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ALLELOPATHY IN THE FIRST STAGES OF SECONDARY SUCCESSION ON THE PIEDMONT OF NEW JERSEY[1]

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ABSTRACT

Ambrosia artemisiifolia (ragweed) is a dominant species in the first year of old field succession but rarely persists for more than two years. Ragweed and *Raphanus raphanistrum* (wild radish), also an early invader, failed to become re-established in plots cleared of second stage perennial vegetation (dominated by *Aster pilosus*), despite the large number of seeds of these primary invaders present in the soil. Edaphic experiments revealed that this pattern of succession was not due to mineral or physical properties of the soil. Field soil from the second stage of succession inhibited the growth and germination of ragweed and wild radish while soil from the first stage had no effect. Inhibitory volatile materials from ragweed were not detected. However, root exudate of ragweed, and shoot extracts of ragweed and aster inhibited the germination and growth of early invaders of abandoned fields. These results indicate that the vegetational change from the first to the second stage of succession may be mediated at least partially by an allelopathic response. Chromatography and bioassay techniques revealed the inhibitory compounds to be phenolic acids, including caffeic and chlorogenic acids.

AMBROSIA ARTEMISIIFOLIA (ragweed) and *Raphanus raphanistrum* (wild radish) have been reported to be the pioneer invaders in old fields that have been abandoned after spring plowing on the piedmont of New Jersey (Bard, 1952; Fleet, 1972; Cocking, 1973). These authors also indicate that the plants in this first stage of succession remain dominant for the first year only and appear only sporadically after that. The investigators characterize the second stage of succession as being dominated by *Aster pilosus* (aster) with quite a diverse group of subordinate species, *Hieracium pratense* (hawkweed) being typical of these. The observations and the allelopathic effects described by a multitude of authors, especially Abdul-Wahab and Rice (1967), Wilson and Rice (1968), Parenti and Rice (1969), and Rice (1964, 1965, 1968, 1972) seem to indicate that this sequence of succession is not caused by direct competition for resources alone.

The term allelopathy has generally come to mean the deleterious effect that one higher plant has on another through the production of chemical retardants (Martin and Rademacher, 1960; Muller, 1965). Molisch (1937) coined the term allelopathy and derived it from two Greek terms meaning "mutual harm." The earliest scientist to suggest allelopathic-like responses was DeCandolle (1832) who investigated *Cirsium*. Sporadic, inconclusive work in this area occurred for the next hundred years until Cook (1921) and Davis (1928) described the allelopathic effects of *Juglans nigra* and identified the inhibitor to be juglone (5-hydroxy-1, 4 napthaquinone). This research initiated a series of investigations in this area. Bonner and Galston (1944) and Bonner (1946) found that two toxic compounds, cinnamic acid and trans-cinnamic acid, are produced by guayule plants. The results of research from the 1940's to the 1960's were of immense value, including that of McCalla and Duley (1948), Evenari (1949), Curtis and Cottam (1950), Keever (1950), Mergen (1959), and Jameson (1961).

The 1960's marked the beginning of an intensive look into allelopathy by such investigators as Muller and Rice. Muller (1965) described an allelopathic phenomenon in California where *Salvia leucophylla* produced volatile terpenes which inhibited such grassland species as *Bromus rigidus* and *Avena fatua*. Rice and his co-workers published many papers including a definitive book on allelopathy (Rice, 1974). Much of Rice's research has dealt with the disappearance of the pioneer weed stage in old field succession, caused in part by phenolic acids.

