AN ABSTRACT OF THE THESIS OF

Karrie I. Rathbone for the Master of Science

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Title : Determining Allelopathic Potential For Two Weed Species In The Flint Hills

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A plant community is comprised in part of individual plants that are tolerant of their neighbors' competitive strategies. Through the process of succession a plant community changes in composition over time. The competitive strategies used in this community can include allelopathy. Allelochemicals are secondary metabolites which inhibit non-tolerant species. In local disturbed communities in the Flint Hills area, two weed species commonly seen in the early stages of succession are Ambrosia trifida and Helianthus maximilliani. Both species flower late in the growing season and are suspected of allelopathy. As these mature plants decompose, the allelochemicals should be broken down into simpler phenolic acids and released into the environment. This plant residue may affect the germinating seeds of the following growing season. Field research determined the species distribution and plant associations of the communities. Results of the field research showed no relationships with Ambrosia trifida. There was a negative association between Helianthus maximilliani and Solidago canadensis. Germination bioassays were performed in the lab with water extracts from the test species. High performance liquid chromatography methods were used to determine the phenolics present in the plant material.

Determining Allelopathic Potential For Two Weed Species In The Flint Hills

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Karrie I. Davidson Rathbone

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Approved for the Biology Division

Committee member

Committee member

Committee member

Major advisor

Approved for the Graduate Council

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I. INTRODUCTION:

Secondary succession is the concept that a plant community is dynamic from a disturbance date to a stable climax. There is a progressive change in the assemblage of plants found together over time. These plants vary according to their strategies on the *r-k* continuum. The rate of succession depends on interspecific competition, disturbances, and community type (Harper, 1977). When a community is disturbed (*e.g.*, as in an agricultural field), the species composition consists of those with life spans and tolerance levels for continued disruption (Bleasdale, 1960).

Plants that are opportunistic and colonize early in succession with high seed production are considered *r*-selected in their strategy. Aggressive annual species make up a large part of the early disturbance flora in the succession process. These annuals are *r*-selected in that they have seasonal, rather than multi-year, life spans and depend on abundant seed dispersal and increased germination for the next season (Ashton and Klingman, 1975). As these annual species become less competitive in a community, they are replaced by (*k*-selected) biennials and perennials that have longer life spans and an extensive root systems, as well as seed dispersion for

competitive success. These plants that are acclimated to a more stable habitat and maintain their populations in sustained equilibrium are termed k-selected. They are density-dependent and rely on perennial root systems. The climax stage of succession is considered to be a community in equilibrium (Horn, 1974). Climax plant communities are comprised of k-selected plants that are tolerant of their neighbors' competitive strategies.

This type of successional exclusion is referred to as the competitive hierarchy, in which there is an interaction of the plants at different environmental levels (Horn, 1976). The earliest phase of succession after a disturbance is the invader weedy stage. The second and third phases include the annual grasses preceding the perennial bunch grasses. Finally, the climax phase of succession, or the most stable stage, slowly dominates the area. The climax species are more density-dependent. In the case of the Osage Cuestas and the Flint Hills area, this is a "true tallgrass prairie" consisting of Andropogon gerardii, Sorgastrum nutans, Andropogon scoparius, and Panicum virgatum (Booth, 1941). This process of succession is not linear; the species present depend on the plant community factors, which refer

back to competitive hierarchy. Many species are present with seed accessibility early in succession, but only become dominant when the other conditions are not favorable to the preceding species. Usually this is the result of a species endurance through competition and increasing community density.

The plant community is a function of several factors: local flora, seed bank accessibility, habitat, time of disturbance, and plant ecological/physiological properties (Mueller-Dombois and Ellenberg, 1974). A fluctuation in any of these biotic and abiotic factors could influence a plant in its distribution, abundance, and interspecific associations. As the environment of the community changes, so does the plant composition (Altieri and Liebman, 1988). Plant species often found together could have an affinity for similar environments. The interaction between differing species relates to their tolerance of a particular assemblage of species in a community.

In local disturbed communities in the Osage Cuestas area, I determined an annual weed species commonly seen in the early stages of succession to be Ambrosia trifida, giant ragweed. Giant ragweed can dominate a disturbance

site and become quite tall if the supply of water is adequate. It flowers in the fall and has bulk seed production. These seeds are usually brought into an area by flooding. A perennial commonly seen in dense stands on disturbed sites is Helianthus maximilliani, Maximillian sunflower (Soule and Piper, 1992; Vuturo, 1971). Both species flower late in the growing season (Barkley, 1983) and are suspected of allelopathy (Rice, 1984).

In the early part of the growing season, these seedlings must tolerate shading and other competitive strategies by early-flowering plants. To be successful, the species must compete efficiently for space, light, and nutrients (Ashton and Klingman, 1975). According to Elroy Rice (1984), there is fierce competition in the early stages of succession; this suggests one of these competitive strategies is allelopathy.

Allelopathy is the chemical interaction of plants involving secondary compounds. These secondary compounds (termed allelochemicals) are mostly simple phenolic acids arranged into a more complex structure as a part of the plant tissue (Rice, 1984). Only plants tolerant of the allelochemical levels in their community can be a prominent part of that community (Muller, 1969). It has

been indicated that allelochemicals are produced by the seedlings to ensure early growth (Tang and Zhang, 1986). Another increase of allelochemicals could be produced just prior to flowering, to promote the plant for pollination (Whittaker, 1969).

Many phenols arise from the shikimic acid pathway as secondary compounds (Chou and Patrick, 1976): cinnamic, p-coumaric, caffeic, ferulic, chlorogenic, protocatechuic, quinic, and gallic acids (Ribereau-Gayon, 1972). This pathway produces aromatic amino acids, including phenylalanine, tyrosine, and tryptophan. These aromatics are the parent materials for the other products resulting from this process. Another major product of secondary compounds is lignin, the rigid structural material of cell walls in plant tissues.

Some allelochemicals have been shown to act to a moderate extent as herbicides (Anderson and Beardall, 1991). Herbicides are toxic to plants because they block the synthesis of amino acids. For growth, plants must produce 20 different amino acids. The properties of an herbicide include causing an end-product inhibition of the pathway enzymes by a build-up of shikimic acid. This feed-back inhibition stops synthesis of the amino acids

and thus stops plant metabolism. The use of these allelochemicals might be a potential alternative for weed control (Altieri and Doll, 1978).

Allelochemicals should be identified directly from the plant material. A quantified chromatography technique used to identify allelochemicals is High Performance Liquid Chromatography (HPLC). The phenolic acids are moved along in a slightly polar mobile phase through a column of a non-polar solid phase, separating the compounds for ultraviolet spectral detection. It is possible to detect the absorbance of double bonds using HPLC with an ultraviolet detector. The secondary plant compounds are separated based on their formula weight, ring construction, and partial polarity of side chains. The simple phenols are in this category by having at least one benzene ring for detection. Retention time in the column and ultraviolet wavelengths are used to identify the peak produced by the compound. Peak height indicates the concentration of the compound based on a standard calibration curve.

My hypothesis for this research was that as the mature plant tissue slowly decomposes, these chemicals should be broken down into simpler phenolic acids and

released into the environment. This plant residue should affect the germinating seeds during the following growing season. Mature plant species found together in a community should be tolerant of each other's breakdown products. The null hypothesis was that all the plant species in a community would be independent from one another, exhibiting no competition resulting from allelochemicals. If germination inhibition exists, then a negative association between the plant species should be observed. The allelochemicals produced should be detectable with the assistance of HPLC.

In this study I observed a natural plant community in which the test species, Ambrosia trifida and Helianthus maximilliani, occurred, and looked for evidence of competition strategies. The objectives of this study were to determine: 1) the distribution of the test species in their study sites; 2) the plant associations of the test species; 3) the effects of their tissue decomposition on associated species; and 4) the identification of simple phenolic acids released from the plant material.

