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THE ECOLOGICAL IMPACT OF ALLELOPATHY IN AILANTHUS ALTISSIMA (SIMAROUBACEAE)[1]

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Compounds inhibitory to the growth of neighboring plant species were found in significant concentrations in the leaves and stems of young *Ailanthus altissima* ramets. The surrounding soil also contained appreciable concentrations of similarly acting toxins. Individuals of neighboring plant species have either incorporated active portions of inhibitory compounds or responded to *Ailanthus* by producing growth-inhibiting substances. Under greenhouse conditions, individuals of neighboring plant species previously unexposed to *Ailanthus* in the field were found to be more susceptible to the *Ailanthus* toxins than individuals previously exposed. Moreover, seeds produced by unexposed populations were also more susceptible to *Ailanthus* toxins than seeds produced by previously exposed populations. These differences demonstrated that the allelochemicals of *Ailanthus altissima* exhibited a measurable impact upon neighboring plant species. Since the progeny of these populations displayed a differential response to *Ailanthus* toxin, this phenotypic difference between the two populations may have a heritable basis.

At the center of the debate over the relevance of allelopathy in plant communities is the issue of ecological importance. Although most would agree that some plant species produce noxious compounds detrimental to the growth of other species, it remains to be demonstrated that such compounds are directly responsible for detectable changes in the species composition of plant communities or the genetic composition of associated plant populations (Rice, 1984). Allelopathy has been defined as the production of compounds by one plant species that influences another species (Newman, 1983), usually in a detrimental manner (Rice, 1984). Deleterious influences include insecticidal (Osbourne et al., 1988), herbicidal (Muller and Muller, 1964), and antipredator (Robbins et al., 1987) results. On occasion, allelopathy has been used to describe positive interactions between plant species (Newman and Miller, 1977). Although it has been a relatively straightforward task to produce a plant extract that is toxic to surrounding species (Gliessman, 1983; Mandava, 1985), toxic compounds have not been shown to induce evolutionary changes in natural field settings. Demonstration of an inhibitory activity of a plant extract is not sufficient to indicate an allelopathic relationship, as an average of one in 40 plant species from Washington State USA, for example, produces a water extract that significantly reduces radicle elongation in at least one of three test species (del Moral and Cates, 1971). Since such extracts contain substances and concentrations of substances that are seldom found in soils, these results indicate, at best, a potential for allelopathic interactions.

The tree of heaven, *Ailanthus altissima* Swingle (Simaroubaceae), was introduced to North America from Asia, quickly spread throughout the United States as an ornamental tree in many northern cities (Steyermark, 1963), and is now distributed across much of the northern hemisphere (Good, 1974). As a sapling, *Ailanthus* reproduces vegetatively and ramets rapidly invade abandoned fields, bridge embankments, roadsides, railroadsides, wastegrounds, and other disturbed habitats (Steyermark, 1963). *Ailanthus* was described by Mergen (1959) to be inhibitory under greenhouse conditions to a variety of tree species. Heisey (1990) subsequently produced inhibitory extracts from several tissues of *Ailanthus*. Our research examines the effects of inhibitory compounds produced by naturally occurring individuals of *Ailanthus* on neighboring plant species. By comparing the responses to *Ailanthus* toxins of individuals previously exposed to *Ailanthus* under natural conditions to those of previously unexposed individuals, one can determine if *Ailanthus* has induced detectable phenotypic differences in neighboring plant populations. In addition, examination of the

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offspring of *Ailanthus*-exposed and unexposed populations allows separation of potential genetic components of these differences from phenotypic components resulting from acclimation of these populations to *Ailanthus*-contaminated environments. These studies will begin to address questions surrounding the possible role of allelochemicals in shaping the evolution of plant communities.

MATERIALS AND METHODS

Plants and extracts—Vegetative tissue (leaf and stem) of *Acer saccharum, Ailanthus altissima, Andropogon virginicus, Platanus occidentalis,* and *Teucrium canadense,* as well as seeds of *A. altissima, Dichanthelium clandestinum* (*Panicum clandestinum*), *Eupatorium rugosum, Platanus occidentalis, T. canadense, Tridens flavus, Verbena urticifolia,* and *Verbesina alternifolia* were collected at the Tyson Research Center, Eureka, Missouri. Additional *A. altissima* and *Liriodendron tulipifera* leaves were collected in St. Louis County, Missouri. Individual *T. canadense* plants growing proximally to *Ailanthus* were collected within 1 m of an *Ailanthus* ramet, while distal individuals were collected 10–15 m from the nearest *Ailanthus* ramet. *Lactuca sativa* var. prizehead seeds were purchased from the Hummert Seed Co., St. Louis, Missouri.

Seed germination and seedling growth were carried out over the winter months in a greenhouse with artificial illumination supplemented to provide 16 hr of light per day. Stems of field-collected plants were freshly cut below leaf nodes and Hormex No. 1 rooting hormone applied prior to placement in moist vermiculite. Field-collected seeds were stratified in moist vermiculite at 4 C for 3 to 5 mo prior to germination. Following germination or rooting on a mist bench, the plants received a single application of Peter's Peat Lite Special Fertilizer (nitrogen diluted to 225 ppm) and were watered daily. Plant extracts were applied at least weekly; dosage varied with the species exposed.