The physiological mechanism of inhibition by phenolic acids has not been completely defined. Rice (1964) believed that phenolic acids inhibit nitrogen-fixing and nitrifying bacteria. Einhellig et al. (1970) found that phenolic acids reduce CO_2 exchange rates. Numerous authors (Sondheimer and Griffin, 1960; Zenk and Muller, 1963; Hare, 1964; Imbert and Wilson, 1970) found that phenolic acids affect hormones and growth regulators. Willemsen and Rice (1972) found phenolic acids in ragweed seed and suggested that some may act as germination inhibitors. Wang, Yang and Chuang (1967) found that almost all agri-

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cultural soils in Taiwan contain phenolic acids that are inhibitory to the growth of young plants. Phenolic acids have been found to alter the flow of carbon from protein and carbohydrates to lipids in rose cell suspension cultures (Danks, Fletcher, and Rice, 1975). This could explain how these compounds inhibit growth and germination.

The above information leads to the hypothesis that allelopthy may be a participating factor in causing the disappearance of the first stage of succession in old fields on the New Jersey Piedmont. The present investigation was designed to test this hypothesis.

MATERIALS AND METHODS—The test species used in this research were ragweed (*Ambrosia artemisiifolia*), wild radish (*Raphanus raphanistrum*), hawkweed (*Hieracium pratense*), and aster (*Aster pilosus*). The ragweed and aster seeds were stratified at least 12 weeks at 5 C before use while the wild radish and hawkweed seeds were stored at room temperature. The plant materials and soil were collected from old fields at the Hutcheson Memorial Forest whose location, climate and soils were described by Bard (1952) and Ugolini (1964).

Field tests—The following experiments were instituted to determine whether the disappearance of ragweed in the second stage of succession is due to competition from overwintering plants or to a thick layer of dead vegetation from the previous year.

A one-hectare previously farmed area at Hutcheson Memorial Forest was plowed and left fallow in the spring of 1972. This area was divided into three equal rectangular fields, two of which were plowed and left fallow in the spring of 1973. One of the two 1973 fields was plowed and left fallow in the spring of 1974. During the times of plowing in 1973 and 1974, 45 plots measuring 1×2 m were delimited in each second and third year field that was not plowed. Fifteen of these plots were left undisturbed, 15 were cleared of all dead vegetation and litter, and 15 were cleared of dead and live vegetation which consisted mostly of rosettes of winter annuals, biennials, and perennials. The vegetation was cleared by careful pulling and cutting the roots with a knife so that the soil surface was disturbed very little. Fifteen control plots were laid out in the freshly plowed fields in 1973 and 1974. Five plots of each plot type in each field were sampled at three times during each summer (June, August, and September) by use of $\frac{1}{2} \times 2$ m quadrants placed in the center of each sample plot. The number of individuals, the cover estimates, and the aboveground dry weight of each species in each quadrant were determined.

Edaphic factors—Thirty 10-cm diam soil samples were taken to a depth of 10 cm in each of the first and second year fields in 1973 and in the first, second, and third year fields in 1974. The soil samples from each of the fields were composited and a subsample of approximately 1 kg from each field was sent to the Rutgers soils department for analysis relative to the *p*H, magnesium, phosphorus, potassium, calcium, nitrate-nitrogen, and texture of the soil samples.

The percent soil moisture was investigated from the first of April until the last of July in each of the three ages of fields. In each field three 4-cm diam samples were taken to a depth of 3 cm. The percent moisture content was calculated following drying at 105 C for 24 hr.

The effect of soil from the three ages of fields on the growth and germination of the test species was determined in the following manner. Soil samples minus litter were collected at random from the upper 2 cm of soil in the three ages of fields in May of 1974. The samples were placed in 15 cm clay pots, and 20 seeds of each test species were planted in each of four replicates of each soil sample. These pots were placed in a growth chamber set at 23.9 C day temperature/18.3 C night temperature with a 16-hr photoperiod (all of the following experiments, except where noted, were conducted under these conditions). The seedlings were thinned to six per pot and after four weeks they were harvested and the dry weights determined. Germination tests were performed by placing 40 g of each of the three ages of soil and 20 ml of distilled H_2O into 15-cm plastic petri plates. Twenty seeds of each test species were planted in each of the four replicates of each soil sample. The seeds were planted in a grid pattern to prevent confusion with seeds already in the soil samples. Germination was recorded after 3 wk.