II. MATERIALS AND METHODS:

A. Field Studies

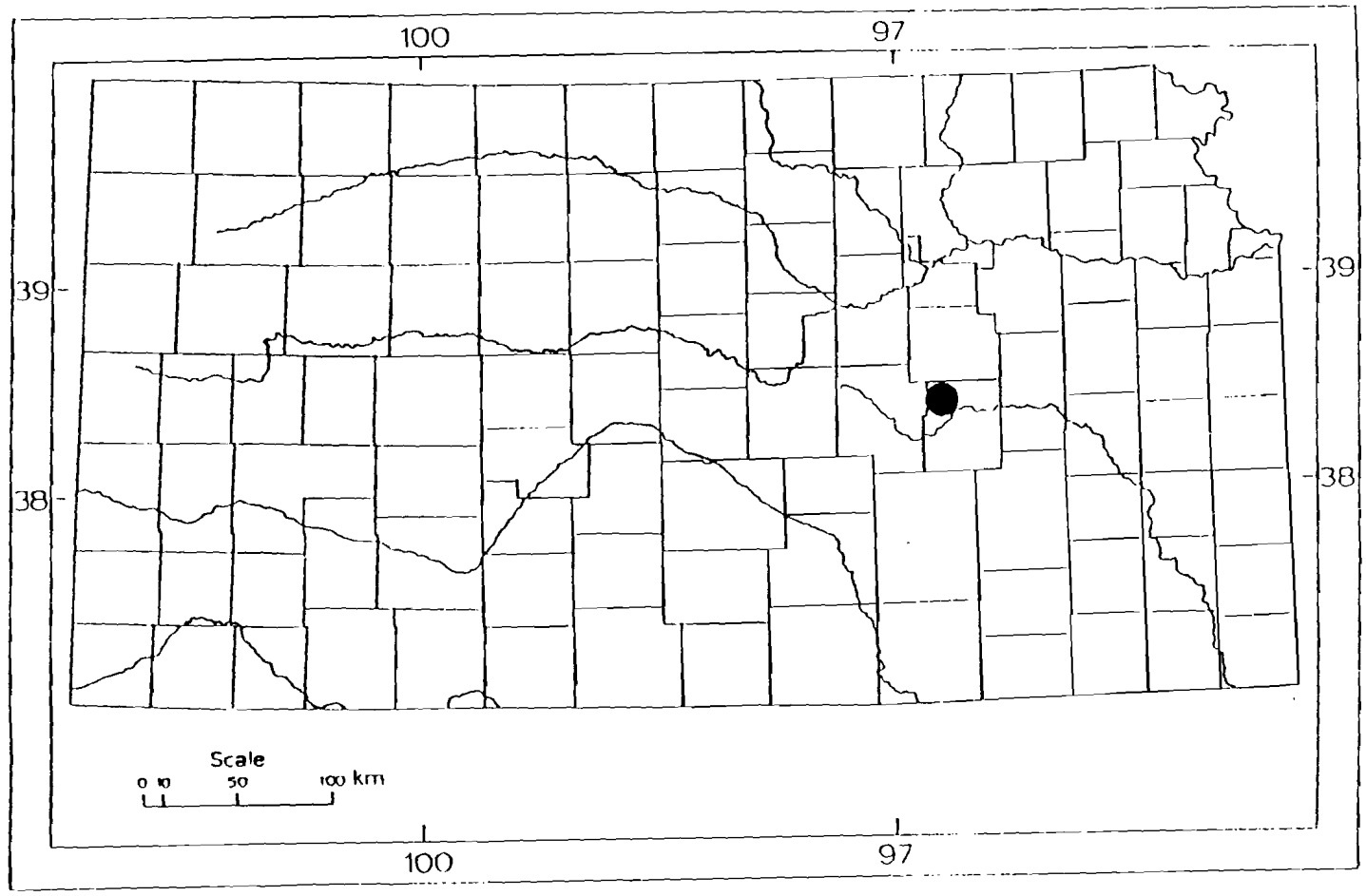
1. Site Location

The sites that were sampled are on the property of Dr. James Mayo, in Chase County, Kansas, five miles west of Elmdale (Figure 1). The first site is bordered on the north by a corn field and on the south by a stream bed. This site has giant ragweed and is often disturbed by flooding. The second site is an abandoned milo field that has not been farmed since 1986. It is bordered on the north by a climax native grassland and on the east by a stream bed and farmland. The west and south edges are bordered by a hardtop road and ditch, the latter of which is shared by the climax grassland. The second site is mostly noted for the presence of Maximillian sunflower. The climax grassland site, characteristic of a tall grass prairie, has never been plowed or grazed, and was sampled for *Ambrosia trifida* and *Helianthus maximilliani*.

2. Spatial Patterning

The distribution of the two test species, giant ragweed and Maximillian sunflower, was assessed using a plotless T-square distance sampling technique described by Ludwig and Reynolds (1986). The sampling method was

Figure 1. Location of study site at Dr. James Mayo's farm, 5 miles west of Elmdale, Kansas in Chase County.

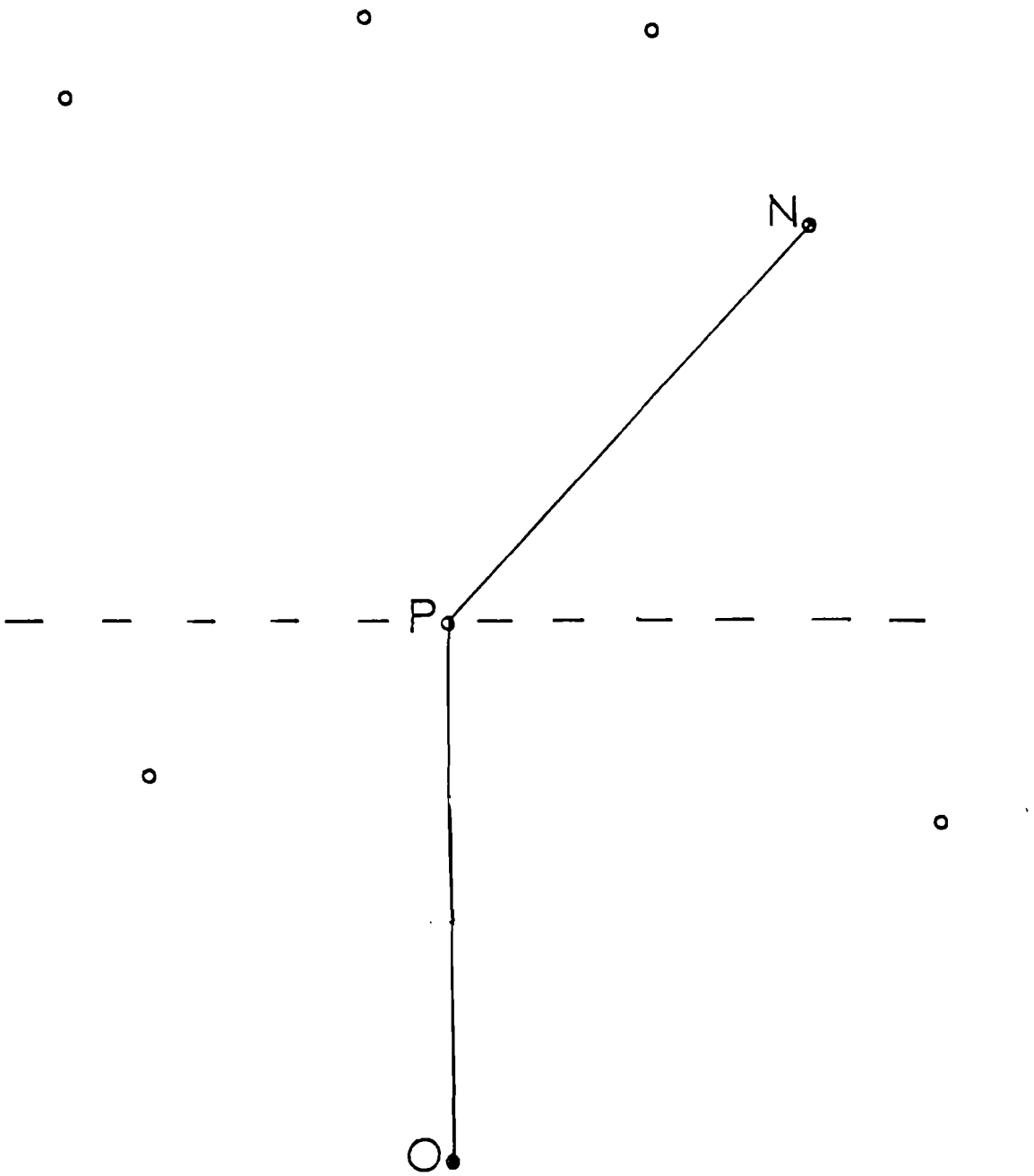

100
97
39
38
39
38
Scale
0 10 50 100 km
100
97

developed for a quick assessment of the vegetation density. This is a powerful method that avoids sampling empty quadrats when the species are sparse by distribution. The sampling points of origin are chosen randomly on a constructed grid. The measure of distance (x) is from the point of origin to the individual plant. At a perpendicular line through the individual, another distance (y) is measured in the same half plane to the nearest neighbor of the same species (Figure 2). An index of spatial pattern (C) and dispersion (I) is determining if the test species is random, clumped, or uniform in its distribution. The index of spatial patterning then can be calculated as a ratio of x^2 and y^2, $C = \text{sum } N_{i=1} [x^2_i/(x^2_i + 1/2y^2_i)]/N$. The value of C is 0.5 for random patterns, <0.5 for uniform distribution, and >0.5 for a clumped distribution. A z statistic is computed for a standard normal distribution to test the significance of any deviation from 0.5, [z = 1.96 at p = 0.05], z = C - 0.5/sqrt(1/(12N)).

