiment, seedlings were grown in hydroponic culture to test the effects of juglone on shoot elongation and dry weight accumulation. Seedlings were grown in sand culture to the first true leaf stage and then transferred to half-strength Hoagland nutrient solutions (Hoagland and Arnon, 1938) containing the same juglone concentrations used in the germination tests. A specially designed static solution hydroponic system (Rietveld, 1982) installed in a growth chamber was used for the growth tests. The growth chamber schedule was set for a 16-hr, 30° C day: 8 hr, 20° C night. Each of the four nutrient-plus-juglone solutions and control (nutrient solution) was represented by four 400-ml culture vessels (replications) containing three seedlings each. The locations of vessels were completely randomized in the growth chamber. Solutions were changed weekly. Tests lasted from 4 to 6 weeks, depending on rates of growth and development of treatment effects. Seedling height was measured initially and repeatedly during the tests. Seedling shoot and root dry weights were determined at the end of each test.

Data were analyzed for significant differences by factorial analysis of variance and Duncan's multiple-range test using the 5% significance level.

To evaluate the effect of juglone concentration on the concurrent time and amount of shoot elongation that occurred during the tests, the speed of shoot elongation (SSE) was calculated as follows:

$$\text{SSE} = \sum \frac{\text{increment since last measurement (mm)}}{\text{time since beginning of test (days)}}$$

A large SSE value indicates early, rapid growth, and a low SSE value indicates late, slow growth. SSE was calculated and summed for each seedling for the duration of the test or period it was alive. Mean SSE of the three seedlings in each replication was subjected to analysis of variance and Duncan's multiple-range test using a 5% significance level.

RESULTS

Seed Germination. Juglone significantly affected percent germination in only 6 of the 14 species tested. Germination was inhibited in four species (Korean lespedeza, sericea lespedeza, autumn olive, and amur honeysuckle) by the 10^{-3} M concentration (Table 1). However, in five other instances (Korean lespedeza and ginnala maple on blotter, amur honeysuckle on blotter and soil, and white oak on soil) germination was enhanced by 10^{-6} M or 10^{-5} M juglone. In most species, germination was similar on the two media; however, germination of Korean lespedeza and autumn olive was inhibited on

TABLE 1. GERMINATION (PERCENT) OF SEED OF 14 SPECIES AT 5 JUGLONE CONCENTRATIONS AND ON 2 GERMINATION MEDIA (EACH VALUE IS THE MEAN OF 4 TRAYS OF 100 SEEDS EACH)

Species	Juglone concentration (M) on blotter					Juglone concentration (M) in soil				
	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³
Crimson clover	89	89	91	94	78	76	77	73	80	82
Crown vetch	58	62	58	61	56	68	63	62	63	60
Hairy vetch	78	78	84	79	81	84	84	95	86	86
Korean lespedeza	67b ^a	77ab	81a	66b	31c	58	62	63	66	66
Sericea lespedeza	66a	72a	69a	70a	52b	85a	72bc	77ab	77ab	61c
Ginnala maple	51b	47b	52b	55b	74a	45	52	54	53	53
Siberian peashrub	71	79	85	84	76	83	87	84	80	81
Russian olive	95	90	92	84	83	64	61	53	51	53
Autumn olive	53a	51a	44a	50a	26b	50	47	47	40	53
Amur honeysuckle	75b	92a	61b	63b	43c	63bc	80a	71b	55c	53c
White oak	18	16	24	13	18	72b	83a	65b	63b	71b
European black alder	25	26	25	22	26	23	21	26	27	24
Eastern white pine	79	60	62	49	60	70	67	60	61	63
Scotch pine	27	36	30	31	24	41	34	40	42	38

^aValues within each row (one test consisting of five juglone concentrations on blotter or soil) followed by the same letter are not significantly different at the 5% level. Tests not followed by a letter showed no significant differences.

