THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

POSSIBLE ROLE OF *Ambrosia psilostachya* ON PATTERNING AND SUCCESSION IN OLD-FIELDS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

BY

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Norman, Oklahoma

1970

POSSIBLE ROLE OF *Ambrosia psilostachya* ON PATTERNING AND SUCCESSION IN OLD-FIELDS

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ACKNOWLEDGMENTS

I would like to thank Dr. Elroy L. Rice for guidance and suggestions as my major advisor, and the other members of my advisory committee, Drs. Norman H. Boke, Simon H. Wender, George J. Goodman and Paul G. Risser for critical reading of the manuscript.

I wish to express my thanks to my wife, Judy, for her understanding and sacrifice in the completion of this research.

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CHAPTER I

INTRODUCTION

Succession in abandoned fields in the prairie areas of central Oklahoma and southeast Kansas includes four major stages: (1) weed stage, lasting 2-3 years; (2) annual grass, dominated by *Aristida oligantha* and lasting from 9-13 years; (3) perennial bunchgrass, which remains for more than 30 years and is dominated by *Andropogon scoparius*; and (4) the climax prairie dominated by *A. gerardi*, *A. scoparius*, *Panicum virgatum*, and *Sorghastrum nutans* (Booth, 1941). Actually some areas near Norman, Oklahoma have been abandoned for 29 years and still have virtually a pure stand of *Aristida oligantha*. The prairie climax is not attained in such fields for a considerable time after abandonment. Savage and Runyon (1937) reported that old-fields in the Southern Great Plains had not reached the climax stage even after 40 years of abandonment.

Nomenclature follows Waterfall (1966) unless authority is given.

The climax composition in an abandoned field in central Kansas had not been attained after 33 years (Tomanek, Albertson, and Riegel, 1955).

Rice, Penfound and Rohrbaugh (1960) studied the nitrogen and phosphorus requirements of Aristida oligantha, Andropogon scoparius and P. virgatum. They found that the order of increasing requirements for nitrogen and phosphorus is the same as the order in which these species invade abandoned fields.

Ambrosia psilostachya (western ragweed) is prominent in the first stage of succession and continues to be present to a lesser degree through all stages. This is in contrast to most species of the first stage which disappear in 1-3 years after abandonment. Their disappearance has been attributed to an allelopathic effect which eliminates many members of the first stage (Abdul-Wahab and Rice, 1967; Parenti and Rice, 1969; Wilson and Rice, 1968).

Rice (1965) found that A. psilostachya produced isochlorogenic acid, chlorogenic acid and a glucose ester of caffeic acid which were inhibitory to nitrogen-fixing and nitrifying bacteria. It was suggested that this inhibitory effect due to A. psilostachya and other species could delay the appearance of the bunch grass stage and the climax stage in old-field succession. Olmsted (unpublished M. S. thesis, Univ. of Okla., 1967) found that isochlorogenic acid and chlorogenic acid inhibited

Bromus japonicus and Amaranthus retroflexus seedlings.

Rice (1968) found that decaying A. psilostachya (1 g/454 g of soil) caused a significant reduction of nodulation in three legume species. Root exudates of ragweed inhibited nodulation and dry weights of the test legumes, and ragweed growing with red kidney beans significantly reduced the nodule number, hemoglobin content of the nodules and dry weights of test plants as compared with the control plants.

With these data in mind, a study was conducted to determine if A. psilostachya has direct allelopathic effects on higher plants, and what the possible effects of the species are on patterning of the vegetation and succession.

CHAPTER II

DESCRIPTION AND LOCATION OF FIELD PLOTS

A field on the south edge of Norman, Oklahoma, abandoned for 25 years, was selected as a study site. In this area it was noted that the pattern of vegetation adjacent to the ragweed was different from that apart from the ragweed. These observations were quantified using 0.25 m^2 quadrats. Clippings were made in quadrats with ragweeds and in quadrats 1 m removed from the first quadrat and in an area which included no ragweed. The second quadrat was located directly west of the first unless the above conditions were not met in that location. The clippings were separated as to species and dry weights were taken. Five sets of quadrats were clipped every two weeks from June, 1969, through October, 1969.

The mean oven-dry weights of the forbs were not statistically different in the quadrats with ragweed as compared with those 1 m away (Table 1). On the other hand, Andropogon ternarius had a statistically significant lower mean oven-dry weight near the ragweed and Leptoloma cognatum had a significantly greater mean dry weight near the ragweed than 1 m away. Tridens flavus also had a higher mean

Table 1. Results of field clippings of species associated with Ambrosia psilostachya.