3. Interspecific Association

The total species composition of the interspecific associations of each site was sampled by using randomly placed meter-square quadrats. This is a measure of how

Figure 2. T-Square distance sampling as described by Ludwig and Reynolds (1986). The distance x is measured between the orgin (o) and the nearest individual (p). The distance y is then measured from point (p) to the nearest neighbor of the same species (N) in the same half-plane.


N
P
O

often two species are found in the same area. An association can exist between two species when they select or avoid the same community because of similar requirements, or they might exhibit an affinity for each other. The study sites were measured at two-meter intervals for axes X and Y and marked into a grid. The minimum area for sampling a grassland is 50-100 m^2. The minimal area for sampling a weedy ecosystem is 25-100 m^2 (Mueller-Dombois and Ellenberg, 1974). The sampling points were randomly selected within the study site (Figures 3 and 4). To make sure enough quadrats were sampled, the data from the quadrat area was used in plotting a species-area curve (Caine, 1938). When the curve plateaued, the maximum plant density had been sampled.

The total species composition of each quadrat was recorded as binary data of either presence (1) or absence (0) (Ludwig and Reynolds, 1986). The plant associations were determined from the binary data in a 2x2 contingency table, and then tested with a Chi-square, to determine if the occurrence of two species together was higher than expected. The Ochiai Index was also used as a measure of test strength for the Chi-square. An interspecific

Figure 3. Position of 50 random quadrats at site #1. Location is determined by a grid marked off at the site in 2 meter sections for x and y axes. The presence of giant ragweed in a quadrat is shown with darkened circles. The triangles show the absence of giant ragweed.

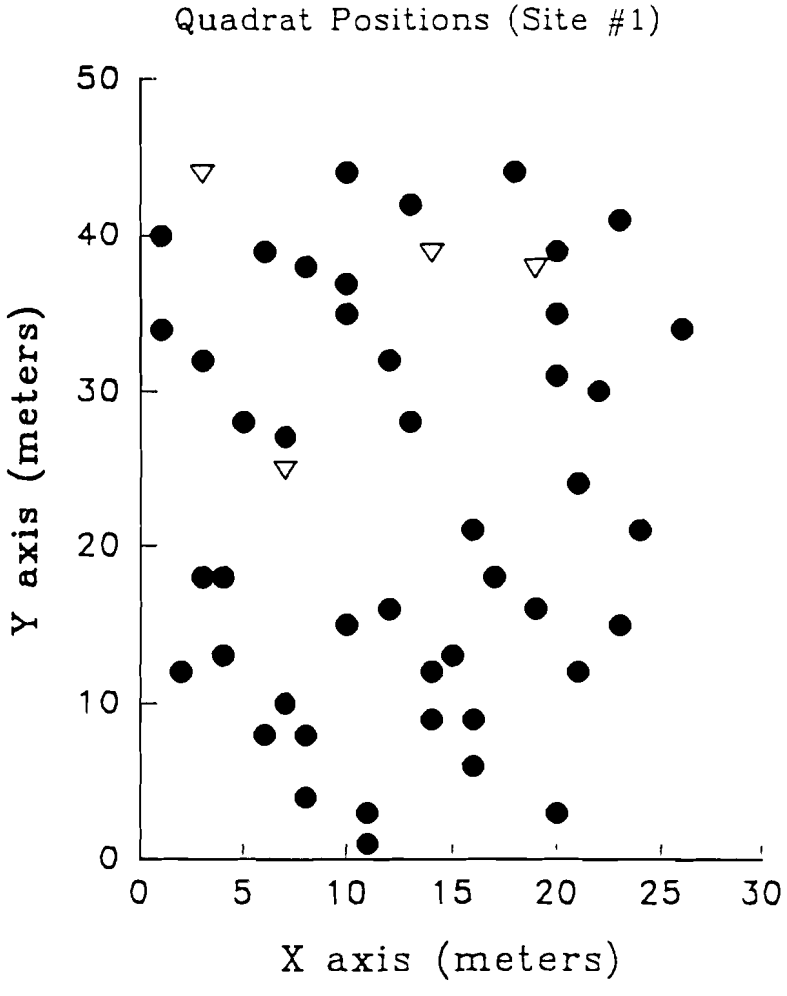
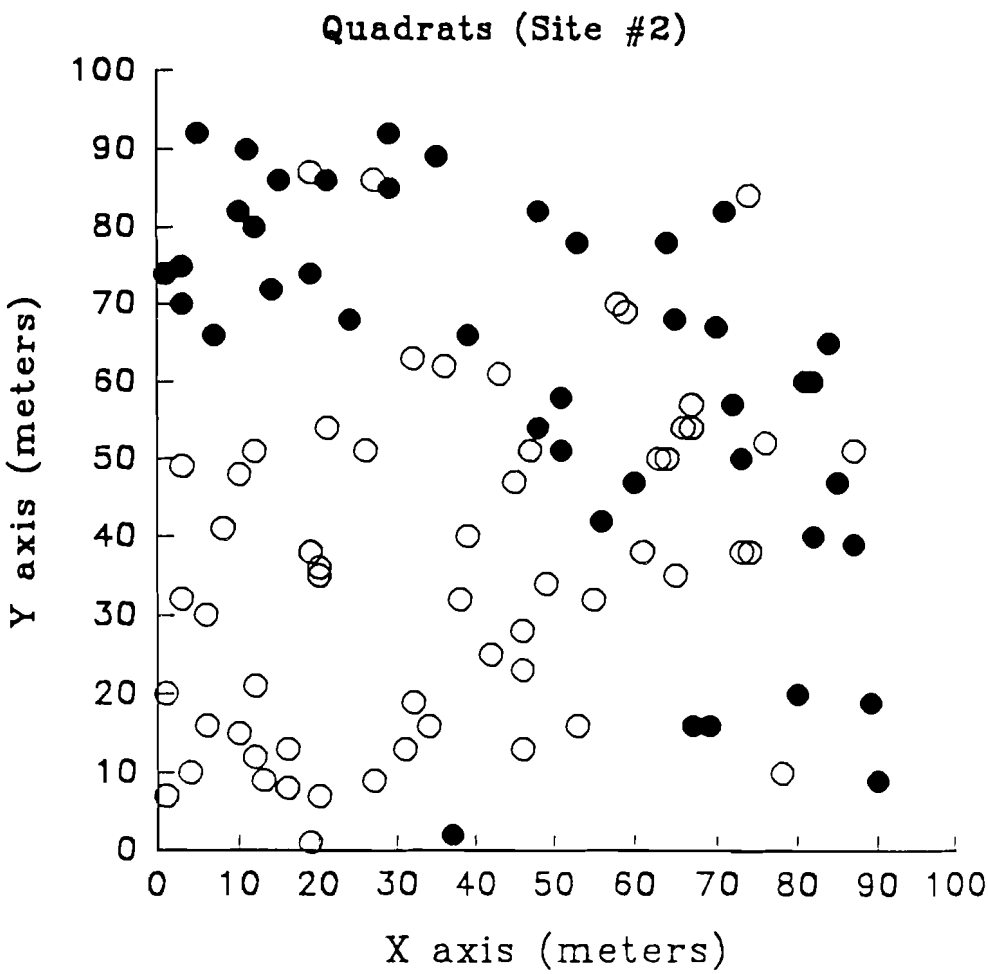

Quadrat Positions (Site #1)
Y axis (meters)
X axis (meters)
0
10
20
30
40
50
0
5
10
15
20
25
30

Figure 4. Position of 100 random quadrats at site #2. Location of the quadrats is determined from a 2 meter increment on x and y axes marked at the site. The presence of Maximillian sunflower in a quadrat is shown with darkened circles. The open circles show the absence of Maximillian sunflower.