TABLE 2. MEAN RADICLE ELONGATION (mm) OF GERMINATING SEED OF 15 SPECIES IN PRESENCE OF VARIOUS JUGLONE CONCENTRATIONS ON 2 MEDIA (EACH VALUE IS THE MEAN OF 10 SEEDS ON EACH OF 4 TRAYS)

Species	Juglone concentration (M) on blotter					Juglone concentration (M) in soil				
	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³
Crimson clover	14b ^a	17a	16ab	17a	5c	6	5	4	5	4
Crown vetch	12a	13a	11a	10a	6b	13a	11ab	10bc	12ab	8c
Hairy vetch	14a	13ab	14a	11bc	10c	13a	13a	10b	13a	7c
Korean lespedeza	34a	29b	27b	26b	9c	22	19	10	18	21
Senecio lespedeza	19a	21a	22a	20a	10b	11	12	13	15	11
Ginnala maple	9a	6b	4c	4c	5bc	8a	8a	3b	4b	4b
Siberian peashrub	16	20	17	17	18	21	18	16	23	21
Russian olive	4	4	4	4	2	7	6	8	5	9
Autumn olive	9a	9a	10a	6b	5b	8a	7a	7a	6ab	4b
Amur honeysuckle	8a	6b	6b	8a	0c	8b	11a	6bc	6bc	5c
White oak	36	34	31	32	30	25	24	24	27	24
White ash	14a	13a	8bc	10ab	4c	8	10	9	9	8
European black alder	14	13	11	11	10	11b	12ab	13a	11b	10b
Eastern white pine	12	10	11	10	12	7	8	7	8	6
Scotch pine	21a	13b	17ab	14b	16ab	9	9	9	11	9

^aValues within each row (one test consisting of five juglone concentrations on blotter or soil) followed by the same letter are not significantly different at the 5% level. Tests not followed by a letter showed no significant difference.

blotter paper but not on soil and germination of white oak acorns was much higher on soil than on blotter paper.

Radicle Elongation. Radicle elongation was more sensitive to juglone than was seed germination—radicle elongation for 11 of the 15 species tested was significantly affected by juglone (Table 2). The higher juglone concentrations (10^{-3} M and 10^{-4} M) were responsible for inhibited elongation in 10 of the 11 species affected (crimson clover, crown vetch, hairy vetch, Korean lespedeza, sericea lespedeza, white ash, scotch pine, ginnala maple, autumn olive, and amur honeysuckle). In three instances (crimson clover on blotter, European black alder on soil, and amur honeysuckle on soil) radicle elongation was stimulated by 10^{-6} M or 10^{-5} M juglone. The herbs as a group were more sensitive to juglone than the woody species. Inhibition of radicle elongation was more common on blotter paper than on soil; however, for unknown reasons, elongation was generally lower on soil than on blotter paper.

Shoot Elongation. All seedlings of all species (except hairy vetch) were killed by 10^{-3} M juglone within a few days after exposure, and seedlings of some species were eventually killed by 10^{-4} M juglone (Table 3). Most seedlings were severely inhibited by 10^{-4} M juglone solutions (Table 3). Shoot elongation of six species (crimson clover, sericea lespedeza, white ash, white

TABLE 3. SHOOT ELONGATION (mm) OF SEEDLINGS OF 13 SPECIES GROWN IN AERATED NUTRIENT SOLUTIONS CONTAINING 5 JUGLONE CONCENTRATIONS

Species	Juglone concentration (M)				
	0	10^{-6}	10^{-5}	10^{-4}	10^{-3}
Crimson clover	57a ^d	57a	29b	8c	0c
Hairy vetch	190a	187a	169a	113b	64c
Korean lespedeza	57ab	66a	56ab	42b	8c
Sericea lespedeza	59a	56a	33b	3c	0c
Ginnala maple	102a	85a	89a	41b	1c
Siberian peashrub	77a	69a	27ab	10b	0b
Russian olive	56a	46a	56a	3b	0b
Autumn olive	47a	40a	32a	9b	1b
Amur honeysuckle	70a	44b	42b	7c	1c
White oak	21a	12a	11a	12a	3b
White ash	57a	56a	42b	28c	0d
European black alder	59a	47a	14b	1b	0b
Yellow poplar	67a	76a	71a	24b	2c

^aValues in each row followed by the same letter are not significantly different at the 5% level.

oak, black alder, and amur honeysuckle) was significantly inhibited by the 10^{-5} M concentration, and that of amur honeysuckle was inhibited by both 10^{-5} M and 10^{-6} M juglone. Only severely inhibited seedlings showed visible chlorosis of leaves and stems; seedlings inhibited by 10^{-6} M and 10^{-5} M juglone showed no visible signs of toxicity. Crown vetch was omitted because of its tendency to form a basal crown rather than elongate.