Plant tissue extracts were made by immersing freshly picked whole leaf and/or stem material in a minimal volume (usually 3–5 ml per g plant material) of water for 2–3 d at 20 C. Extracts were decanted, passed through Whatman® 3MM filter paper, and stored at 4 C for up to 1 yr. For comparative assays, extracts were normalized for dry weight of plant material per volume eluant. Although other extraction methods exist (Kaminsky and Muller, 1977; Rice, 1984), this procedure more closely reproduced the field conditions responsible for leaching of water-soluble toxins from plants than those involving elevated temperatures, pulverization of plant material, or various organic solvents. When necessary, the eluants were passed through Nalge 0.45-μm filter sterilizers to remove microbial contaminants.

Soil samples—A 36-m linear transect was established through a large *Ailanthus* clone at the Tyson Research Center, Eureka, Missouri. While the ramets themselves were quite young, *Ailanthus* had been present at the site for more than 10 yr and had been sporadically mown. Soil was removed to a depth of 10 cm under the surface debris every 2 m along the transect. The distance to the nearest *Ailanthus* ramet as well as its height and diameter were recorded for the NE, NW, SE, and SW quadrants at each sample point. To facilitate removal of plant material, the soil samples were air dried for several days. Plant debris was removed by passing the soil through a 2-mm wire mesh and by inspection. One hundred ml of distilled water was twice passed through a 400-ml volume of soil, filtered through Whatman 3MM paper, and stored at 4 C for up to 6 wk.

Toxicity bioassay—Two Whatman 3MM filter disks were placed in an inverted 90-mm petri dish and saturated with 5 ml of the appropriate eluant. *Lactuca* seeds were utilized to quantify the inhibitory potential of plant extracts due to their ability to germinate quickly (usually within 1 d) and grow rapidly under laboratory conditions. Twenty to 30 *Lactuca* seeds were scattered on the paper and allowed to germinate at 23 C under ambient light conditions. Additional water was added daily to prevent desiccation. After 4–8 d, the seedlings were stored at −20 C and radicle lengths were measured with Toshiba digital calipers within 1 wk. Both distilled water and *Acer* extract were used as negative controls, and assays were run in triplicate.

RESULTS AND DISCUSSION

Toxicity—*Lactuca* seeds were germinated on water extracts of various Missouri plant species including *Ailanthus altissima* (Table 1). Of the plant species tested, only the extracts from *Ailanthus* tissues inhibited the germination and growth of *Lactuca* relative to a distilled water control ($P < 0.01$, t-test). The fact that the eluants from tissues of other species affected neither germination nor radicle growth of *Lactuca* seedlings indicated that a high concentration of solutes in a leaf leachate of this sort was neither beneficial nor detrimental under our

TABLE 1. *Effect of plant extracts on the germination and growth (radicle length ± SD) of* Lactuca *seedlings germinated on water extracts made from different plant species*

Treatment	Germination (%)	Sample size	Radicle length (mm)
Water	100	50	14.40 ± 3.51
Acer saccharum	100	50	14.25 ± 2.91
Platanus occidentalis	100	50	15.04 ± 3.02
Liriodendron tulipifera	98	50	11.83 ± 2.51
Andropogon virginicus	100	50	14.57 ± 2.55
Ailanthus altissima (leaf)	60	60	3.12 ± 0.86**
A. altissima (stem)	73	73	4.25 ± 0.96**

** Significantly different from water control, $P < 0.01$, t-test.

assay conditions. Figure 1 presents the inhibition of growth of *Lactuca* radicles when exposed to *Ailanthus* extract relative to those exposed to *Acer* extract. After the first 3 d, the rate of growth of *Lactuca* radicles exposed to *Acer* extract (slope = 7.77 ± 0.46, $r^2 = 0.99$) was significantly greater ($P < 0.01$) than the rate of growth of radicles exposed to *Ailanthus* extract (slope = 1.57 ± 0.07, $r^2 = 0.99$). These data exclude the possibility that the small size of *Lactuca* radicles observed in the first experiment (Table 1) resulted solely from an initial delay of normal developmental processes. Rather, *Ailanthus*-exposed seedlings grew consistently slower than unexposed individuals. These results are consistent with those of Mergen (1959) and Heisey (1990), who demonstrated that inhibitory compounds existed in and presumably were produced by the vegetative tissues of young *Ailanthus altissima* plants. Although an assay of this sort may be affected by the availability of toxins to germinating seeds (Weidenhamer, Morton, and Romeo, 1987), similar results were obtained utilizing a larger number of seeds per plate in the *Lactuca* assay, indicating that availability of toxins to any one seedling was not influenced by toxin uptake by surrounding individuals.

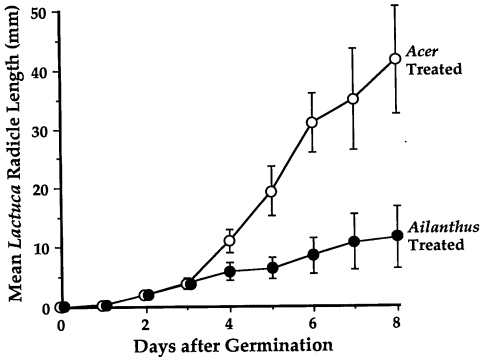

Fig. 1. The inhibitory activity of *Ailanthus* extract relative to *Acer* extract. *Lactuca* seeds were germinated in the presence of either eluant; 20–25 samples in each of three replicate plates were measured and discarded daily. Error bars indicate 1 SD.