Species	Mean oven-dry weight in g/0.25 m^2 with Standard Error	
	Quadrats[1]	
	A	B
Andropogon ternarius	0.97±0.31	6.37±1.19[a]
Aristida oligantha	2.14±0.63	2.89±0.64
Bromus japonicus	1.22±0.50	2.48±0.97
Erigeron canadensis	0.66±0.36	0.55±0.43
Haplopappus ciliatus	0.47±0.27	0.59±0.42
Leptoloma cognatum	1.26±0.35	0.43±0.12[a]
Rudbeckia hirta	0.32±0.11	0.26±0.09
Tridens flavus	1.03±0.34	0.56±0.30

[1]Quadrat A includes the ragweed plants.
Quadrat B starts 1 m from quadrat A.

[a]Significant difference among quadrats.

dry weight near the ragweed but not at the 0.05 level of significance. Amaranthus retroflexus and Digitaria sanguinalis were not sampled in this area since they disappear usually shortly after abandonment of the field from cultivation.

CHAPTER III

EXPERIMENTATION AND RESULTS

Effects of Ragweed on Soil Reaction and Mineral Content. To determine if the growth patterns in the field were due to mineral or physical properties, ten soil collections were taken to a depth of 30 cm at each of the indicated distances from the ragweed. These were air-dried, passed through a 2 mm sieve and the pH was determined by the glass electrode method (Piper, 1942). The soil was then ground to pass a 0.5 mm sieve, and analyzed for organic carbon by the chromic acid digestion method (Piper, 1942), total phosphorus by the method of Shelton and Harper (1941), and total nitrogen by the macro-Kjeldahl method (Bremner, 1965). Apparently ragweed caused no significant differences in pH or mineral content near it (Table 2).

Effects of Ragweed on Soil Moisture. Ten soil samples were taken to a depth of 30 cm within 0.25 m of several ragweed plants and ten at a distance of at least 1 m from the same plants in July 1969. The moisture content was determined and the percentage moisture was calculated on the basis of the oven-dry weight. The mean

Table 2. Comparison of mineral and physical properties of soils around A. psilostachya.[a]

Distance of Soil Sample from Ragweed Plants	Moisture	pH	Organic Carbon[b] %	Total Nitrogen %	Total Phosphorus %
Within 0.25 m	5.3±0.30	6.66	0.48±0.03	0.047±0.003	0.012±0.0005
Greater than 1 m	6.2±0.51	6.58	0.50±0.04	0.050±0.006	0.014±0.0005

[a]No significant differences were found.

[b]Walkley and Black organic carbon values.

percentage soil moisture was not significantly affected by the presence of the ragweeds (Table 2).

Of course, all soil factors were not analyzed but moisture, nitrogen and phosphorus are generally most likely to be deficient in the area studied. Even though there were no significant differences in the mineral factors studied, the possibility of differential ion and water uptake by the ragweed and test species was not eliminated. Therefore field and soil studies were initiated to study this possible competitive relationship.

Effects of Field Soil on Test Species. In July (1969) and January (1970) a series of eight soil samples, minus litter, was taken within 0.25 m of several ragweed plants and another series of eight was taken at a distance of 1 m or greater from the same plants. The samples were collected with a sharp-nosed shovel and placed in 5 inch plastic pots. Seeds of test species collected from abandoned fields near Norman were planted in their respective pots and grown in the greenhouse. Germination was recorded at the end of two weeks after which the plants were thinned to the five largest plants per pot, allowed to grow for 3 more weeks, and dry weights were then determined. Field soil taken in July from near the ragweed plants proved to be significantly stimulatory to Amaranthus retroflexus, Andropogon ternarius, B. japonicus, D. sanguinalis, L. cognatum, R. hirta and T. flavus (Table 3). However, soil

Table 3. Effects of field soils previously in contact with ragweed roots on germination and growth of test species.

Species	Date Soil Taken	Mean Dry Weight, mg with Standard Error		Germination % of Control
		Control	Test	
Amaranthus	July	42±8.0	95±10.0[a]	130
retroflexus	January	15±1.8	9± 0.8[a]	100
Andropogon	July	25±1.4	33± 2.1[a]	80
ternarius	January	10±0.6	10± 0.5	66
Aristida	July	31±1.8	26± 1.7	100
oligantha	January	11±0.5	11± 0.5	105
Bromus	July	22±1.1	46± 3.3[a]	112
japonicus	January	17±1.0	14± 0.6	97
Digitaria	July	56±6.2	117± 7.7[a]	101
sanguinalis	January	17±1.8	41± 4.3[a]	94
Erigeron	July	13±1.0	13± 0.8	92
canadensis	January	8±0.6	7± 0.6	85
Haplopappus	July	18±1.2	20± 1.9	77
ciliatus	January	10±0.5	7± 0.5[a]	83
Leptoloma	July	20±1.9	36± 1.8[a]	90
cognatum	January	13±1.0	15± 1.1	114
Rudbeckia	July	16±0.8	25± 1.6[a]	83
hirta	January	8±0.7	6± 0.4[a]	130

Table 3 (Continued)

Species	Date Soil Taken	Mean Dry Weight, mg with Standard Error		Germination % of Control
		Control	Test	
Tridens flavus	July	27±1.6	43± 3.2[a]	102
	January	6±0.8	8± 1.4	100

[a]Dry weight significantly different from control at 0.05 level or better.

collected in January prior to the onset of germination in the field and after accumulation of ragweed leaves significantly inhibited A. retroflexus, H. ciliatus and R. hirta but significantly stimulated D. sanguinalis. Leptoloma cognatum and T. flavus were stimulated also but not at the 0.05 level of significance.