The pattern and degree of juglone effects on speed of shoot elongation were similar to those on total shoot elongation (data not presented). In all species, 10^{-3} M and 10^{-4} M juglone retarded shoot elongation abruptly at the beginning of the test, while the inhibitory effects (if any) of 10^{-5} M and 10^{-6} M juglone occurred gradually during the tests.

Dry Weight. In several species, plant dry-weight accumulation was more sensitive to juglone than was shoot elongation, i.e., more juglone concentration levels were significantly different from the control (Table 4). Dry weight of white oak roots was not significantly affected by juglone. In nearly all of the remaining species, concentrations of 10^{-4} M and greater significantly inhibited dry weight accumulation. In five species (crimson clover, crown vetch, sericea lespedeza, black alder, and amur honeysuckle) concentrations of 10^{-5} M and greater significantly reduced both shoot and root dry weight accumulation. Dry weight of sericea lespedeza and autumn olive shoots and amur honeysuckle roots was significantly reduced by all concentrations of juglone. In three species (Siberian peashrub, Russian olive, and yellow poplar) shoot dry weight of seedlings grown in 10^{-6} M or 10^{-5} M juglone was higher than in the control, but not significantly so. Juglone had no significant effect on the shoot/root weight ratio (data not shown).

DISCUSSION

Every species tested was found to be sensitive to juglone. Because the species and growth processes responded differently to juglone concentration, some means of assessing overall species sensitivity was needed. Therefore, the summary of species sensitivity to juglone shown in Table 5 is based on the following rationale: (1) the number of concentrations that were significantly inhibitory compared to the control is an indicator of species sensitivity; (2) it is unlikely that 10^{-3} M juglone occurs commonly under natural conditions (see below), so the 10^{-3} M concentration was omitted; (3) species sensitivity during the establishment phase depends on whether they were grown from seed or planted; and (4) following establishment, the principal effects (if any) of juglone will be on plant growth. Thus two rankings of species sensitivity are presented—one based on toxicity to seed germination and radicle elongation and one based on toxicity to seedling shoot elongation and dry weight accumulation. The former ranking applies to establishment from seed, while

TABLE 4. SHOOT AND ROOT DRY WEIGHT (mg) OF 14 SPECIES GROWN IN AERATED NUTRIENT SOLUTIONS AS AFFECTED BY JUGLONE CONCENTRATION

Species	Juglone concentration (M)				
	0	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³
Shoot dry weight					
Crimson clover	64a ^a	54a	32b	12bc	7c
Crown vetch	163a	166a	28b	10b	6b
Hairy vetch	51a	52a	36a	17b	11b
Korean lespezeza	34a	31a	18b	25ab	3c
Sericea lespezeza	60a	42b	17c	5d	4d
Ginnala maple	108a	180a	177a	70b	8c
Siberian peashrub	185a	229a	100ab	31b	9b
Russian olive	99a	83a	131a	8b	7b
Autumn olive	98a	54b	34bc	6c	4c
Amur honeysuckle	277a	164ab	108bc	24c	9c
White oak	434a	334ab	256ab	206b	212b
White ash	475a	381a	201a	81bc	25c
European black alder	90a	60a	13b	5b	4b
Yellow poplar	317a	376a	341a	88b	5b
Root dry weight					
Crimson clover	18a	20a	9b	4b	4b
Crown vetch	71a	70a	13b	2b	2b
Hairy vetch	21a	23a	19a	9b	7b
Korean lespezeza	7a	8a	5a	6a	2b
Sericea lespezeza	16a	15a	6b	2b	2b
Ginnala maple	29a	19a	21a	5b	3b
Siberian peashrub	74a	64ab	21bc	7c	1c
Russian olive	12ab	13ab	24a	3b	3b
Autumn olive	17a	14a	10ab	2b	2b
Amur honeysuckle	62a	28b	24b	4b	3b
White oak	425	334	310	340	313
White ash	77a	72a	53a	22b	12b
European black alder	31a	23a	4b	2b	2b
Yellow poplar	84a	87a	67ab	19bc	2c

^aValues in each row followed by the same letter are not significantly different at the 5% level.