To test the influence of *Ailanthus* toxins on native species, seeds were collected from plants growing near or around *Ailanthus* clones and germinated in the greenhouse as described in the Materials and Methods section. When applied to the germinated seedlings, *Ailanthus* extract considerably inhibited growth (Table 2). In addition, germination (50% reduction) and inflorescence number (37% reduction relative to individuals of similar size) were also adversely affected in these species (data not shown). However, while individuals exposed to *Ailanthus* were much smaller than unexposed individuals, as observed for *Lactuca,* the average number of leaf pairs per plant did not significantly differ between exposed and unexposed seedlings (Table 2). These data suggest that plants exposed to *Ailanthus* were following normal patterns of development but were smaller due to the presence of *Ailanthus* toxins. The timing of developmental events, such as the production of leaf-pairs, had not been affected.

The toxins in *Ailanthus* could operate in at least two different ways. The magnitude of the inhibitory effect may be proportional to the concentration of the toxins in the environment. Alternatively, inhibition of germination and growth may be evident only if the toxins were present in concentrations greater than some threshold amount. Above this level, the magnitude of inhibition would be independent of environmental concentration. Figure 2 demonstrates the increased inhibitory activity of *Ailanthus* extract with increasing concentration. After 4 d growth, both the germination of the *Lactuca* seeds ($r^2 = 0.79$) and radicle length of the seedlings ($0.91 < r^2 < 0.99$ for various logarithmic and semilogarithmic transformations) were significantly correlated with the concentration of *Ailanthus* extract in the applied eluant. These data do not indicate a threshold concentration at which the *Ailanthus* compounds become inhibitory; rather, inhibition of germination and growth increase with concentration. Therefore, even moderate

TABLE 2. *Effect of plant extracts on the germination and growth (height ± SD) of field-collected plant seeds*

Species	*Acer* extract	*N*	*Ailanthus* extract	*N*	*t*
	Mean seedling height (cm)				
Ailanthus altissima	57.39 ± 7.49	50	55.23 ± 7.08	50	1.15
Eupatorium rugosum	57.70 ± 7.76	80	14.48 ± 4.63	80	42.73**
Dichanthelium clandestinum	5.31 ± 1.07	50	3.52 ± 1.11	50	8.15**
Platanus occidentalis	2.21 ± 0.31	31	1.10 ± 0.22	8	11.46**
Teucrium canadense	4.48 ± 1.55	50	1.05 ± 0.41	8	14.44**
Tridens flavus	14.53 ± 1.76	50	7.48 ± 4.41	80	12.77**
Verbesina alternifolia	11.72 ± 2.21	25	2.09 ± 0.84	10	18.67**
Verbena urticifolia	2.56 ± 0.46	30	0.73 ± 0.21	30	19.84**
	Mean number of leaf pairs				
T. canadense	4.50 ± 0.57	50	4.55 ± 0.50	11	1.06
V. alternifolia	3.84 ± 0.37	25	3.60 ± 0.49	10	1.40
V. urticifolia	2.87 ± 0.43	30	2.87 ± 0.43	30	0.00

** *Ailanthus* and *Acer* treatments are significantly different, $P < 0.001$, *t*-test.

concentrations of *Ailanthus* toxins in the soil may have a physiological effect on neighboring plant species. Moreover, inhibition of germination and radicle growth appear to be substantially different. While the inhibition of seed germination exhibits a linear relationship with toxin concentration, the inhibition of radicle growth is more properly described by nonlinear functions. While many physiological explanations are possible, these relationships suggest that either a single compound exhibits more than one mode of action, or multiple compounds are responsible for *Ailanthus*' inhibitory effects.

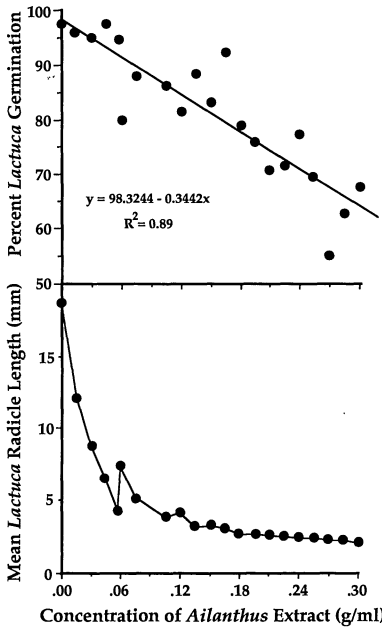

Fig. 2. Effect of concentration on the toxicity of *Ailanthus* extract. Twenty to 30 *Lactuca* seeds in each of three replicate plates were germinated with various concentrations of *Ailanthus* extract. Final radicle lengths were measured after 4 d growth.

Field transect—Possible modes of transmission of toxins from *Ailanthus* to neighboring individuals include 1) the action of precipitation in the leaching of toxins from leaves and stems to the ground below, 2) the leaching of toxins from decaying root material and fallen stems and leaves, and 3) seepage of toxins from roots and shoots into the soil. By all methods, neighboring individuals could be exposed to toxins present in the soil. When quantified by the *Lactuca* bioassay described in Materials and Methods, the toxicity of *Ailanthus* tissue is substantially reduced during periods of high rainfall and moderate temperatures (Table 3). Correspondingly, periods of little precipitation and elevated temperatures result in a substantial increase of toxicity of *Ailanthus* tissues. These data are consistent with the models of toxin transmission described above and suggest that precipitation may contribute to the mobilization of the inhibitory compounds present in *Ailanthus* tissues. Alternatively, volatile inhibitors of plant growth have been described (Elmer, 1932; Muller and Muller, 1964; Muller, Muller, and Haines, 1964) and have been found in the soil (del Moral and Muller, 1970). Since volatile allelopathic compounds may be most effective under arid conditions (Rice, 1984), these data are also consistent with this model of transmission.