Quadrats (Site #2)
Y axis (meters)
X axis (meters)
0
10
20
30
40
50
60
70
80
90
100

association was recorded as positive, negative, or none.

4. Plant Material Collection

At the end of the growing season, plant material samples were taken of Ambrosia trifida and Helianthus maximilliani. Leaves and stems were clipped and deposited into labeled paper bags. The samples were placed in the drying ovens at 20 degrees Celsius for analysis at a later time. Seeds of representative plants at the study sites were harvested for germination assays in the lab. The seeds were put into zip-lock bags with moist sand and placed in the refrigerator for future germination tests. The moist sand is necessary to provide water for imbibing the seed. The refrigerator mimics the winter coldness to break seed dormancy.

B. Laboratory Studies

1. Water Extracts

After the plant material was dried, the samples were ground in a quaker mill and passed through a 0.13-mm screen. These ground samples were stored in sealed plastic containers at room temperature. Differing concentrations of water extracts (w/v) were obtained by combining a weighed amount of sample material with a standard amount of distilled water, and shaken for 24

hours in 250 ml flasks. Amounts measured were 0.129 g, 0.189 g, 0.025 g, 0.5 g, 0.75 g and 1 g of giant ragweed and Maximillian sunflower in 150 ml distilled water, making concentrations of 0.5, 0.75, 1, 2, 3, and 4, respectively. The extracts were then vacuum-filtered through medium/coarse porosity filter paper (Bhowmik and Doll, 1982). These filtered water extracts were then used for the germination assays. They were stored between applications at 5 degrees Celsius. New water extracts were made up after five days of applications to avoid microbial degradation of the extract.

2. Germination

Germination tests are very important in determining the allelopathic activity of a species. Native seeds from the study sites were used for the association assays. Purchased agronomic seeds of wheat and corn were used to show sensitivity. Controls of distilled water were used for all tests.

Twenty-five seeds of each species to be tested were placed on absorbent paper in a section of the appropriate germination tray (Copeland and McDonald, 1985). The corn assays only allowed for 12 seeds per section because of size. Forty milliliters of each Ambrosia trifida water

extract concentration was added to the appropriate absorbent material for each seed type. Additional treatments were added and recorded as needed to keep the seeds from drying out. This procedure was repeated using the Helianthus maximilliani water extract concentrations for each seed type.

There were four sections in each germination chamber, each with a different aqueous extract concentration (0, 0.5, 0.75, and 1.0) per extract type. Because of a lack of response, additional tests were run with higher concentrations of 0, 2, 3, and 4 of Ambrosia trifida and Helianthus maximilliani extracts. Each seed type had multiple trials run for each extract type, depending on response. Each test was allowed to run for five days. The percent germination was recorded daily as a percent of the control (Tang and Zhang, 1986). The mean shoot length was calculated and recorded on the fifth day for each section under seed type and extract type.

The raw data were entered and analyzed using statistical analysis package (SAS) on the Internet. The significance of the germination data was determined with a nested model I ANOVA (analysis of variance) (Zar, 1984).

Methanol extractions were used for phenol

identification. Unknown extracts were compared to known phenols suspected of allelopathy, using High Performance Liquid Chromatography (HPLC) methods (Waterman and Mole, 1994).

3. Methanol Extractions

Methanol extractions were used for phenol identification. Unknown extracts were compared to known phenols suspected of allelopathy, using High Performance Liquid Chromatography (HPLC) methods (Waterman and Mole, 1994). The plant material (0.300g) was weighed and packed into glass pipettes. Lipids in the samples were removed by pouring 15 milliliters of methylene chloride through each column, in 5 milliliter aliquots. The solution collected was then dried to a constant weight to determine the lipid composition from previously consistently-dried test tubes. Lipids can be damaging to an HPLC column, which is made up of 5-micron-sized particles. The plant material was then washed with 80 milliliters of methanol to extract the phenolic acids. This methanol extract was filtered through a 0.1 millimeter-pore vacuum filter and stored in a aluminum foil-wrapped glass bottle in the refrigerator.

4. HPLC

For HPLC procedures, a C-18 reversed-phase column was used. The column dimensions were 125 x 4 mm in diameter with 5um particle size. In the reversed-phase method, the most polar component elutes first. The mobile phase was a water (polar) and methanol (moderately polar) mix with the flow rate at 2.5 ml/min. The ultraviolet wavelengths of purchased phenolic acids were determined by testing with a spectrophotometer. According to their spectra, the highest absorbance, on the up slope, was recorded for each phenolic stock solution. The best ultraviolet wavelength for reading was determined to be 220nm for total phenolic detection. Optimization tests were run on the unknown extracts with the HPLC to determine the best mobile phase and method. More methanol decreases the t_R (retention) time (Cresser and Marr, 1983). The mobile phase of methanol and water was adjusted for the best peak performance and separation of the unknown extracts. The retention times were adjusted so that all analytes were eluted in 20 minutes or less. The range was set to about twice the expected height of the largest peak.

The stock solutions of suspected phenols were prepared by weighing each chemical into a tared, 250 ml

volumetric flask filled with 70% methanol. Stock solutions were mixed until dissolved. Standards were prepared at differing concentrations with micropipettes (0, 1, 2, 3, and 4 microliters) of the suspected phenols to plot a calibration curve based on peak height. The different concentrations of standards were injected, based on the unknown extract method and mobile phase. Peak heights were recorded in centimeters and absorbance units. The concentration of the unknown was computed by dividing the average peak height of the unknown by the average height of the standard, and then multiplying the total by the standard concentration $\{C_x = C_s (H_x/H_s)\}$. By lowering the concentration of the unknown, a detection limit of the unknown was found; three injections of the lowest unknown concentration were followed by three injections of the lowest concentration of standard. A detection limit is determined by dividing the standard deviation by the sensitivity and multiplying by three $\{C_L = 3(S_b/m)\}$. At a relatively high pH, the organic acids disassociate, therefore 0.25% phosphoric acid was added to keep the pH constant at 3 (Schroeder, 1993).

III. RESULTS

A. Field Studies

1. Spatial Patterning

The index of spatial patterning for each test species was determined for the distribution, using the T-square technique. If C is >0.5, then it is clumped. If it is <0.5, then it is uniform. If it is equal 0.5, then the species is random by distribution in the population. The index for giant ragweed at 80 sample points was 0.387 with a z statistic at 10.446 and was considered to be uniform in the population. The Maximillian sunflower index at 30 sample points was 0.767 with a z statistic of 13.62, and was categorized as clumped in the population.

2. Species Area Curve

Plant species lists were recorded for each site based on the random quadrats (see Tables 1 and 2). From this, a species area curve was developed. Site 1 leveled off at 27 species and site 2 leveled off at 17 species per 30 quadrats (Figures 5 and 6). Site three had about 17 species for 30 quadrats but did not completely level off (Figure 7).

3. Interspecific Association

The top five species of greatest occurrence were used

Table 1. Species list and occurance for the community sites observed in 200 quadrats.

SPECIES	Intermediate site(#2)	Climax site(#3)
Helianthus maximiliani	42	1
Helianthus annuus	5	1
Ambrosia trifida	1	0
Ambrosia artemesifolia	19	0
Solidago missouriensis	78	4
Bromus japonica	78	0
Artemisia ludoviciana	9	9
Erigeron strigosus	25	0
Cirsium undulatum	1	0
Aster ericoides	3	55
Bromus tectorum	3	0
Solanum carolinense	1	0
Sorghastrum nutans	1	62
Asclepias syriaca	1	0
Conyza canadensis	1	0
Eupatorium altissimum	1	0
Achillea millefolium	1	2
Amorpha canescens	0	31
Euphorbia nutans	0	5
Salvia pitcherii	0	19
Baptisia bracteata	0	1
Eupatorium perfoliatum	0	3
Polytaenia nuttallii	0	2
Panicum virgatum	0	19
Andropogon gerardii	0	83
Andropogon scoparius	0	83
Ratibida columnifera	0	17
Cassia chamaeerista	0	3
Kuhnia eupatorioides	0	3
Rosa arkansana	0	1
Rhus glabra	0	1
Helianthus tuberosus	0	1
Desmanthus illinoensis	0	0

Table 2. Species list for the disturbance site at 50 quadrant observations.