TABLE 5. SUMMARY OF NUMBER OF JUGLONE CONCENTRATIONS INHIBITING^a GROWTH PROCESSES TESTED^b

Species	Germination		Radicle elongation		Sensitivity ranking	Shoot elongation	Dry weight		Sensitivity ranking
	Blotter	Soil	Blotter	Soil			Shoot	Root	
Crimson clover	0	0	0	0	5	2	2	6	3
Crown vetch	0	0	0	1	4	c	2	4	—
Hairy vetch	0	0	1	1	3	1	1	3	6
Korean lespedeza	0	0	3	0	2	1	0	2	7
Sericea lespedeza	0	1	0	0	4	2	3	7	2
Ginnala maple	0	0	3	2	1	1	1	3	6
Siberian peashrub	0	0	0	0	5	1	1	4	5
Russian olive	0	0	0	0	5	1	1	3	6
Autumn olive	0	0	1	0	4	1	3	5	4
Amur honeysuckle	0	0	2	0	3	3	2	8	1
White oak	0	0	0	0	5	0	1	1	8
White ash	c	c	1	0	—	2	1	4	5
Yellow poplar	c	c	c	c	—	1	1	3	6
European black alder	0	0	0	0	5	2	2	6	3
Eastern white pine	0	0	0	0	5	c	c	—	—
Scotch pine	0	0	2	0	3	c	c	—	—

^a Differs significantly from control (0 concentration) at the 5% level; 10^{-3} M concentration omitted.

^b Two rankings of species sensitivity to juglone are presented: one based on toxicity to seed germination and radicle elongation, and one based on toxicity to shoot elongation and dry weight accumulation. Species not included in all of the tests are summarized, but their degree of sensitivity is not ranked with the other species.

^c Species not included in test.

the second ranking applies to establishment and subsequent growth of planting stock.

When the 10^{-3} M concentration was not considered, few significant effects of juglone on seed germination and radicle elongation remained. These growth processes were less sensitive to juglone in most species than were shoot elongation and dry weight accumulation. Ranking of the species in order of their seed germination and radicle elongation sensitivity to juglone from most sensitive to least sensitive was as follows (Table 5): ginnala maple > Korean lespedeza > hairy vetch = amur honeysuckle = Scotch pine > crown vetch = sericea lespedeza = autumn olive > crimson clover = Siberian peashrub = Russian olive = white oak = European black alder = eastern white pine. Establishing sensitive species that are normally grown from seed (e.g., Korean lespedeza and hairy vetch) should be avoided near existing walnut trees.

Shoot elongation and dry weight accumulation were growth processes most commonly and conspicuously inhibited by juglone. Ranking of the species according to their shoot elongation and dry weight accumulation sensitivity to juglone from most sensitive to least sensitive was as follows (Table 5): amur honeysuckle > sericea lespedeza > crimson clover = European black alder > autumn olive > white oak > hairy vetch = ginnala maple = Russian olive = yellow poplar > Korean lespedeza > white oak. This ranking of species sensitivity would be more applicable than the seed germination-radicle elongation sensitivity ranking to estimating the compatibility of species coestablished with walnut.

In contrast to the growth inhibition by high juglone concentrations, in some instances low juglone concentrations (10^{-6} M and 10^{-5} M) apparently promoted seed germination and/or seedling growth. The responses were due to the exceptional growth of a few individual seedlings. These seedlings were either more resistant to juglone because of their vigor or were more vigorous because of their immunity to juglone. We reported similar stimulatory responses in previous research on juglone effects on the growth of coniferous seedlings (Funk et al., 1979b).