To detect inhibitory compounds, soil samples were collected along a 36-m linear transect through an *Ailanthus* clone, and eluants of the soil samples were prepared as described in Materials and Methods. Figure 3 summarizes the average characteristics of the *Ailanthus* ramets present along the transect. The ramets were large and dense at the center of the clone and

TABLE 3. *Relationship between the toxicity of* Ailanthus *tissue and environmental variables*

Year	Mean daily rainfall (mm)[a]	Mean maximum temperature (C)	Drought index[b]	*Ailanthus* toxicity[c]
1988	1.18	29.21	6.14	High
1987	3.09	28.79	3.34	Moderate
1989	2.46	26.23	0.52	Low

[a] Environmental variables were recorded daily at the Tyson Research Center, Eureka, MO. Data from 1 May to 30 September were averaged for each year.

[b] The drought index is a function of average daily temperature and mean daily rainfall, formulated for prairie environments (Walter, 1973). Values above 4.0 indicate harsh drought conditions. Values of 8–10 represent the harshest droughts on record.

[c] *Ailanthus* tissues were collected from several locations from 1 May to 30 September of each year, and the toxicity of extracts was measured by the *Lactuca* assay. Toxicity was measured as the inhibition of *Lactuca* radicle growth as follows: High: 0%–15% normal growth; Moderate: 30%–60% normal growth; Low: 80%–100% normal growth.

small and scarce toward the edge. We conclude that the density of *Ailanthus* tissue is greatest at the center of the clone (transect point 0), and predict that the levels of *Ailanthus* toxins in the soil there should be higher. A correlation between the increased local density of *Ailanthus* tissue and an increased inhibitory activity of the immediately surrounding soil would demonstrate the presence of an *Ailanthus*-associated toxin in the soil.

Figure 4 presents the inhibitory activity of extracts of soil samples as functions of the average characteristics of the immediate *Ailanthus*. The correlation between inhibitory activity of the soil samples and the average diameter of the nearest *Ailanthus* ramets was significant ($r^2 = 0.69$, $P < 0.05$). Since the diameter of an *Ailanthus* ramet is proportional to its biomass, the inhibitory activity of the soil extracts is correlated to the density of *Ailanthus* tissue in any given area. While there were individually significant correlations between the inhibitory activity of the soil samples and other measures of the local density of *Ailanthus* tissue, significant improvement was not seen by the inclusion of these additional variables into a multivariate model ($r^2 = 0.77$). While some researchers have speculated that plant toxins may not be present in the soil in sufficient quantities to inhibit surrounding plants (Kaminsky, 1981), we conclude that *Ailanthus* toxins were present in the soil at this site in sufficient concentrations to detrimentally affect the growth of neighboring plant species. Since the inhibitory effects of *Ailanthus* compounds decrease with lower concentrations (Fig. 2), the allelopathic impact of *Ailanthus* would be limited to its immediate environment. (Transect point 0 was excluded from the analysis due to aberrant behavior [Fig. 4], and some foreign substance may have been present in the soil sample that had influenced

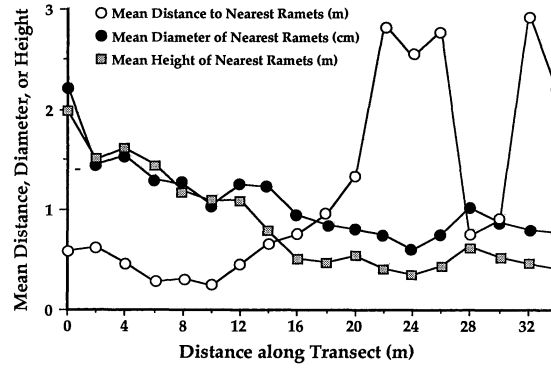

Fig. 3. Characteristics of nearest *Ailanthus* ramets growing in the NE, NW, SE, and SW quadrants at each point along a 36-m linear transect through a young clone.

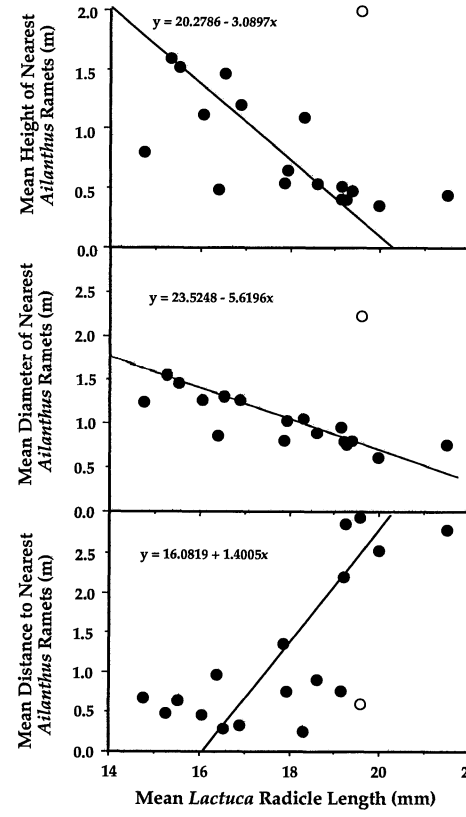

Fig. 4. Toxicity of soil along the transect described in Fig. 3. *Lactuca* seeds were germinated in the presence of extracts made from soil collected along the transect. Radicle lengths were measured after 4 d growth. Radicle length is plotted against the mean height of, diameter of, and distance to the nearest *Ailanthus* ramets from that point. The unfilled markers represent the transect 0 data points (see text).