Residual ragweed and radish seeds were separated from the soil by sifting three 100-g subsamples of soil from each age of field through wire mesh screens. The number of seeds collected were then counted.

Effect of water extracts on germination and growth—Extracts of ragweed and aster were prepared in a manner modified from Neill and Rice (1971). Shoots of ragweed and aster were air-dried and ground in a Wiley Mill. Twenty grams of ground plant material were boiled for 5 min in distilled H_2O and the extract was filtered through Whatman No. 3 filter paper. The filtrate and the control were adjusted to *p*H 6 with HCl. The osmotic potential of these solutions was determined by the use of a standard "osmette."

Thirty seeds of each test species were placed on germination blotting paper in $11 \times 11 \times 2.5$ cm closed plastic boxes and 12 ml of ragweed extract, aster extract, and distilled water were added to the appropriate boxes (four replicates for each treatment). Germination was recorded after 3 wk.

TABLE 1. *Effect of field manipulations on ragweed, wild radish, aster and hawkweed frequency, cover, and biomass in 1 m² plots*

Field age	Sample Time	Cleared of Litter			Completely Cleared			Undisturbed		
		Freq. (%)	Cover (%)	Bio. (g)	Freq. (%)	Cover (%)	Bio. (g)	Freq. (%)	Cover (%)	Bio. (g)
Ragweed										
1st year	June	—	—	—	—	—	—	50.0	5.0[b]	8.8[b]
	Aug.	—	—	—	—	—	—	100.0	60.0[b]	241.6[b]
	Sept.	—	—	—	—	—	—	100.0	48.0[b]	363.1[b]
2nd year	June	20	1.2	0[a]	80	3.0	.2	0	0[a]	0[a]
	Aug.	42	1.2[a]	103.4[a]	17	1.0[a]	16.0[a]	.1	1.0[a]	9.2[a]
	Sept.	5	.1[a]	1.3[a]	5	.1[a]	5.7[a]	2.0	.1[a]	0[a]
3rd year	June	20	1.0	2.8	30	1.3	.2[a]	0	0[a]	0[a]
	Aug.	0	0[a]	0[a]	0	0[a]	0[a]	0	0[a]	0[a]
	Sept.	0	0[a]	0[a]	0	0[a]	0[a]	0	0[a]	0[a]
Wild Radish										
1st year	June	—	—	—	—	—	—	100.0	99.0[b]	97.9[b]
	Aug.	—	—	—	—	—	—	0	0	0
	Sept.	—	—	—	—	—	—	0	0	0
2nd year	June	32	2.5[a]	2.2[a]	100.0	5.0[a]	.6[a]	0	0[a]	0[a]
	Aug.	0	0	0	0	0	0	0	0	0
	Sept.	0	0	0	0	0	0	0	0	0
3rd year	June	10	.1[a]	.1[a]	45.0	.4[a]	.2[a]	25.0	.1[a]	3.5[a]
	Aug.	0	0	0	0	0	0	0	0	0
	Sept.	0	0	0	0	0	0	0	0	0
Aster										
1st year	June	—	—	—	—	—	—	0	0	0
	Aug.	—	—	—	—	—	—	5.0	.5	2.3
	Sept.	—	—	—	—	—	—	0	0	0
2nd year	June	20	3.0	9.1	24	4.0	8.0	17.0	5.0	8.5
	Aug.	40	2.5	21.4[a]	20	1.0	8.4	20.0	2.0	49.3[a]
	Sept.	20	37.0[a]	47.4[a]	10	2.0	2.1	68.0	50.0[a]	47.9[a]
3rd year	June	100	30.0[a]	114.8[a]	95	19.0	15.8	100.0	55.0[a]	76.7[a]
	Aug.	100	35.0	108.6	100	58.0	113.8	100.0	62.0	168.8[a]
	Sept.	100	90.0	310.4[a]	68	5.0	151.5	100.0	95.0[a]	306.6[a]
Hawkweed										
1st year	June	—	—	—	—	—	—	0	0[b]	0[b]
	Aug.	—	—	—	—	—	—	8.0	.5	1.3
	Sept.	—	—	—	—	—	—	0	0[b]	0[b]
2nd year	June	42	2.5[a]	6.3[a]	58	2.3[a]	2.9[a]	33.0	2.5[a]	3.4[a]
	Aug.	44	2.0	.9	38	1.0	.4	50.0	1.2	2.2
	Sept.	0	0	0	0	0	0	25.0	2.5[a]	1.6[a]
3rd year	June	35	3.0[a]	4.5[a]	63	4.0[a]	1.6	40.0	2.5[a]	2.8
	Aug.	60	4.0	3.1	35	4.5	6.2	35.0	3.1	4.0
	Sept.	45	3.1[a]	6.3[a]	40	4.0[a]	7.4[a]	55.0	5.0[a]	7.2[a]