SPECIES	Disturbance site(#1)
Rumex altissimus	
Verbena stricta	11
Desmanthus illinoensis	7
Polygonum pensylvanicum	5
Asclepias syriaca	1
Cuscuta sp.	1
Rumex crispis	10
Chenopodium album	1
Viola sp.	1
Hordeum jubatum	2
Galium aparine	1
Apocynum cannabinum	11
Amaranthus retroflexus	0
Helianthus tuberosus	1
Carex sp.	5
Verbena hastuta	9
Elymus canadensis	5
Sorghum halepense	2
Plantago sp.	2
Lamium amplexicaule	3
Ambrosia trifida	46
Helianthus annuus	2
Aster ericoides	1
Circsium undulatum	0
Solidage missouriensis	16
Solanum carolinense	0

Figure 5. Species area curve for site #1 as determined by the number of different species found in random quadrats.

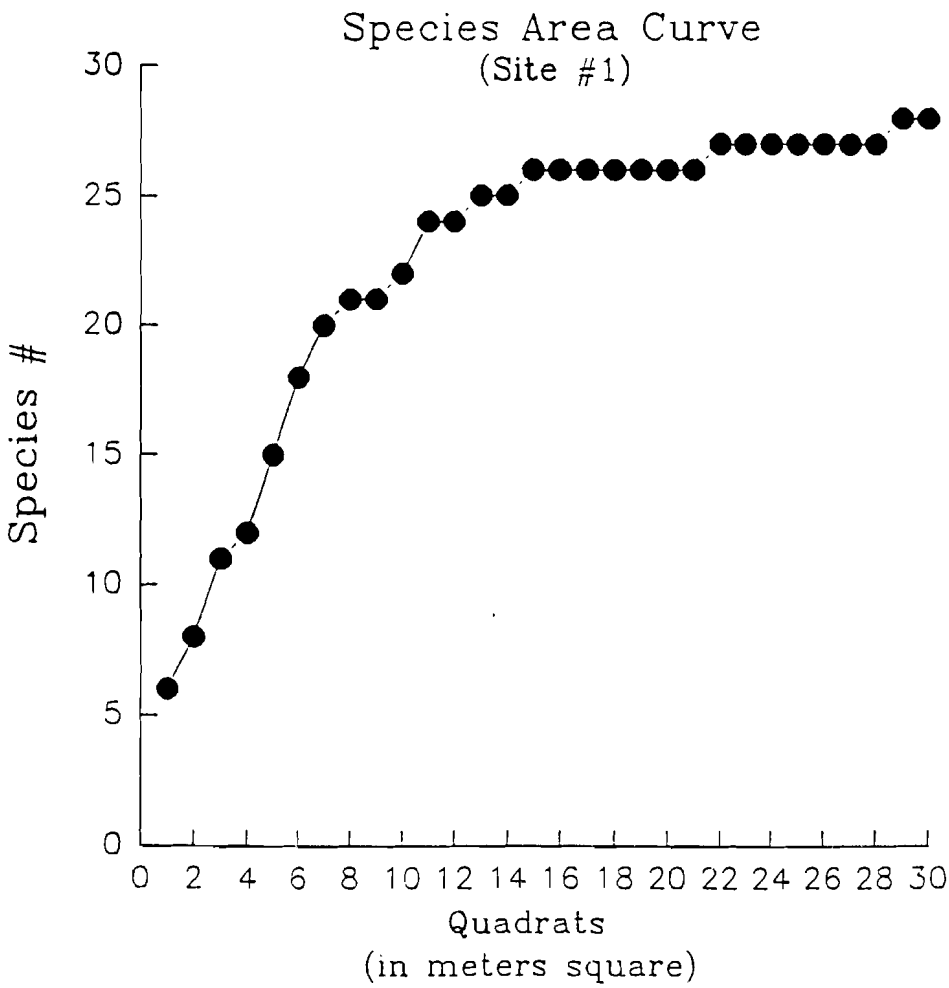

Species Area Curve
(Site #1)
Species #
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Quadrats
(in meters square)

Figure 6. Species area curve for site #2 as determined by the number of different species found in random quadrats.

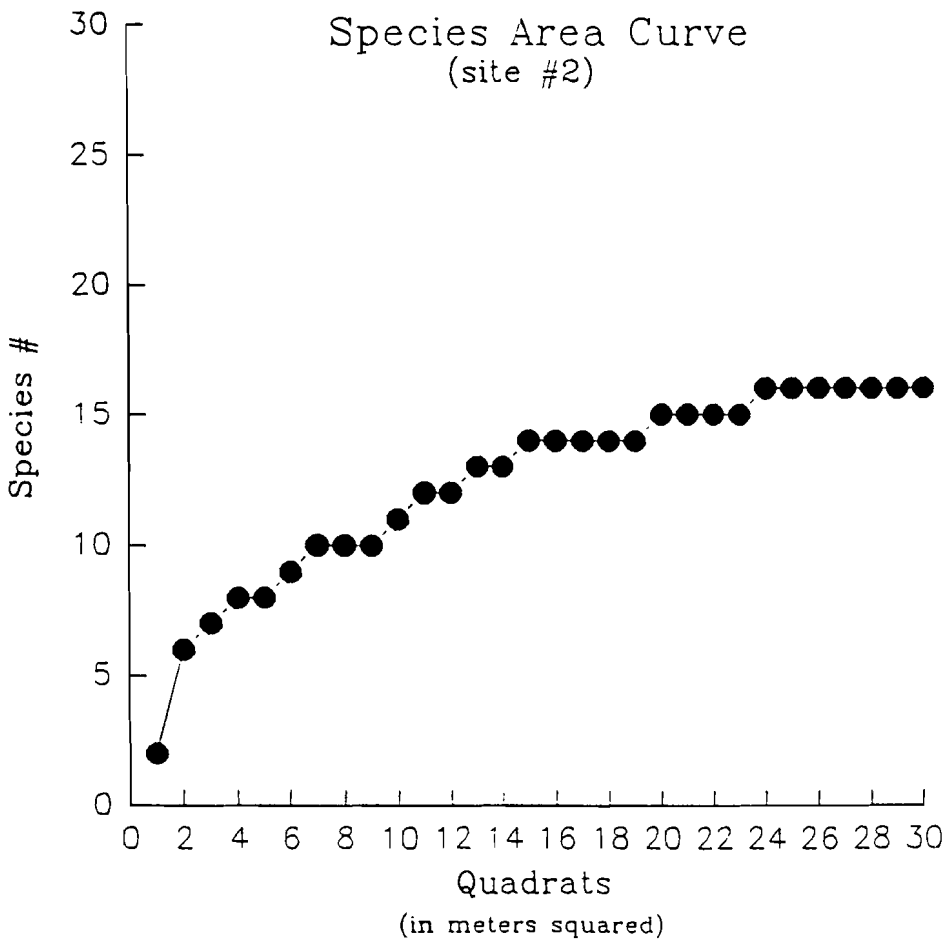
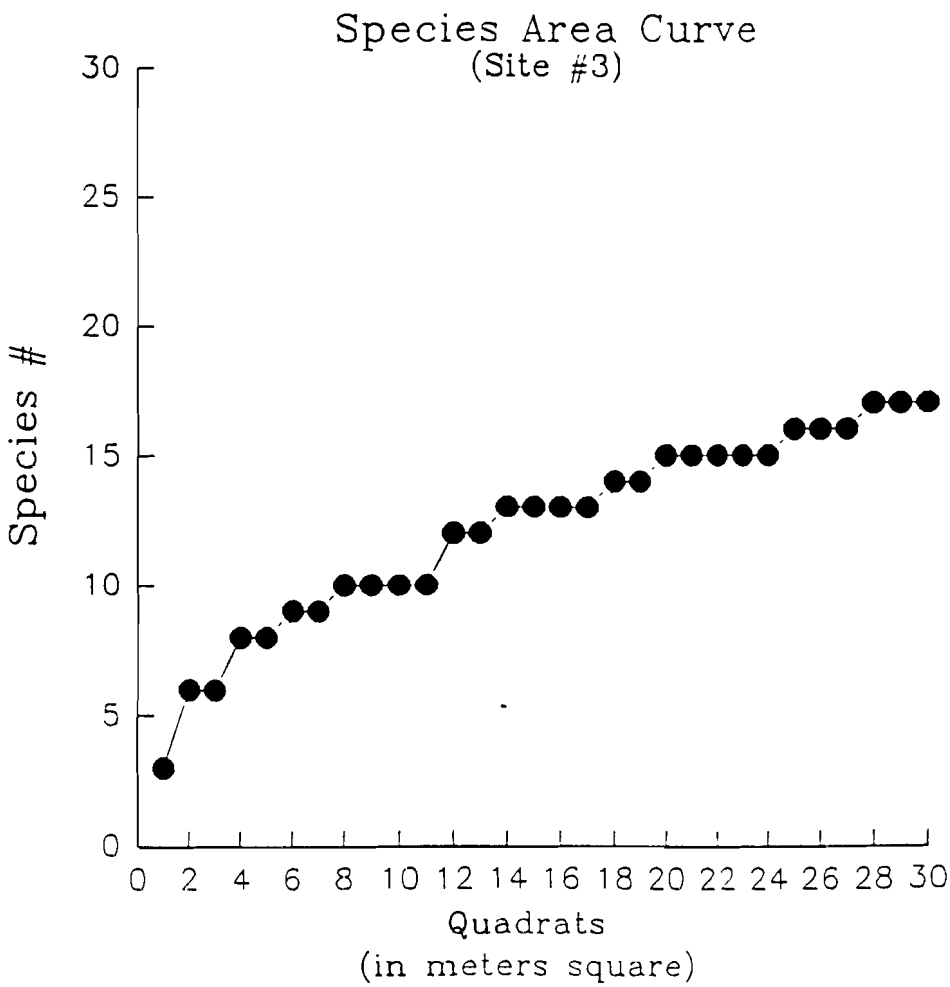