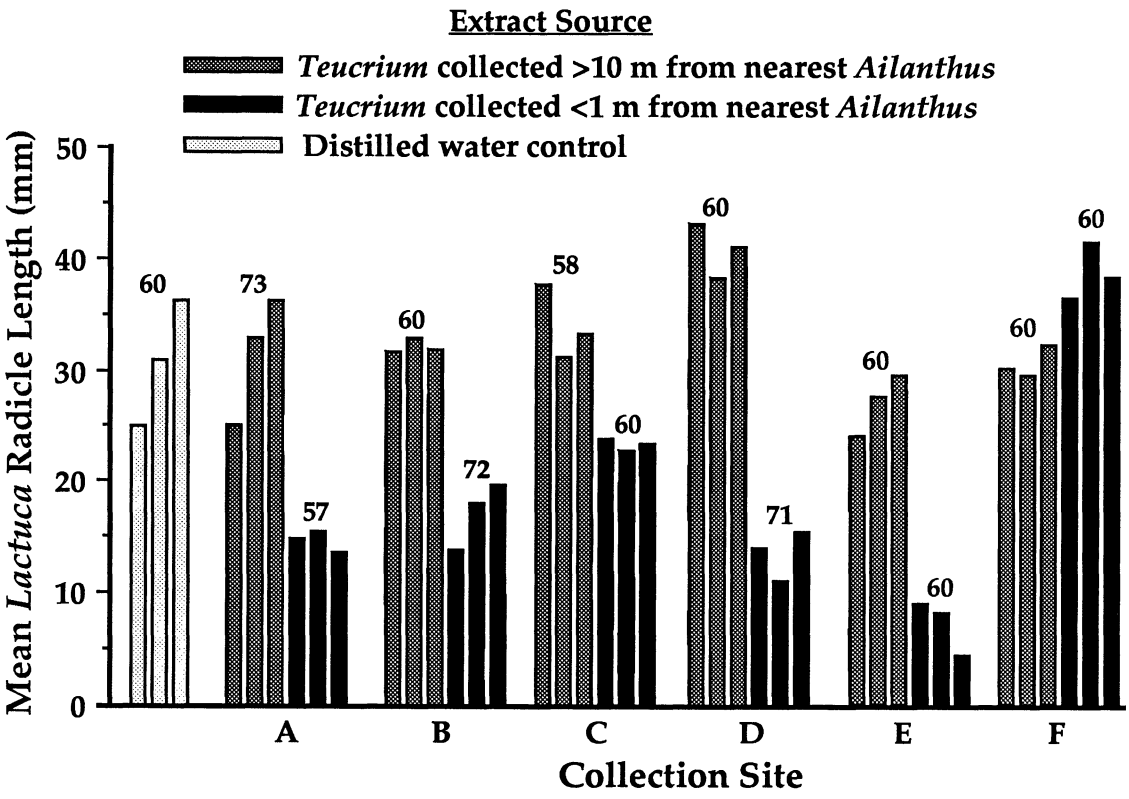

Fig. 5. *Ailanthus*-induced toxicity of *Teucrium canadense*. Extracts were made as described from samples of *Teucrium* growing proximally (<1 m) and distally (>10 m) to *Ailanthus* clones. *Lactuca* seeds were germinated in the presence of these extracts; radicle lengths were measured after 4 d growth. Numbers above groups indicate sample sizes; bars represent independently treated replicates.

the assay. While the elimination of this sample improved correlation [with all data points, $r^2 = 0.50$], the exclusion of no other data point similarly improved the model.)

Transmission of the toxic compounds—To demonstrate the uptake of *Ailanthus* toxins from the soil by neighboring plants, paired samples of *Teucrium canadense* growing proximally (<1 m) and distally (>10 m) to *Ailanthus* clones were collected from six sites. Analysis of the field transect indicates these distances should place each paired sample inside (<1 m) and outside (>10 m) *Ailanthus*' sphere of inhibitory influence. To minimize other differences in local environment, the paired samples from each site were collected no more than 20 m apart. Extracts were made from individuals representative of each *Teucrium* population and their inhibitory activities were assayed on *Lactuca* seeds as described in Materials and Methods. The data presented in Fig. 5 indicate that the plants growing in *Ailanthus*-contaminated soil contain concentrations of inhibitory compounds not present in plants growing in an adjacent *Ailanthus*-free environment ($P < 0.05$). We conclude from this correlation that the toxic principle of *Ailanthus* has been transmitted to *Teucrium* through the soil. Although unlikely, *Ailanthus* toxins may have stimulated *Teucrium* to produce detectable levels of inhibitory compounds. If this is the case, then the toxicity of the neighboring *Teucrium* reflects an environmental stress associated with *Ailanthus*. In either case, *Teucrium* individuals have responded to the presence of *Ailanthus* in their environment. In addition, while the uptake of at least portions of a variety of toxic compounds from the soil has been demonstrated using radiolabel incorporation studies (Blackman et al., 1959; Foy, 1961; Glass and Bohm, 1971; Shann and Blum, 1987), our methods suggest that if *Ailanthus* toxins were incorporated in the *Teucrium* individuals, it exists in an active state following uptake and is possibly sequestered in the recipient plant's tissues. It should be noted that these individuals were collected following the first precipitation after several months of above-average temperature (August 1988, see Table 3). There-

TABLE 4. *Inhibition of seedling growth of* Lactuca *germinated in the presence of standardized water extracts made from* Ailanthus *leaves collected from ramets and trees of various sizes*

Ailanthus circumference (cm)	Inflorescence	*Lactuca* radicle length (mm)		
		N	Mean	SD
2.8	Absent	60	6.0**	1.3
2.8	Absent	60	3.3**	0.6
2.9	Absent	60	3.7**	0.6
4.2	Present	60	34.9	3.6
6.3	Present	60	32.3	3.9
14.5	Present	60	31.4	3.5
18.2	Present	60	36.3	3.9
33.3	Present	60	33.5	4.5
61.0	Present	60	31.9	3.6
Water control		60	31.0	3.0

** Significantly shorter than water control, $P < 0.001$, *t*-test.

fore, the concentration of toxins in the soil and the rate of water uptake by *Teucrium* individuals may have been unusually high, leading to unusually high exposure of these plants to *Ailanthus* toxins.