There were some interesting correlations with field patterns. L. cognatum and T. flavus, which grew better near ragweed in the field, were stimulated by soil collected near ragweed during both sampling periods. On the other hand, Andropogon ternarius which grows very poorly near ragweed in the field was significantly stimulated by the soil collected near ragweed in July and was not affected by the soil collected near ragweed in January.

The results to this point suggested, therefore, that the ragweed plants were producing organic compounds that stimulated some plants and inhibited others. Experiments were designed to investigate this possibility.

Effects of decaying ragweed leaves on test species. Seeds of test species often associated with ragweed were germinated in 4-inch glazed pots containing soil (6:4:1 - soil: sand: peat) mixed with 1 g air-dried powdered leaf material per 454 g of soil, or 1 g of air-dried peat moss in control pots. Rice (1968) determined that mature stands of A. psilostachya produced more than 1 g of air dry weight

of leaves per 454 g of soil to the depth of plowing, the top 17 cm. Eight pots were run in the control and test series for each species. Percentage germination of the greenhouse grown plants was determined after 14 days, the plants were thinned to the five largest ones per pot, and the oven-dry weights of the seedlings were taken after three more weeks growth.

The air-dried leaves inhibited growth of Andropogon ternarius seedlings but significantly stimulated Aristida oligantha, B. japonicus, H. ciliatus and R. hirta (Table 4). All other species were stimulated to some degree. Rice (1968) also found some stimulation in growth of three legume species when decaying material of A. psilostachya was added to soil.

This experiment was repeated using old leaves that had aged on the ragweed plant and were starting to drop from it. These leaves produced contrasting results, with significant inhibition in dry weights occurring in six of the test species (Table 5). The percentage germination of Amaranthus retroflexus was reduced appreciably as was that of H. ciliatus.

A similar experiment was conducted by placing 0.5 g of old ragweed leaves (over-wintered on plant) on the soil surface of the test series. For the control, 0.5 g of peat moss was used. The percentage germination was recorded after two weeks, the plants were thinned to the five largest

Table 4. Effects of decaying ragweed (air-dried fresh leaves) on seedling growth.

Species	Exp. No.	Mean Dry Weight of Seedlings, mg		Germination % of Control
		Control	Test	
Amaranthus	1	468.7±36.4	560.2±48.7	88
retroflexus	2	586.5±62.6	687.1±70.2	60
Andropogon	1	51.7± 4.3	12.1± 1.6[a]	110
ternarius	2	35.2± 2.4	21.4± 1.9[a]	92
Aristida	1	55.7± 3.3	105.2± 5.2[a]	84
oligantha	2	75.9± 7.8	105.5± 5.9[a]	86
Bromus	1	43.8± 3.1	102.8± 9.8[a]	98
japonicus	2	121.3± 6.1	143.4± 6.9[a]	100
Digitaria	1	153.8±16.9	160.7±22.8	102
sanguinalis	2	170.3± 7.2	175.1± 7.9	115
Erigeron	1	13.7± 1.3	14.2± 1.2	70
canadensis	2	12.3± 0.9	13.2± 0.9	70
Haplopappus	1	19.7± 1.7	57.1± 5.9[a]	136
ciliatus	2	25.9± 3.2	29.7± 3.3	100
Leptoloma	1	94.0± 6.3	114.1± 8.6	127
cognatum	2	106.1± 9.0	114.7± 7.7	97
Rudbeckia	1	30.0± 2.9	92.8± 8.2[a]	193
hirta	2	43.9± 4.3	68.1± 6.2[a]	77
Tridens	1	375.9±24.5	386.0±27.2	137
flavus	2	376.0±35.3	387.6±24.8	78

[a]Dry weight significantly different from control at 0.05 level or better.

Table 5. Effects of decaying ragweed leaves (over-wintered on plant) on seedling growth and germination.