[a] Indicates a significant difference from 1st year undisturbed plots for the same month (cover and biomass only).
[b] Indicates a significant difference from 2nd and 3rd year undisturbed plots for the same month (cover and biomass only).

The effect of these extracts on the growth of the seedlings of the test species was also determined. Both extracts and the distilled water were diluted 50% by double-strength Hoagland's solution (Hoagland and Arnon, 1950). Six 7-day-old seedlings of the test species were placed in sterile perlite and watered for 14 days with the above solutions (six replicates for each treatment). The results were recorded as dry weight of the shoots and roots of the test species.

Effect of volatile materials on seed germination —Thirty seeds of ragweed or radish were placed on moist filter paper in 10-cm open petri plates which were suspended by nylon string in 8-liter closed fishbowls. The control fishbowls contained

TABLE 2. *Results of the soil nutrient tests for each of the three ages of fields*

Sample		pH	Mg ppm	P ppm	K ppm	Ca ppm	Nitrate ppm	Texture
Collected 1974	1st Year	5.1	30	10	27	115	14	Loam
	2nd Year	5.2	45	10	51	143	12	Loam
	3rd Year	5.1	67	10	46	160	12	Loam
Collected 1973	1st Year	4.9	50	10	72	132	16	Loam
	2nd Year	5.0	56	10	36	132	10	Loam

150 g of moist cotton. The test fishbowls contained 150 g of freshly crushed ragweed leaves. This type of apparatus allows rapid diffusion of volatile materials but avoids direct contact between the test seeds and the plant materials. The results were recorded in percent germination.

Root exudates—The effect of root exudates from ragweed on the growth of the test species was determined by a stairstep apparatus modified from Wilson and Rice (1968). Six columns were used, each with six 10-cm plastic pots. Each two columns had an 8-liter reservoir of distilled H_2O on the step above it. The water flow came from this reservoir and was divided equally into two columns by a Y tube. All six streams of distilled H_2O flowed down the stairstep columns out of the bottom of one pot into the top of the next lower pot until the liquid collected in an 8-liter reservoir below each of the two columns. At the bottom of the stairstep three pumps were programmed to recycle the liquid daily. Evaporation and transpiration loss was replaced daily, and during the four weeks of each experiment each pot was flushed eight times with 100 ml of Hoagland's solution. This experiment was done in the greenhouse and all the plants were grown in washed sterile sand. The control consisted of two columns in which the test species alternated with pots containing only sand, and the four test columns consisted of ragweed alternating with the test species. There were three plants per pot giving a total of nine test plants per column.