The sample of *Teucrium* growing adjacent to *Ailanthus* that did not demonstrate significant toxicity (sample F in Fig. 5) was collected beneath a mature *Ailanthus* tree with few young ramets growing in the vicinity. The data suggest that *Ailanthus* toxins were present in lower concentrations in the soil at this site. Moreover, extracts prepared from whole *Ailanthus* fruits did not demonstrate any inhibitory activity (data not shown), and the adult *Ailanthus* tissue did not produce the strong odor characteristic of younger plants. These results led us to believe that mature trees produced lower concentrations of inhibitory compounds than invading ramets. To test this relationship, leaves were collected from *Ailanthus* saplings and trees of various sizes. Extracts were made and inhibitory activities were assayed on *Lactuca* seeds as described in Material and Methods. The data suggest that while nonflowering ramets produce significant concentrations of inhibitory compounds, leaves from mature trees do not (Table 4, $P < 0.001$). While Heisey (1990) produced toxic extracts from tissues of mature trees, including seeds removed from the fruits, the levels of inhibition of these extracts were not compared to those of extracts made from saplings.

Our data support the hypothesis that young, invading *Ailanthus* clones produce greater amounts of inhibitory compounds than adult trees. Once established, there may be less advantage to produce allelopathic compounds for competitive or antipredator purposes, and their biosynthesis may be interrupted. Alternatively, the inhibitory compounds may interfere with the development of *Ailanthus* seeds. While we have not directly tested this hypothesis, the germination of *Ailanthus* seeds and growth of *Ailanthus* seedlings were not affected by the presence of extremely high concentrations of *Ailanthus* toxins derived from yearling ramets (Table 2). Moreover, while there were not detectable concentrations of toxins in *Ailanthus* fruits, *Ailanthus* seedlings produced significant concentrations of inhibitory compounds immediately following germination. These data do not indicate that the development of young *Ailanthus* plants is influenced by its own toxins.

Differential response of neighboring species to Ailanthus toxins—Although *Teucrium canadense* individuals are exposed to and possibly take up *Ailanthus* toxins, it has not been demonstrated that the toxins have had any ecological or evolutionary impact on natural populations of *Teucrium.* This would be accomplished by eliciting a differential response to the *Ailanthus* toxins by *Teucrium* individuals that had been previously exposed to the toxins relative to previously unexposed individuals. If previously exposed plants were more tolerant of *Ailanthus,* these individuals would represent a population altered by exposure to toxic soil. The populations growing beyond the influence of *Ailanthus* clones would not have experienced this selection. Such a difference would reflect either acclimation of *Ailanthus*-exposed individuals to the toxins or an underlying genetic difference between exposed and unexposed populations.

Although an attractive possibility, competitive exclusion in itself does not demonstrate allelopathy. While early studies of species distributions implied allelopathic interactions (Culpepper, 1633; see also Rice, 1984), modern studies generally look beyond species distribution studies as evidence for allelopathy. From observation of several *Ailanthus* clones, it is apparent that some species have been excluded from *Ailanthus*-infested areas. However, it is not clear that *Ailanthus*-produced toxins are responsible for the distribution of plant species in these regions. While the *Ailanthus* compounds may be responsible, other factors such as shading, water availability, or nutrient concentrations may contribute to the spatial distribution of these species. These factors are not relevant when examining populations of the same species growing proximally and distally to *Ailanthus* clones. By comparing the responses of individuals growing proximally to *Ailanthus* clones to those of distally located individuals, one can determine if *Ailanthus* has