Species	Exp. No.	Mean Dry Weight, mg with Standard Error		Germination % of Control
		Control	Test	
Amaranthus	1	136± 8.3	135± 8.2	72
retroflexus	2	118±11.9	110± 7.0	67
Andropogon	1	14± 0.9	12± 0.8	100
ternarius	2	15± 1.4	12± 0.9	91
Aristida	1	32± 0.5	25± 1.4[a]	87
oligantha	2	90± 6.2	67± 5.2[a]	80
Bromus	1	69± 4.6	53± 3.4[a]	92
japonicus	2	78± 4.7	69± 4.6	99
Digitaria	1	207±25.4	126± 9.5[a]	99
sanguinalis	2	74± 3.6	34± 2.7[a]	90
Erigeron	1	24± 1.8	12± 1.1[a]	98
canadensis	2	9± 0.9	4± 0.4[a]	92
Haplopappus	1	39± 6.3	26± 5.0	55
ciliatus	2	36± 5.6	26± 4.5	80
Leptoloma	1	156±15.2	84± 7.9[a]	91
cognatum	2	20± 2.1	11± 0.8[a]	87
Rudbeckia	1	9± 0.9	12± 1.4[a]	87
hirta	2	11± 0.8	13± 1.0	80
Tridens	1	104±17.1	84±18.3	98
flavus	2	62± 5.7	52± 4.0	107

[a]Dry weight significantly different from control at 0.05 level or better.

per pot, allowed to grow for an additional 3 weeks and dry weights were taken.

Growth of B. japonicus and H. ciliatus was significantly stimulated, whereas seed germination and growth of L. cognatum were reduced (Table 6).

Effect of leaf leachate on test species. Leaf leachate was collected by spraying a mist of cistern water over the ragweed. The leachate was used to water pots containing a soil mixture (6:4:1 - soil: sand: peat) and seeds of the test species. Control pots set up in the same manner were watered with equal amounts of cistern water. Eight control and eight test pots were planted for each species. The percentage germination was recorded two weeks after planting, the plants were thinned to five plants per pot, allowed to grow for three more weeks, and dry weights were then determined.

The leaf leachate increased the germination in E. canadensis L., H. ciliatus, R. hirta and L. cognatum (Table 7). Germination of Amaranthus retroflexus and Aristida oligantha was reduced slightly. The dry weights of L. cognatum and T. flavus were increased approximately 50 mg per plant. Digitaria sanguinalis and Aristida oligantha were also significantly stimulated. The leachate significantly reduced the dry weights of seedlings of H. ciliatus and B. japonicus in at least one experiment for each species.

Table 6. Effects on germination and growth of test species of A. psilostachya leaves (0.5 g) placed on the surface of the soil.

Species	Exp. No.	Mean Dry Weight, mg with Standard Error		Germination % of Control
		Control	Test	
Amaranthus	1	11±0.8	12±0.9	101
retroflexus	2	10±0.7	12±1.4	91
Andropogon	1	11±0.8	11±0.7	108
ternarius	2	11±0.7	10±0.8	100
Aristida	1	12±0.5	13±0.7	98
oligantha	2	13±0.7	12±0.7	92
Bromus	1	19±0.9	22±1.0[a]	102
japonicus	2	19±1.2	18±0.8	98
Digitaria	1	18±1.4	22±1.8	128
sanguinalis	2	20±1.7	22±1.2	114
Erigeron	1	6±0.5	5±0.3	103
canadensis	2	6±0.4	6±0.5	96
Haplopappus	1	9±0.6	11±1.0[a]	131
ciliatus	2	8±0.6	11±1.0[a]	98
Leptoloma	1	13±2.2	7±0.6[a]	72
cognatum	2	13±1.8	8±0.8[a]	80
Rudbeckia	1	6±0.7	8±0.7	100
hirta	2	6±0.5	7±0.9	92

Table 6 (Continued)

Species	Exp. No.	Mean Dry Weight, mg with Standard Error		Germination % of Control
		Control	Test	
Tridens flavus	1	11±1.1	11±0.7	100
	2	11±1.1	12±1.0	87

[a]Dry weight significantly different from control at 0.05 level or better.

Table 7. Effects of leaf leachate on germination and seedling growth.

Species	Exp. No.	Mean Dry Weight, mg with Standard Error		Germination % of Control
		Control	Test	
Amaranthus	1	31± 4.7	26± 4.3	88
retroflexus	2	65± 5.4	66± 5.2	60
Andropogon	1	101±18.8	98± 6.4	110
ternarius	2	111± 7.9	106± 7.0	92
Aristida	1	45± 1.9	51± 2.3[a]	86
oligantha	2	35± 8.4	39± 6.7	84
Bromus	1	40± 2.7	33± 2.1[a]	98
japonicus	2	38± 2.3	37± 1.8	90
Digitaria	1	437±21.8	522±39.2[a]	115
sanguinalis	2	463±22.2	509±36.4	102
Erigeron	1	24± 3.1	18± 1.6	142
canadensis	2	30± 3.4	23± 3.2	110
Haplopappus	1	44± 3.4	33± 2.5[a]	136
ciliatus	2	46± 5.1	46± 3.0	112
Leptoloma	1	134±16.4	189±20.2[a]	127
cognatum	2	128±17.6	183±20.4[a]	103
Rudbeckia	1	25± 2.4	26± 1.7	193
hirta	2	47± 4.1	49± 7.0	102
Tridens	1	130±10.5	182±11.3[a]	115
flavus	2	164±11.7	198± 8.8[a]	100

[a]Dry weight significantly different from control at 0.05 level or better.