Biological activity and characterization of inhibitors—Twenty grams of freshly ground air-dried ragweed shoots were boiled for 5 min in 150 ml of 80% ethanol. A similar solution of aster was prepared. These solutions were filtered through Whatman No. 1 filter paper. The filtrates were evaporated to dryness and washed with chloroform to remove the lipids and chlorophyll. The residue was dissolved in 80% ethanol and made to volume (5 ml). The extracts were streaked on a 15-cm front on Whatman 3 MM chromatography paper and developed using the descending technique in 20% isopropanol. A control of 80% ethanol was also run. Inspection with UV light revealed three light blue fluorescent bands for both species on the test chromatograms. These bands appeared yellow-green under UV light after exposure to NH_3. After the Rf's of the bands were recorded, they were cut out, placed in petri dishes with moist filter paper, and 30 ragweed seeds were placed in each petri plate. The areas of the chromatograms which did not fluoresce were similarly treated. Results were recorded in percentage germination and seedling radicle length. Chromatograms that were run as mentioned above were dipped in the following reagents: diazotized p-nitraniline and ferric chloride-potassium ferricyanide (Smith, 1969). A second series of chromatograms were prepared by spotting 25 microlitres of known phenolic acids on Whatman No. 1 paper. The papers were developed by the descending technique using N-butanol-acetic acid-water (60:10:20), BAW, for the first dimension and 6% aqueous acetic acid,

TABLE 3. *Effect of the three ages of field soils on the percent germination and growth (mean mg dry weight/plant shoots) of the test species*

Soil Source	Test Species							
	Ragweed		Wild Radish		Aster		Hawkweed	
	Germ.	Growth	Germ.	Growth	Germ.	Growth	Germ.	Growth
1st year field	66	51.4	72	52.9	90	4.7	42	2.3
2nd year field	50[a]	32.5[a]	72	37.4[a]	85	5.8	25[a]	2.2
3rd year field	33[a]	28.5[a]	57[a]	57.9	70[a]	6.3	10[a]	2.0

[a] Indicates significant difference from 1st year soil (95% confidence level).

TABLE 4. *Effect of extracts of ragweed and aster on the percent germination and growth (mean mg dry weight/plant shoots and roots) of the test species*

Test Species	Solution								
	Control			Ragweed Extract			Aster Extract		
		Growth			Growth			Growth	
	Germ.	Shoots	Roots	Germ.	Shoots	Roots	Germ.	Shoots	Roots
Ragweed	84	33.5	11.0	67[a]	19.9[a]	6.2[a]	48[a]	14.6[a]	3.6[a]
Wild radish	65	44.5	6.5	34[a]	22.1[a]	2.9[a]	42[a]	13.5[a]	1.7[a]
Aster	56	2.3	1.8	66	2.3	8.3[a]	60	1.5	5.3[a]
Hawkweed	76	5.6	2.0	62	5.4	2.4	65	3.1[a]	1.7

[a] Indicates a significant difference from the control (95% confidence level).

AA, in the second dimension. The chromatograms were viewed with short- and long-wave UV light, the fluorescing spots were marked, the Rf's were recorded, and the correlation between the unknown and standard spots was determined. Those unknowns having positive correlations with the standards, the standards, and the corresponding Rf from the control were eluted in 80% ethanol. The absorption spectra of the unknowns were compared to the standards by use of a Beckman DB-G spectrophotometer.

Statistical analysis—The numerical results obtained in these experiments were subjected to a statistical analysis that consisted of an ANOVA test (Hewlett-Packard, 1971). Ninety-five percent was considered significant for the F test.

RESULTS—Field treatments produced very similar results in the two years of sampling (1973 and 1974). Therefore, only the 1974 data are presented (Table 1). Ragweed and wild radish cover, and biomass were the same in the second and third year fields regardless of the type of treatment the plot received. Also, the growth of ragweed in August and September, and wild radish in June resulting from the soil treatment types in the second and third year collections were significantly less than the first year controls. There was a significant reduction of aster in all three completely cleared plots compared to the undisturbed and cleared of litter plots. The vegetational response to the removal of aster was a decrease in plot biomass rather than an increase in an alternate species. There was a significant increase in hawkweed in the second year fields and the increase was affected very little by the field treatments.