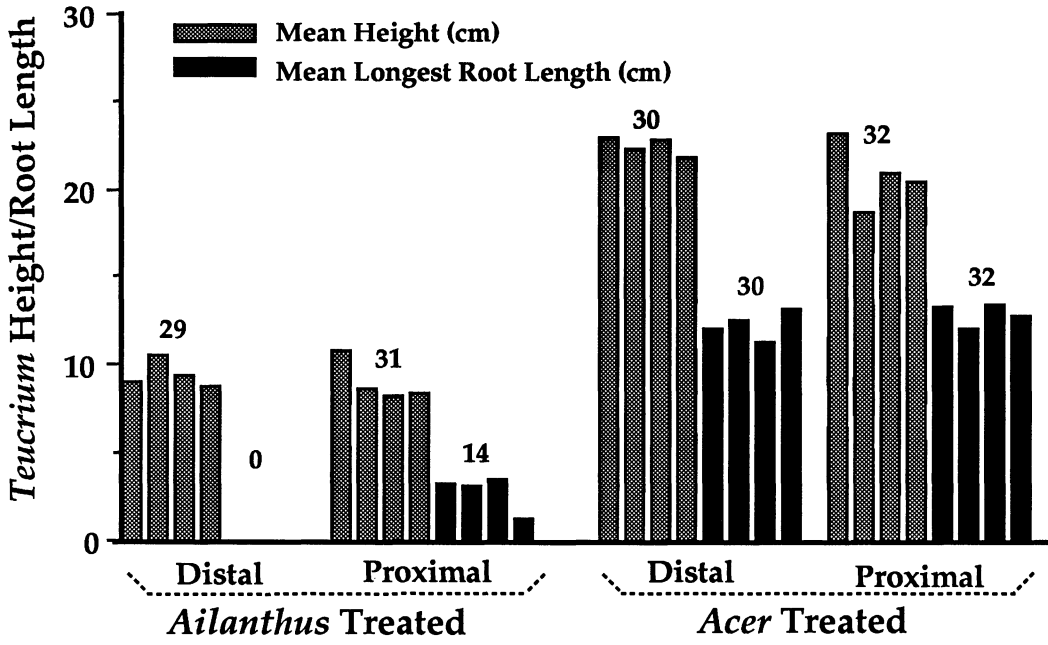

Fig. 6. Cuttings of *T. canadense* growing proximally (<1 m) and distally (>10 m) to an *Ailanthus* clone were rooted in the presence of extracts from either *Acer* or *Ailanthus*. Heights and root lengths were measured after 3 wk growth. Numbers above groups indicate sample sizes; bars represent independently treated replicates.

induced detectable phenotypic differences in these populations. In this manner, investigation is focused on the effect of plant toxins on a neighboring plant species independent of additional environmental variables.

To elicit this differential response, individuals of *Teucrium canadense* growing proximally (<1 m) and distally (>10 m) to *Ailanthus* were collected, rooted in the greenhouse as described in Materials and Methods, and exposed to either an *Ailanthus* or *Acer* extract. After 3 wk, proximal and distal plants exposed to *Acer* extract did not differ significantly in height or root length (Fig. 6). In contrast, when exposed to *Ailanthus* toxins in the greenhouse, 45% of the individuals previously exposed to *Ailanthus* sprouted roots; roots were absent from previously unexposed individuals (Fig. 6). When *Teucrium* was allowed to grow roots prior to exposure to plant extracts in the green-

TABLE 5. *Differential response (seedling height ± SD) of seeds collected from plants growing proximally (<1 m) or distally (>10 m) to* Ailanthus *ramets to plant extracts*

Sample	Extract	Seedling height (cm)				
		Proximal	*N*	Distal	*N*	*t*
Dichanthelium clandestinum	*Acer*	5.31 ± 0.18	50	5.38 ± 0.71	4	0.179
	Ailanthus	3.52 ± 1.11	50	3.93 ± 0.60	4	1.189
	Reduction	33.7%		27.0%		
Teucrium canadense (Population 1)	*Acer*	4.42 ± 0.95	50	4.83 ± 1.55	50	1.597
	Ailanthus	1.63 ± 0.46	11	1.05 ± 0.41	8	2.89**
	Reduction	63.1%		78.3%		
Teucrium canadense (Population 2)	*Acer*	4.97 ± 1.19	50	5.32 ± 1.19	50	1.47
	Ailanthus	2.37 ± 1.17	23	1.02 ± 0.38	5	4.57**
	Reduction	52.3%		80.8%		
Teucrium canadense (Population 3)	*Acer*	11.66 ± 3.12	42	10.85 ± 2.76	27	1.13
	Ailanthus	4.57 ± 2.71	71	1.43 ± 1.15	61	8.90**
	Reduction	60.8%		86.8%		
Tridens flavus	*Acer*	12.91 ± 1.91	50	14.53 ± 1.77	50	4.85**
	Ailanthus	8.70 ± 2.63	80	7.48 ± 4.41	80	2.14**
	Reduction	32.6%		49.5%		