Species Area Curve
(site #2)
Species #
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Quadrats
(in meters squared)

Figure 7. Species area curve for site #3 as determined by the number of different species found in random quadrats.


Species Area Curve
(Site #3)
Species #
30
25
20
15
10
5
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Quadrats
(in meters square)

for statistical testing using a Chi-square for plant associations. The contingency table had one degree of freedom with a probability of 0.05, calculating the Chi-square at 3.84. If the Chi-square is greater than 3.84, then the null hypothesis of independent association is rejected. A contingency table was constructed for a test of association for two species determining the expected frequencies. There are four cells in the table, in which a is the number of quadrats where both species (A and B) occur together. The number of quadrats where species A is present and species B is absent is cell b. The number of quadrats where species B is present and species A is absent is cell c, and cell d is where both species are absent. The total number of quadrats sampled is N. The expected values are calculated based on the null hypothesis that the species are independent of each other, i.e., no association.

There are two types of associations that can be determined using the Chi-square. A positive association occurs if the observed value for cell a is greater than the expected value for cell a; the two species were found together more often than expected. A negative association is shown if the observed value for cell a is less than the

Table 3. Species association for giant ragweed (Ambrosia trifida) and the top 5 species as determined from random quadrats at site #1. The VR is the variance ratio to test an index of association. The expected value for the VR is 1.0. If the VR is greater than 1.0 then there is a possible positive association. If the VR is less than 1.0 then there is a possible negative association. The statistic W is computed to test whether deviations from 1.0 are significant.

Site # 1 (Ambrosia trifida) VR = 1.00 , W = 50.04			
SPECIES	**ASSOCIATION**	**CHI-SQUARE**	**OCHIAI**
Solidago missouriensis	negative	0.163	0.522
Polygonum pensylvanicum	negative	1.049	0.405
Bromus japonicus	positive	1.822	0.885
Carex sp.	negative	0.337	0.304
Rumex crispis	negative	0.780	0.430

Table 4. Species association of Maximillian sunflower (Helianthus maximilliani) and the top 5 species determined by random quadrats at site #2. The VR is the variance ratio to test an index of association. The expected value for the VR is 1.0. If the VR is greater than 1.0 then there is a possible positive association. If the VR is less than 1.0 then there is a possible negative association. The statistic W is computed to test whether deviations from 1.0 are significant.

Site #2 (Helianthus maximilliani) VR = 0.80, W = 80.18			
SPECIES	ASSOCIATION	CHI-SQUARE	OCHIAI
Ambrosia artimeisifolia	positive	0.278	0.319
Artemisia ludoviciana	positive	0.024	0.206
Solidago missouriensis	negative	18.358	0.419
Bromus japonicus	positive	12.540	0.699
Erigeron strigosus	negative	6.623	0.154

expected value for cell a; the two species were not found together as expected. The strength of the association was determined for each pair using a Ochiai index. This index is zero for no association and one for a maximum association. The Ochiai index is based on the geometric mean. The association was biased if the frequency for a cell was less than one or if one species was in all quadrat sites.

An association for giant ragweed with cheat grass was found to be positive with a Chi-square of 1.822 and a Ochiai index of 0.885 (Table 3). The associations for ragweed were biased because of its dominant uniform nature. Associations found for Maximillian sunflower were positive with cheat grass (Bromus japonicus) with a Chi-square of 12.540 and a Ochiai index of 0.669. A strongly negative association was exhibited with goldenrod (Solidago missouriensis), with a Chi-square of 18.358 and a Ochiai index of 0.419 (Table 4).

Variance ratios (VR) were calculated for each site. This ratio is an index of overall association in the community. Accepting the null hypothesis that the community had no overall associations, VR = 1. If the VR > 1 then there is a possible positive association. If the

VR < 1 then there is a possible negative association. A W statistic is calculated to test whether deviations from 1 are significant, W = (N)(VR). This statistic is also based on the null hypothesis of no association. To indicate no association then the W statistic lies in the chi-square limits. The variance ratio for site #1 is 1.00 with a W statistic of 50.04. Site #2 has a variance ratio of 0.80 and a W statistic of 80.18.

B. Laboratory Studies

1. Germination

The germination percentage for wheat was between 80 and 100%, whereas for corn it was 50 to 60% (Figures 8 and 9). These agronomic species showed consistent germination and demonstrated no statistically significant difference among extract treatment groups. The native seeds showed great variability in seed germination. The Maximillian sunflower exhibited germination from 0 to 40% (Figure 10). The cheat grass germination was between 20 and 60% (Figure 11). The Maximillian sunflower showed no significant autotoxicity with its own extract at the p = 0.5 level. The Maximillian sunflower with the giant ragweed extract treatment was also not significant at the p = 0.5 level. The cheat grass with the Maximillian sunflower extract

Figure 8. The percent germination of wheat seed with differing concentrations of Maximillian sunflower (MS) and Giant ragweed (GR) extracts.