Edaphic factors—Soil moisture varied from 9 to 23% over the sampling period and there was no significant difference between the moisture content of the three ages of fields on a given sampling date.

No dramatic differences in soil nutrients were found to exist between the first, second, and third year soils (Table 2). The 1974 collection shows a very slight (45 ppm) increase in calcium but this is not reflected in the 1973 collection. Both years exhibited a slight increase in magnesium (37 ppm and 6 ppm). The results for *p*H, phosphorus, potassium, and texture show little change during the first three years of succession.

A count of the seeds in the top 2 cm of the first year soil revealed an average of 16 wild radish seeds and 8 ragweed seeds per dm^2 (222 g of soil), while both the second and third year soils averaged 28 wild radish seeds and 28 ragweed seeds per dm^2 (222 g of soil).

All of the test species had significantly less germination in the third year soil compared with the first year soil (Table 3). Only ragweed and hawkweed had less germination in the second year soil compared with the first year soil.

The growth of ragweed was significantly reduced by the second and third year soils (Table 3). The growth of wild radish was significantly reduced by the second year soil but not third year soil. The growth of hawkweed and aster was not significantly affected by any of the soils.

Effect of water extracts on germination—Ragweed extract significantly reduced ragweed seed germination, averaging 17% less than the control (Table 4). Ragweed germination was even more reduced by the aster extract, averaging 24% less germination than the control. Although both aster and ragweed extracts significantly reduced radish germination, ragweed extract reduced radish germination 31% below that of the control while there was only a 23% reduction of radish germination in aster extract. These tests showed that neither of the extracts had a significant effect on the germination of hawkweed or aster.

Effect of water extracts on growth—Aster and ragweed extract significantly reduced the shoot and root growth of ragweed (Table 4). Ragweed growth was reduced by a third when watered with ragweed extract and almost a half when watered with the aster extract. Wild radish showed an even more dramatic response for both shoots and roots,

TABLE 5. *Effect of root exudates of ragweed on growth (mean mg dry weight/plant) of the test species*

	Test Species							
	Ragweed		Wild Radish		Hawkweed		Aster	
Solution	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots
Ragweed exudate	219.8[a]	223.3[a]	105.5	42.9	6.3	2.5	1.8	1.2
Control	376.6	316.6	117.5	32.2	5.7	2.3	1.8	1.4

[a] Indicates significant difference from the control (95% confidence level).

exhibiting a greater than 50% reduction in ragweed extract and an even more severe reduction of growth in the aster extract. Aster extract caused a significant reduction in shoot growth of hawkweed but ragweed extract did not. Ragweed and aster extract promoted the root growth of aster but did not affect the shoot growth. The osmotic concentration of the aster solution was 18 milliosmoles, the ragweed extract, 20 milliosmoles, and the Hoagland's solution was 16 milliosmoles.

Volatile inhibitors—Freshly crushed ragweed plants had no significant effect on the germination of ragweed or wild radish. The average germination for ragweed and wild radish in the test chambers never varied more than 3% from the control.

Root exudates—The growth of the shoots and roots of ragweed was inhibited by the root exudates of ragweed (Table 5). It was found that ragweed root exudates were not inhibitory to any of the other test species.

Effect of ethanol extract fractions on radicle growth and germination—Table 6 indicates that there were two regions inhibitory to root growth and germination on the chromatograms of ragweed extract. The first region (Rf .64–1) caused a 30% reduction in germination and as much as a 60% reduction in the average radicle length of ragweed. The second region (Rf .30–.49) was slightly less inhibitory. The aster extract produced one region of inhibition (Rf .49–1) that significantly reduced both radicle length and germination of ragweed. Radicle growth in this region of inhibition generally averaged about ⅛ the growth of the control.