** Proximal and distal populations are significantly different, $P < 0.01$, *t*-test.

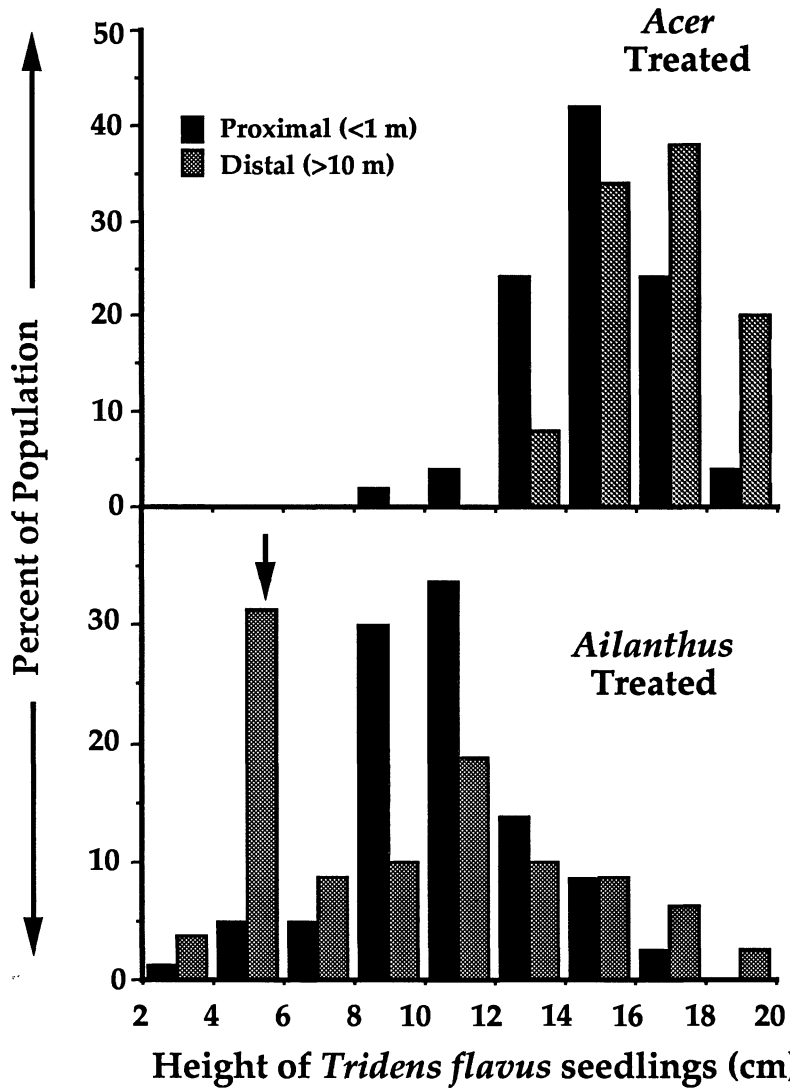

Fig. 7. *Tridens flavus* seeds were collected from plants growing proximally (<1 m) and distally (>10 m) to *Ailanthus* ramets. Seeds were stratified, germinated, and exposed to *Ailanthus* or *Acer* extracts. The arrow designates highly susceptible individuals (see text).

house, *Teucrium* previously exposed to *Ailanthus* in the field were significantly larger than *Teucrium* previously unexposed to *Ailanthus* ($P < 0.05$, data not shown). In both cases, populations previously exposed to *Ailanthus* were more tolerant of *Ailanthus* toxins than previously unexposed populations. We conclude that there is a phenotypic difference between these two populations correlated with the presence of *Ailanthus altissima* in the environment. This phenotypic difference may be due to an underlying genetic difference between the two populations induced by the selective pressure of *Ailanthus* toxins, or it may be an expression of phenotypic plasticity, indicating an acclimation of these individuals to the *Ailanthus* toxins. To distinguish genetic change from phenotypic plasticity, it is necessary to test the responses of seeds collected from the two populations to *Ailanthus* toxins. If the progeny of individuals from the proximal and distal populations also exhibit differential responses, it is likely that there is a genetic difference between these two populations, barring carryover of maternal factors within the seed. If differential responses are not evident, the previously observed phenomenon was likely to have been the result of acclimation of the *Teucrium* individuals to the presence of *Ailanthus* in the environment.

Seeds of *Dichanthelium clandestinum, Teucrium canadense,* and *Tridens flavus* were col-

lected from fruiting plants growing proximally (<1 m) and distally (>10 m) to *Ailanthus* clones, stratified, and germinated in the greenhouse as described in Materials and Methods. Following germination, *Ailanthus* extract was applied. For all samples except *D. clandestinum,* seeds from plants previously unexposed to the *Ailanthus* toxins were more adversely affected than seeds from previously exposed plants (Table 5). These results imply that the seeds of individuals previously exposed to *Ailanthus* are, on average, better suited for growth in *Ailanthus*-contaminated environments. In addition, it is clear that this differential response is not a phenotype resulting from previous exposure of the individual to the toxin. Since the response is correlated with the exposure of the maternal plant to *Ailanthus,* genetic factors are indicated. Figure 7 presents the corresponding data for *Tridens flavus.* Although both proximal and distal populations are inhibited by *Ailanthus* toxins, the distal population includes a class of highly susceptible individuals not present in the proximal population (designated by the arrow in Fig. 7). The lack of these highly susceptible individuals from the proximal population of *Tridens* is the primary factor contributing to the statistical difference between these the two populations. Presumably, individuals bearing this phenotype are less suited for growth and reproduction in the proximal environment and are selected against by the allelopathic compounds. Correspondingly, the genotypes responsible for that phenotype would be removed from the gene pool of the proximal population. In conclusion, *Ailanthus* present in the environment is apparently responsible for altering the genetic makeup of certain neighboring plant species. Other species, such as *Dichanthelium clandestinum,* may remain unaffected by *Ailanthus* toxins due to physiological factors or properties of the surrounding populations, such as population structure, neighborhood size, genetic variability, and rate of gene flow.

In summary, we have demonstrated the presence of compounds in *Ailanthus altissima* that inhibit the germination and growth of associated plant species. These compounds are present in the soil in detectable concentrations and are transmitted to individuals of neighboring plant species. These plants may have incorporated active portions of the *Ailanthus* inhibitory compounds. Individuals growing adjacent to *Ailanthus* produce progeny that are, on average, better suited for growth in *Ailanthus*-infested environments than progeny of previously unexposed plants. These data indicate that the allelochemicals produced by *Ailanthus altissima* ramets have tangible effects on neighboring species. In addition to influencing the spatial distribution of plant species, allelochemicals may provide an environmental stress that contributes to genetic change within associated plant populations.

Although *Ailanthus* may significantly influence surrounding plant populations, this inhibition may not yield a competitive advantage. Quite apart from the existence of an allelopathic interaction remain questions regarding the origin and maintenance of the allelochemical system. While one effect of *Ailanthus* toxins is to inhibit the germination and growth of neighboring plants, allelochemical production may primarily serve insecticidal or antipredator functions. These pressures may lead to the selection of an allelochemical defense with secondary herbicidal activity. A competitive advantage of allelopathic *Ailanthus* individuals in natural populations remains to be demonstrated.

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