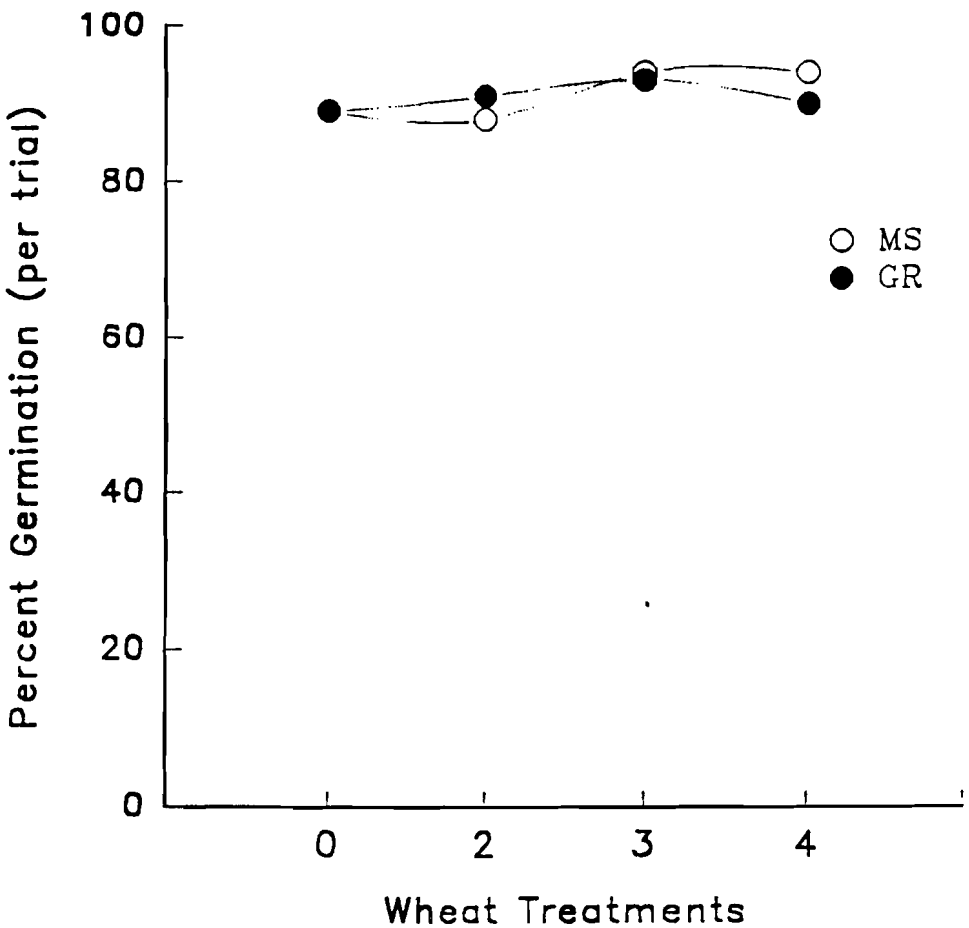

100
80
60
40
20
0
Percent Germination (per trial)
MS
GR
0
2
3
4
Wheat Treatments

Figure 9. Percent germination of Corn seed at differing concentrations of Maximillian sunflower (MS) and Giant ragweed (GR) extracts.

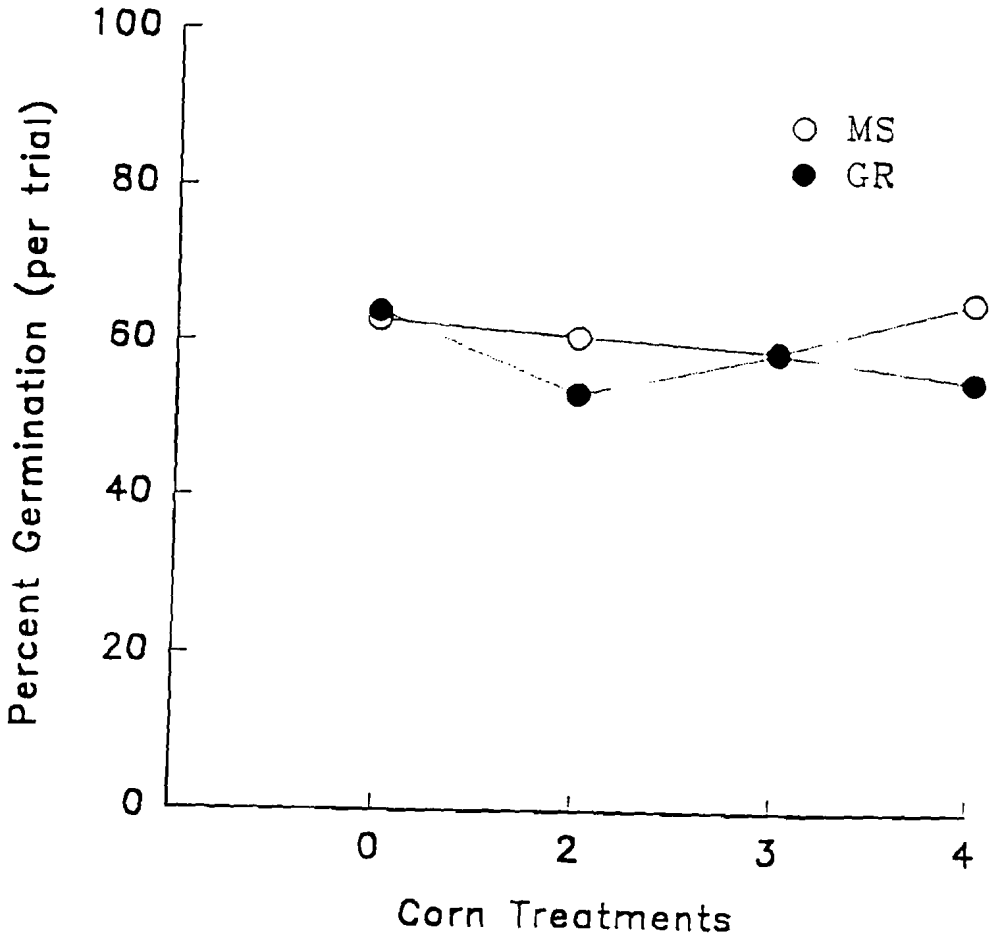

100
80
60
40
20
0
Percent Germination (per trial)
MS
GR
0
2
3
4
Corn Treatments

Figure 10. Percent germination of Maximillian sunflower seed in differing concentrations of Maximillian sunflower (MS) and Giant ragweed (GR) extracts.

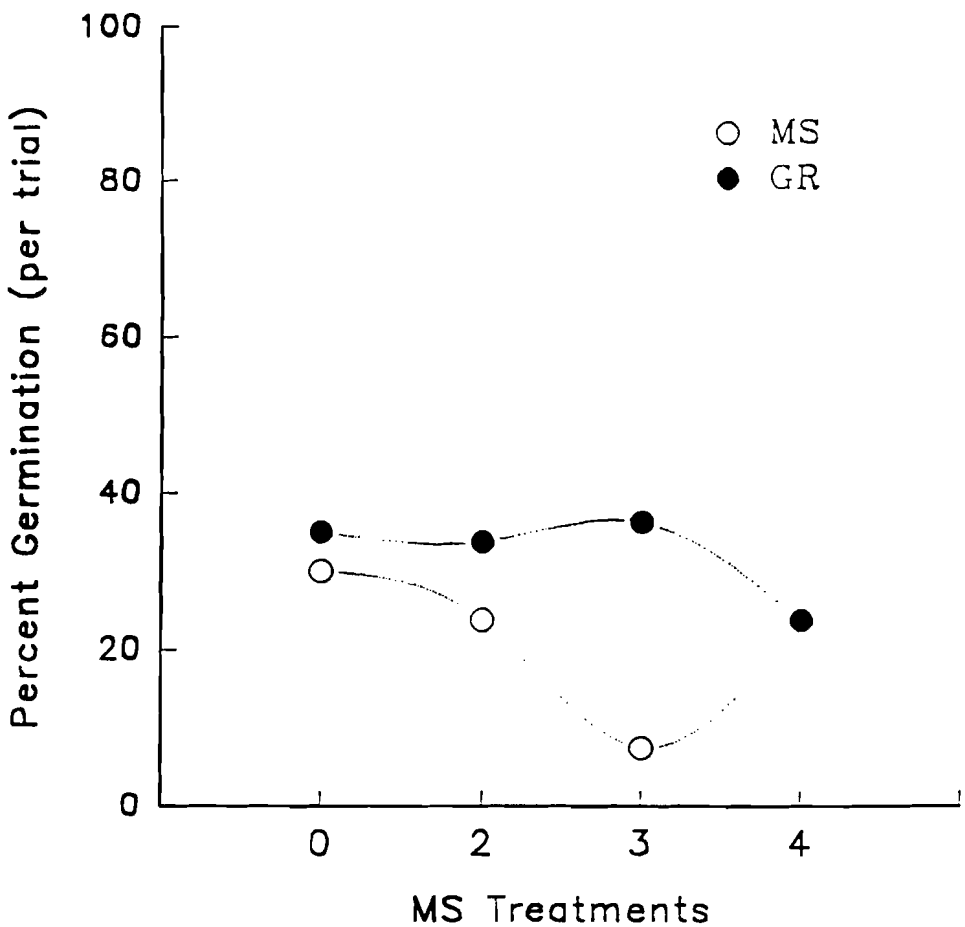
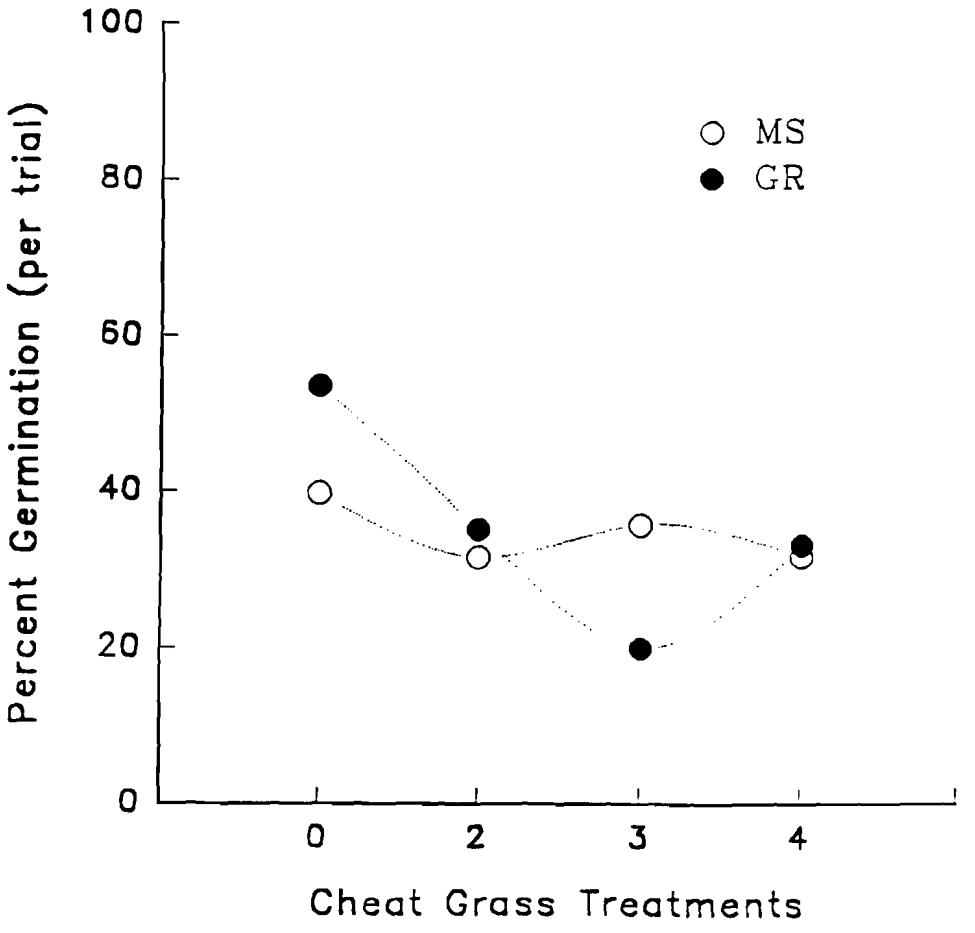