Identification of inhibitory compounds—The regions of inhibition exhibited positive results for phenolic acids with the tests used (UV light, diazotized p-nitraniline reagent, ferric chloride-potassium ferricyanide reagent, and NH_3). Bidirectional descending chromatography revealed 21 phenolic acid spots under UV light in ethanol extracts of aster and 18 in ethanol extracts of ragweed. Two spots, which were present on both aster and ragweed chromatograms and seemed larger and more intense than the others, were chosen for identification. A series of laboratory standard phenolic acids were chromatographed and their Rf values compared to the unknowns. Close Rf approximation was found between one of the unknowns and chlorogenic acid (Table 7). The other unknown correlated quite closely with caffeic acid. The absorption spectrum of the suspected chlorogenic acid and the suspected caffeic acid were very similar to their corresponding standards.

DISCUSSION—Bard (1952), Small et al. (1971) and others have described the changes in plant composition during the first two stages of succession on the New Jersey Piedmont and found them to follow a somewhat uniform pattern. The results from the undisturbed fields (Table 1) reinforce these findings. Ragweed and wild radish (annuals) dominate the first stage of succession which was found to last only a year; aster, a perennial, becomes the dominant in the second stage of succession.

Removal of the overwintering perennials and litter (which denudes the ground) caused the second successional stage fields to be first successional stage fields on the basis of competing vegetation and litter, but second successional stage fields on the basis of edaphic factors. Re-establishment of ragweed and wild radish did not occur in these fields (Table 1) which indicated that the vegetation was responding as though it were in a second successional stage field. The failure of re-establishment was not due to a lack of residual seeds in the soil. This information suggested that direct competition was not the only factor limiting ragweed and wild radish survival in the second stage of succession. Raynal and Bazzaz (1975) found that the removal of competing perennials caused a moderate increase in ragweed germination in second and third year fields. However, ragweed was less dominant in first year fields in Illinois than it was in the present study.

No significant difference in soil moisture and no growth limiting changes in soil nutrients (Table 2) were found between the first and second stages of succession in New Jersey old fields. However, second year soil (second successional stage) inhibited the growth of ragweed and wild radish, and the germination of all the test species was inhibited by the third year soil (Table 3). These

TABLE 6. *Effect of portions of chromatograms of ragweed and aster ethanol extract on germination and radicle growth of ragweed*

Aster Extract			Ragweed Extract		
Rf	Radicle length (mm)	Germination %	Rf	Radicle length (mm)	Germination %
Control	32	95	Control	32	95
.79–1.0	6[a]	57	.79–1.0	10[a]	62
.64–.79	6[a]	67	.65–.79	11[a]	67
.49–.64	7[a]	87	.49–.65	29	87
.35–.49	22	75	.30–.49	20[a]	75
.15–.35	27	85	.15–.30	33	85
.00–.15	32	85	.00–.15	32	85

[a] Indicates significant difference from control.

findings suggested that allelopathic agents in the soil could be one of the mechanisms involved in the vegetation change from the first to the second stage of succession. This conclusion was reinforced by Quinn (1974) who reported that *Convolvulus sepium*, a common component of the old fields at Hutcheson Memorial Forest, caused allelopathic inhibition of associated species and possibly of itself. Neill and Rice (1971) also found that soil associated with *Ambrosia psilostachya* inhibited the growth and germination of annual weeds.

Ragweed and aster exert the dominant influence in the first and second stages of succession on the Piedmont of New Jersey (Bard, 1952), indicating that one or both of these plants are potential producers of allelopathic chemicals. The volatile inhibitor test showed no air-borne inhibitors from ragweed. Water extracts of ragweed and aster were found inhibitory to ragweed and wild radish germination, and to growth (Table 4). These extracts were generally not found to be inhibitory to aster and hawkweed germination and growth. The results from the stairstep apparatus experiment showed ragweed root exudate inhibitory to ragweed growth (Table 5), and chromatograms of ragweed and aster ethanol extracts revealed inhibitory regions (Table 6). These results suggest the occurrence of allelopathic substances which restrict growth and germination of first stage annuals in the second successional stage soil and that both ragweed and aster produce phenolic compounds capable of causing this effect.