Data from these short-term tests are useful for comparing species sensitivity to juglone and the relative sensitivity of different growth processes. However, biological assays are insufficient as a means of identifying the occurrence of allelopathy and the concentrations of chemicals responsible for allelopathy in the field (Stowe, 1979). Although natural juglone concentrations have not been measured in the field and are therefore unknown, it is unlikely that 10^{-3} M juglone occurs in soil under field conditions. Aqueous solutions of that concentration were difficult to prepare in the laboratory, and some juglone usually precipitated out in hydroponic culture. Perry (1967) found no inhibition of oxygen uptake in bean or tomato plants from 5×10^{-7} M to 10^{-5} M juglone. Between 10^{-5} M and 3×10^{-4} M, the inhibition rose rapidly with the log of juglone concentration. The results of this experiment

generally agree with Perry's findings, although growth of two species (*sericea* *lespedeza* and *amur* honeysuckle) was inhibited by 10^{-6} M juglone. Thus, the allelopathic effects observed in the field are most likely the result of longer-term exposures to moderate juglone concentrations. The experiments also suggest that moderate and low juglone concentrations may inhibit (or stimulate) growth for a period of time before any obvious symptoms of toxicity appear.

Although every species tested was sensitive to juglone under short-term laboratory conditions, the occurrence of allelopathy under field conditions depends on three factors: (1) the sensitivity of associated species to juglone; (2) size and density of walnut trees; and (3) soil and climate conditions that control the disposition of juglone.

Species undoubtedly vary in their sensitivity to black walnut juglone, as evidenced by Brook's extensive surveys of the association frequencies of 218 species with walnut (Brooks, 1951), and the results of the experiments reported here. Brook's species lists include only four of the species that were tested in this study: white oak, white ash, yellow poplar, and white pine. Although Brooks stated that he found no evidence of antagonism between walnut and those species in natural stands, his tables show that white oak and yellow poplar occur less frequently within the crown projection of black walnut. For planted stands, however, the literature contains several reports of phytotoxicity to white pine (Wiant and Ramirez, 1974), Scotch pine (Schreiner, 1949), and other conifers. Pines are often planted with or near walnut and are especially sensitive to juglone (Funk et al., 1979b; Rietveld, 1981). I know of no published reports of phytotoxic effects from walnut on the other species tested in this study.

The occurrence of allelopathy is distinctly different when species are planted near existing large walnut trees than when even-aged mixtures of species, including walnut, are planted. In the former case, the toxic effects may appear within months or a few years, while in the latter case they may take much longer to appear because the walnut trees must grow to sufficient size and density to have a significant chemical effect on the environment. From a survey of 41 mixed, even-aged walnut plantations (Rietveld, 1981), a buildup period of approximately 12–25 years is needed for walnut trees to reach a sufficient size to produce and release enough juglone to have noticeable allelopathic effects. However, it is unknown when and for how long toxicity occurred before symptoms appeared because moderate juglone concentrations may inhibit growth without visible injury.

The principal factor affecting the length of the buildup period appears to be soil characteristics. The speed and completeness of decomposition and immobilization depend on a variety of factors including soil type, moisture and oxygen content, soil reaction, presence of decomposing organisms, concentration of juglone, and juglone's resistance to microbial attack.

Because sterile soil was used in the germination experiments, the reduced allelopathic effects on soil, although variable, can be attributed to fixation of juglone by soil colloids. In a survey of mixed, even-aged walnut plantations, soil drainage was consistently related to the occurrence of allelopathy (Rietveld, 1981). Toxicity occurred earlier on imperfectly drained soils but occurred later or not at all on well-drained soils.

Fisher (1978) found a strong relation between soil moisture and the allelopathic activity of walnut trees. As soil moisture increased, the amount of extractable juglone and allelopathic activity increased. High soil moisture creates anaerobic reducing conditions unfavorable for the chemical and biological oxidation of juglone, and thus allows juglone to accumulate. Although no direct evidence of microbial breakdown of juglone exists, it seems most probable that it occurs (Fisher, 1978). Soil conditions also influence growth rates and vigor, which in turn determine tree size and juglone production by walnut and are related to susceptibility of associated species to juglone toxicity. Thus, it appears that walnut allelopathy is more likely to occur on imperfectly drained soils where chemical and microbial oxidation is restricted and plant vigor is lower. Allelopathy may not occur at all, even in sensitive species, if they are growing vigorously on well-drained soils.

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