Effects of root exudate on test species. The effects of ragweed root exudate on the test species were investigated using an experimental design modified from Martin and Rademacher (1960). Seedlings of 14 day-old test plants were placed in 4-inch pots filled with washed quartz sand. The experimental group consisted of alternating pots of 3-4 ft high ragweed plants and test plants. The control group was composed of all test plants. Complete nutrient solution (Hoagland and Arnon, 1950) was placed in reservoirs at the bottom of a staircase structure, and was pumped to other reservoirs at the top of the apparatus. The solution dripped from the upper reservoir into pots of the control and test series by gravitation to the lower reservoirs. The solutions were then pumped back to the upper reservoirs. The nitrate activity was monitored in the second experiment with E. canadensis, L. cognatum, A. ternarius and R. hirta and the nitrate activity was maintained the same in the test series as in the control series. The circulation of solution continued for 4 hours a day for 14 days at which time the test species were harvested and dry weights were taken.

This experiment was designed to eliminate competition for light, minerals and water between the ragweed and the test species; thus all mutual effects were excluded except for the interaction of root exudates. The term exudate is used in a broad sense here to refer to chemicals

which get out of the root cells in any fashion. The exudate caused a significant reduction of growth in all but three test species in at least one experiment (Table 8). Dry weight of T. flavus was significantly increased by the exudate in both trials, and of Aristida oligantha in one trial.

Effects of volatile materials from ragweed. On calm days a distinctive odor can be detected from a mature stand of ragweed. An experiment was designed (modified from Muller, 1966) to test the effects of this volatile material on early growth of Amaranthus retroflexus and B. japonicus.

Seeds of B. japonicus and A. retroflexus were placed on filter paper in Petri dishes and then placed in 10 cm desiccator chambers. The control chambers contained 5 g of peat moss placed below the Petri dish and the test chambers held 5 g of air-dried ragweed. This allowed circulation of volatile materials without physical contact between the test seeds and plant materials. The Petri papers were moistened with distilled water, the chambers sealed, and the seeds were germinated in the dark at room temperature. The length of the first leaf in B. japonicus and of the hypocotyl of A. retroflexus were measured at the end of one week. The hypocotyl length of A. retroflexus and leaf length of B. japonicus were significantly reduced in the test chambers as compared with the controls (Table 9).

Table 8. Effects of ragweed root exudate on seedling growth.

Species	Exp. No.	Mean Dry Weight, mg with Standard Error	
		Control	Test
Amaranthus	1	49± 8.7	18± 3.4[a]
retroflexus	2	19± 1.2	14± 1.7
Andropogon	1	28± 1.6	24± 2.1
ternarius	2	24± 1.2	14± 1.5[a]
Aristida	1	96±10.9	137± 9.4[a]
oligantha	2	79± 6.2	52± 4.5[a]
Bromus	1	52± 4.4	29± 3.9[a]
japonicus	2	120± 7.6	102± 9.2[a]
Digitaria	1	118±12.2	76±18.6
sanguinalis	2	137± 7.8	48± 6.0[a]
Erigeron	1	3± 0.1	2± 0.2[a]
canadensis	2	3± 0.3	2± 0.2[a]
Haplopappus	1	34± 2.7	38± 3.6
ciliatus	2	33± 2.4	31± 3.1
Leptoloma	1	57± 5.4	50± 5.7
cognatum	2	47± 4.4	37± 3.5
Rudbeckia	1	6± 0.5	3± 0.2[a]
hirta	2	4± 0.4	3± 0.3
Tridens	1	44± 4.7	77±10.7[a]
flavus	2	51± 3.2	83± 8.2[a]

[a]Dry weight significantly different from control at 0.05 level or better.

Table 9. Effects of volatile materials from A. psilostachya leaves on growth of test seedlings.

Species	Exp. No.	Length of First Leaf (Bromus) or Hypocotyl (Amaranthus), mm with Standard Error	
		Control	Test
Amaranthus retroflexus	1	16.1± 1.3	2.8± 0.3[a]
	2	8.1± 0.9	3.6± 0.7[a]
Bromus japonicus	1	25.8± 1.3	12.2± 0.4[a]
	2	33.4± 1.0	26.9± 1.0[a]

[a]Length significantly different from control at 0.05 level or better.

Biological activity and characterization of inhibitors. Ten grams of over-wintered western ragweed leaves were immersed in 100 ml boiling water for 10 minutes after which the extract was then filtered and 100 λ were spotted on acid washed Whatman 3 MM chromatographic paper. The papers were developed by the descending technique in n-butanol-acetic acid-water (63:10:27, v/v), BAW, in the first dimension and 6% aqueous acetic acid, 6% AA, in the second dimension. The developed chromatograms were viewed with short (2537 Å) and long (3360 Å) UV light and two absorption spots were noted and marked (Table 10).