Rice and his co-workers have found many specific phenolic compounds capable of acting as allelopathic agents in old field succession. Wilson and Rice (1968) found isochlorogenic and chlorogenic acid to be the allelopathic agents in *Helianthus annuus*. Rice (1965) found that *Ambrosia psilostachya* produced isochlorogenic acid, chlorogenic acid, and a glucose ester of caffeic acid. In the present study, ragweed and aster were found to produce chlorogenic acid and caffeic acid (Table 7) in addition to a number of other unknown phenolic acids. The presence of a large number of phenolic acids in the extracts of ragweed and aster could possibly produce an additive or synergistic allelopathic effect.

The conclusions reached in this research can be summarized in five general statements. (1) The rapid disappearance of the annual stage of succession and concomitant establishment of the perennial stage is a vegetational response greater than should be expected by direct competition. (2) Field manipulation studies revealed that this seral response is not due to direct competition but primarily to some soil factor. (3) Edaphic studies showed that the soil factor was probably an allelo-

TABLE 7. *Chromatography of phenols from aster and ragweed*

Compound	Rf's on Whatman #1		Fluorescence under U.V.		Maximum Absorption (NM)
	BAW	6%	–NH3	+NH3	
Chlorogenic acid	50	67	Light blue	Yellow-green	310
Suspected ragweed chlorogenic acid	52	63	Light blue	Yellow-green	312
Suspected aster chlorogenic acid	49	67	Light blue	Yellow-green	310
Caffeic acid	76	29	Faint blue	Light blue	306
Suspected ragweed caffeic acid	72	25	Faint blue	Light blue	305
Suspected aster caffeic acid	79	31	Faint blue	Light blue	307

pathic agent. (4) The greenhouse and laboratory studies indicated that ragweed and aster extracts were more inhibitory to plants of the first stage of sucession than to those in the second stage. (5) The chromatograms of extract from ragweed and aster revealed numerous phenolic acids, two of which are known plant inhibitors. These findings make it seem almost certain that the change from the first stage of succession to the second is, at least in part, an allelopathic mediated response.

One of the allelopathic mechanisms demonstrated was the inhibition of germination. Plants present in the second stage of succession may exhibit adaptations to this. Three adaptations are quite obvious in hawkweed and aster: (1) absence of a response to the extracts inhibitory to the first stage plants; (2) a perennial habit, subsequently not needing yearly germination success; (3) vegetative reproduction (Bach, 1975). How can the seemingly adverse characteristic of autotoxicity be selected for in an annual like ragweed? Perhaps the direct autotoxic effect on the plant itself is an unnatural side effect, and the advantage offered by autotoxicity lies in the inhibition of seed germination by the inhibitory compounds which may induce seed dormancy. Willemsen (1975) suggests that seed dormancy may be induced by many factors which inhibit germination. Dormancy induction in seeds of annuals during the transition between the first and second stage of succession would be advantageous since they are generally considered to be competitively inferior to the perennials of the second stage. This indicates that the transition would occur independent of allelopathy, but perhaps not as rapidly. The effect on the annuals may continue through the second stage of succession since aster also inhibits their germination. This mechanism along with seed burial would allow for the maintenance of a population of viable annual weed seeds in the soil. Thus, allelostasis would seem a more appropriate term than allelopathy but only further research can determine this. Lastly, it should be noted that two plants (ragweed and aster) and also a third, *Convolvulus sepium* (Quinn, 1974), are in all probability affecting old field succession on the New Jersey Piedmont by releasing allelopathic compounds. A question still remaining is the relative importance of allelopathy in the overall successional pattern.

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