To determine the biological activity of the absorption areas they were eluted with 50% aqueous ethanol, evaporated to dryness, and taken up in 4 ml of 0.05 M phosphate buffer (pH 5.8). The solution was added to a Petri plate containing a 5 cm disc of washed Whatman 3 MM chromatography paper and 100 Amaranthus palmeri (a primary invader in abandoned fields) seeds. A similar sized piece of paper was cut from a blank chromatogram developed in BAW-6% AA, eluted, the eluate was dried and taken up in 4 ml of buffer to serve as the control. The seeds were kept in darkness at 25 C and percentages of germination were noted at 24 hour intervals for one week. The biological activity of the absorption zones expressed as percent of control germination was: Unknown No. 1, 17; Unknown No. 2, 104.

Table 10. Chromatography of possible phytotoxins from decaying Ambrosia psilostachya leaves.

Compound	R_f's on Whatman No. 1[a]				Fluorescence[b]		Reagent Colors[b,c]		
	BAW	6% AA	IAW	IBW	Long UV	Short UV	P-Nit.	Sulfan. Acid	$FeCl_3$ $K_3Fe(CN)_6$
Unknown 1	.87	.03	.04	.91	Abs	Abs	br	yel	bl
Unknown 2	.80	.03	.03	.83	Abs	Abs	br	yel	bl

[a] Refer to text for solvent systems. R_f's are average of many runs.

[b] abs, absorption; br, brown; yel, yellow; bl, blue.

[c] Diazotized p-nitroaniline (Bray et al. 1950), diazotized sulfanilic acid (Bray et al. 1950), ferric chloride-potassium ferricyanide (Smith 1960, p. 234).

The phytotoxin, Unknown No. 1, and Unknown No. 2 were run in one dimension on Whatman No. 1 paper in four different solvent systems: BAW; 6% AA; isopropanol-ammonia-water (200:10:20, v/v), IAW; and isopropanol-n-butanol-water (140:20:40, v/v), IBW. The R_f's in the various solvent systems and colors with UV light and various reagent tests did not coincide with data from available known compounds (Table 10).

The inhibitory compound was eluted, evaporated to dryness *in vacuo*, taken up in 10 ml of 0.5 N HCl, refluxed one hour, cooled and extracted with two half volumes of ether. The ether fraction was evaporated to dryness and taken up in 3 ml absolute ethanol. The water fraction was evaporated three times to dryness to remove the HCl and taken up in 3 ml 40% ethanol. The water fraction was chromatographed on Whatman 3 MM paper in BAW followed by IBW and the chromatograms were dipped in a benzidine sugar reagent (Smith, 1960) with negative results. Both the water and ether fractions were chromatographed on Whatman 3 MM paper in BAW followed by 6% AA. The papers were observed under UV light and then dipped in diazotized sulfanilic acid, diazotized p-nitraniline and ferric chloride - potassium ferricyanide and examined for reacting spots. No spots were visible under UV and all reagent tests were negative.

Some phenolic acids are destroyed by acid hydrolysis

so the Unknown No. 1 was hydrolyzed with 2N NaOH under N_2 for 2 hr at room temperature. The hydrolyzed material was passed through an Amberlite IR-120 (H^+ form) column to remove sodium ions and then was ether extracted with 2 half volumes of ether. The ether fraction was evaporated to dryness and taken up in 3 ml absolute ethanol. The water fraction was evaporated three times to dryness to remove the HCl and taken up in 3 ml 40% ethanol. The water fraction was chromatographed on Whatman 3 MM paper in BAW followed by IBW, and the chromatogram was dipped in the benzidine sugar reagent with negative results. Both ether and water fractions were subsequently chromatographed on Whatman 3 MM paper in BAW followed by 6% AA. The papers were observed under UV light and then dipped in either ferric chloride-potassium ferricyanide or sulfanilic acid and examined for reacting spots. No spots were found on the chromatograms of the ether fractions but four spots were found on the chromatograms of the water fractions (Table 11). Apparently Unknown No. 1 is made up of a complex molecule with ester linkages. Identification of the compounds resulting from hydrolysis was attempted but not accomplished.

The water extract from over-wintered ragweed leaves was acidified to 2.5 with 0.5 N HCl and extracted with two half volumes of ether. The ether fraction was evaporated to dryness and taken up in 3 ml absolute ethanol. The

Table 11. Chromatography of NaOH hydrolyzed phytotoxin (Unknown No. 1 in Table 10) from decaying ragweed leaves.

Compound	R_f's on Whatman 3MM[a]		Fluorescence[b]		Reagent Colors	
	BAW	6% AA	Long UV	Short UV	$FeCl_3$ $K_3Fe(CN)_6$	Sulfan Acid
Fraction 1	.58	.88	abs	abs	none	none
Fraction 2	.43	.77	abs	abs	bl	none
Fraction 3	.25	.84	bl	bl	none	none
Fraction 4	.11	.93	abs	abs	none	none

[a] See text for solvent systems. R_f's are average of 3 runs.

[b] See Table 10 for symbols.

ether fraction was then chromatographed on Whatman 3 MM paper in methylisobutyl ketone: formic acid: water (14:3:2, v/v) KFW, in the first dimension followed by IAW in the second dimension. Three compounds that streaked in KFW were located with the sulfanilic acid reagent. Two yellow streaks (R_f's in IAW, .32 and .63) and a peach colored streak (R_f in IAW, .86) were noted, and one of these (R_f, .63) was eluted with 50% ethanol. The eluate was evaporated to dryness *in vacuo* and the residue was taken up in 2 ml 0.05 M phosphate buffer (pH 5.8) and the *Amaranthus palmeri* bioassay was run as previously described. The biological activity of the streak expressed as percent germination of the control was 20. The identity of the inhibitory compound was not determined. An inhibitory compound which appears to be similar to this has been found in the root exudate of young sterile seedlings of *Aristida oligantha* by Dr. E. L. Rice in this laboratory (personal communication). Both inhibitors appear to give similar breakdown products after acid hydrolysis. Work toward characterization and identification is continuing.

CHAPTER IV

DISCUSSION

Ambrosia psilostachya which exists throughout all stages of old-field succession in Oklahoma was found to play a role in delaying the appearance of the bunch grass stage and climax prairie by reducing the population of nitrogen-fixing bacteria (Rice, 1968) and nitrogen-fixing blue-green algae (Parks and Rice, 1969).

The present study indicates that growth of certain species of the early stage of succession is inhibited by ragweed while other species are stimulated. The fact that field soils produced different growth patterns even though they contained similar amounts of minerals suggests that the patterns were due to organic compounds which escaped into the soil from the ragweed and not to a competitive mechanism. Experimentation supported this hypothesis. The concept of plants being a closed system has been set aside and recent work indicates that organic and inorganic compounds can escape from living plants as well as from decaying material (Morgan and Tukey, 1964; Muller, 1966; Rovira, 1956; and Woods, 1960).

Andropogon ternarius which exhibited reduced growth

near ragweed in the field was significantly inhibited by root exudate and decaying material of ragweed. Leptoloma cognatum which was stimulated by ragweed in the field had reduced growth in decay experiments but was stimulated by field soil and leaf leachate. Tridens flavus, also stimulated in field sampling, was stimulated by field soil, leaf leachate and root exudate.

The overall effect of ragweed on the vegetation pattern is probably caused by the interaction of substances from the living plant in the form of leaf leachate, root exudate or volatile materials with the effect of the decaying ragweed. Whether this influence on the vegetation pattern is direct or indirect, it can be a significant one as indicated in Andropogon ternarius, L. cognatum and T. flavus. If growth and germination of species associated with ragweed were stimulated this would give the species a competitive advantage for available water, minerals and light. This advantage could be recognized in the reproductive potential and seed production of the associated species which in turn could be a major factor in establishing the pattern of vegetation near the ragweed. If associated species are inhibited the situation is reversed.

The fact that inhibitors have been isolated from air-dried leaves suggests that A. psilostachya is potentially an inhibitory species. It is apparent, however, that the compounds escaping from over-wintered ragweed

leaves are different from those found in air-dried leaves, but the allelopathic activity still exists for certain species. Although decaying material (1 g/ 454 g soil) inhibits growth of test species it is probable that the amount of decaying material in the field soil is less than the amount used, because some of the leaves persist on the plants into March and April of the following growing season. Moreover, many of them blow away from the parent plant after falling. In the case of T. flavus and L. cognatum the decaying material apparently was not the primary factor in establishment of growth patterns. It is known however that some inhibitory compounds can cause stimulation at low concentrations and it is possible that this mechanism could be partially responsible for their increased growth near ragweed. Leaf leachate of ragweed, however, probably accounts primarily for the stimulation of these species in the field. Root exudate could certainly accentuate the stimulation of T. flavus. The poor growth of Andropogon ternarius near ragweed in the field was no doubt due to inhibition by root exudate and to some extent decaying leaves of ragweed. Even the decaying air-dried fresh leaves of ragweed which stimulated most species, inhibited A. ternarius.

Laboratory tests indicate that A. psilostachya probably helps eliminate Amaranthus retroflexus, B. japonicus, E. canadensis and H. ciliatus from the first stage of

succession. On the other hand, Aristida oligantha, the dominant of the second stage of succession, is either not affected or stimulated generally by ragweed. Ragweed may also delay the entrance of the bunchgrass stage as A. ternarius, a characteristic bunch grass following Aristida oligantha (Smith, 1940), is inhibited. It is doubtful that volatile materials from ragweed are of significance in establishing patterns in vegetation as Oklahoma winds would disperse and remove the volatile materials. However, this aspect merits further investigation.

CHAPTER V

SUMMARY

Ambrosia psilostachya (western ragweed) is a characteristic species found in the first stage of old-field succession and it persists through the later stages. Previous workers found that *A. psilostachya* was inhibitory to the nitrogen-fixing algae and bacteria as well to the nitrifying bacteria. The present study was conducted to determine if *A. psilostachya* has allelopathic effects on higher plants, and what the effects of the species are on the pattern of vegetation. Field studies indicated a different pattern of vegetation around the ragweed, and initial experiments suggested that the patterns were not due to mineral and physical properties nor to competition. The root exudate, leaf leachate and decaying leaves of ragweed inhibited many of the early invaders of abandoned fields. *Andropogon ternarius*, a bunch grass, was inhibited by decaying material and root exudate of *A. psilostachya*. *Tridens flavus* and *Leptoloma cognatum*, two species found more commonly near ragweed in the field, were stimulated by leaf leachate and field soil collected near ragweed. *Tridens flavus* was stimulated, also, by root exudate of

ragweed. The differential patterns created by the ragweed were probably due to the interaction of organic compounds released by leaf leachate, root exudate and decaying materials. The possibility of volatile materials from ragweed affecting growth of associated plants was suggested. Two phytotoxins were isolated from overwintered ragweed leaves but attempts to identify them were not successful.

LITERATURE CITED

Abdul-Wahab, A.S. and E.L. Rice. 1967. Plant inhibition by Johnson grass and its possible significance in old-field succession. Bull. Torrey Bot. Club 94: 486-497.

Booth, W.E. 1941. Revegetation of abandoned fields in Kansas and Oklahoma. Amer. J. Bot. 28:415-422.

Bray, H.G., W.V. Thorp, and K. White. 1950. The fate of certain organic acids and amides in the rabbit. 10. The application of paper chromatography to metabolic studies of hydroxybenzoic acid and amides. Biochem. J. 46:271.

Bremner, J.M. 1965. Total Nitrogen. In Methods of soil analysis, part 2, p. 1149-1176. Black, C.A. ed. Amer. Soc. Agron., Madison, Wisc.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Cir. 347.

Martin, P. and B. Rademacher. 1960. Studies on the mutual influences of weeds and crops. In The biology of weeds. p. 143-152. Ed. J.L. Harper. Oxford: Blackwell.

Morgan, J.V. and H.B. Tukey Jr. 1964. Characterization of leachate from plant foliage. Plant Physiol. 39:590-593.

Muller, C.H. 1966. The role of chemical inhibition (allelopathy) in vegetational composition. Bull. Torrey Bot. Club 93:332-351.

Parenti, R.L. and E.L. Rice. 1969. Inhibitional effects of Digitaria sanguinalis and possible role in old-field succession. Bull. Torrey Bot. Club 96:70-78.

Parks, J.M. and E.L. Rice. 1969. Effects of certain plants of old-field succession on the growth of blue-green algae. Bull. Torrey Bot. Club 96: 345-360.

Piper, C.S. 1942. Soil and plant analysis. The University of Adelaide, Adelaide, Australia. 368 p.

Rice, E.L., Wm. T. Penfound and L.M. Rohrbaugh. 1960. Seed dispersal and mineral nutrition in succession in abandoned fields in central Oklahoma. Ecology 41:224-228.

Rice, E.L. 1965. Inhibition of nitrogen-fixing and nitrifying bacteria by seed plants. IV. The inhibitors produced by Ambrosia elatior L. and Ambrosia psilostachya DC. Southwestern Natur. 10:248-255.

_____. 1968. Inhibition of nodulation of inoculated legumes by pioneer plant species from abandoned fields. Bull. Torrey Bot. Club 95:346-358.

Rovira, A.D. 1956. Plant root excretions in relation to the rhizosphere effect. Plant and Soil 7:178-194.

Savage, D.A. and H.E. Runyon. 1937. Natural revegetation of abandoned farm land in the central and southern Great Plains. International Grassland Congress. Aberystwyth, Great Britain, Rept. Sect. 1 (Grassland Ecology): 178-182.

Shelton, W.R. and H.J. Harper. 1941. A rapid method for the determination of total phosphorus in soil and plant material. Iowa State Coll. J. Sci. 15: 408-413.

Smith, C.C. 1940. Biotic and physiographic succession on abandoned eroded farmland. Ecol. Monogr. 10: 421-484.

Smith, I., Ed. 1960. Chromatographic and electrophoretic techniques. Vol. I. Chromatography. Interscience Publishers, Inc. N. Y.

Tomanek, G.W., F.W. Albertson and A. Riegel. 1955. Natural revegetation on a field abandoned for thirty-three years in central Kansas. Ecology 36:407-412.

Waterfall, U.T. 1966. Keys to the flora of Oklahoma. Third ed. Published by the author. Stillwater, Oklahoma.

Wilson, R.E. and E.L. Rice. 1968. Allelopathy as expressed by Helianthus annuus and its role in old-field succession. Bull. Torrey Bot. Club 95:432-448.

Woods, F.W. 1960. Biological antagonisms due to phytotoxic root exudates. Bot. Rev. 26:546-569.