100
80
60
40
20
0
Percent Germination (per trial)
MS
GR
0
2
3
4
MS Treatments

Figure 11. Percent germination of Cheat grass seed in differing concentrations of Maximillian sunflower (MS) and Giant ragweed (GR) extracts.


100
80
60
40
20
0
Percent Germination (per trial)
MS
GR
0
2
3
4
Cheat Grass Treatments

treatment was significantly different from the control at the p = 0.5 level. The cheat grass with the giant ragweed treatment was not significantly different from the control at the p = 0.5 level. The germination studies concerning radicle length of the agronomic species of corn and wheat were inconclusive. The native seeds showed such variability in germination that calculation of variances was not conducted.

2. HPLC

HPLC chromatograms compared the methanol extracts of giant ragweed (Ambrosia trifida) and Maximillian sunflower (Helianthus maximilliani) at the same range (0.16), mobile phase (38% methanol : 62% water : 0.25% phosphoric acid), and the same U.V. wavelength (220 nm). The Maximillian sunflower had a concentration of simple phenolic acids about five times higher than the giant ragweed (Figure 12). The retention time value of protocatechuic acid at 3.3 minutes, matches the largest peak in the Maximillian sunflower chromatogram (Figure 13). The mobile phase consisted of 38% methanol : 62% deionized water : 0.25% phosphoric acid.

A calibration curve was figured for protocatechuic acid at a range of 0.16 (appendix). The corresponding

Figure 12. HPLC Chromatogram of Maximillian sunflower and giant ragweed extracts at the same range (0.16), mobile phase (38% methanol : 62% water : 0.25% phosphoric acid) and the same U.V. detection (220nm).

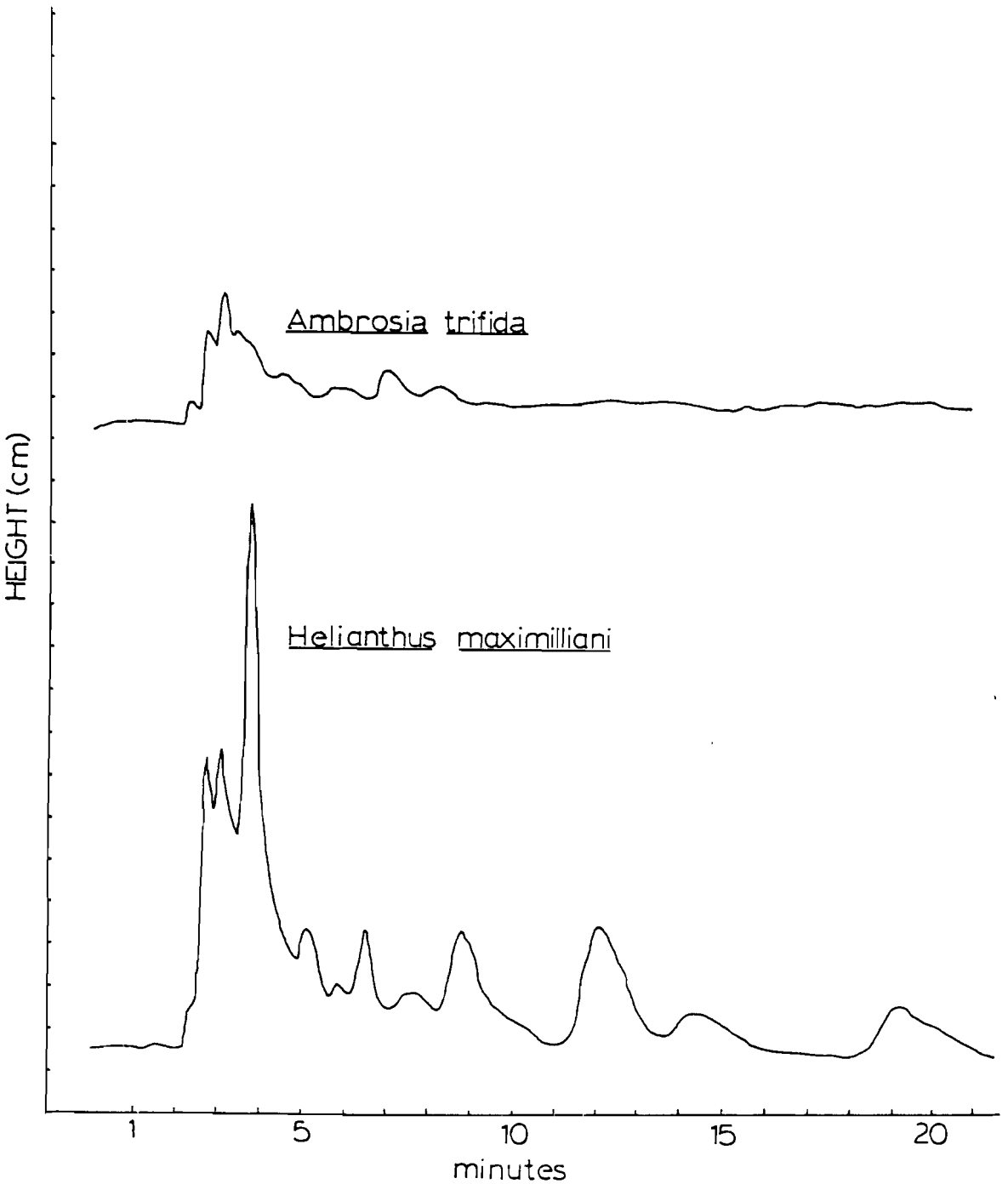

HEIGHT (cm)
Ambrosia trifida
Helianthus maximilliani
1
5
10
15
20
minutes

Figure 13. HPLC Chromatogram of Maximillian sunflower extract, identifying protocatechuic acid with a standard retention time of 3.3 minutes. The method had a range = 0.16, and a mobile phase of 38% methanol : 62% deionized water : 0.25% phosphoric acid.

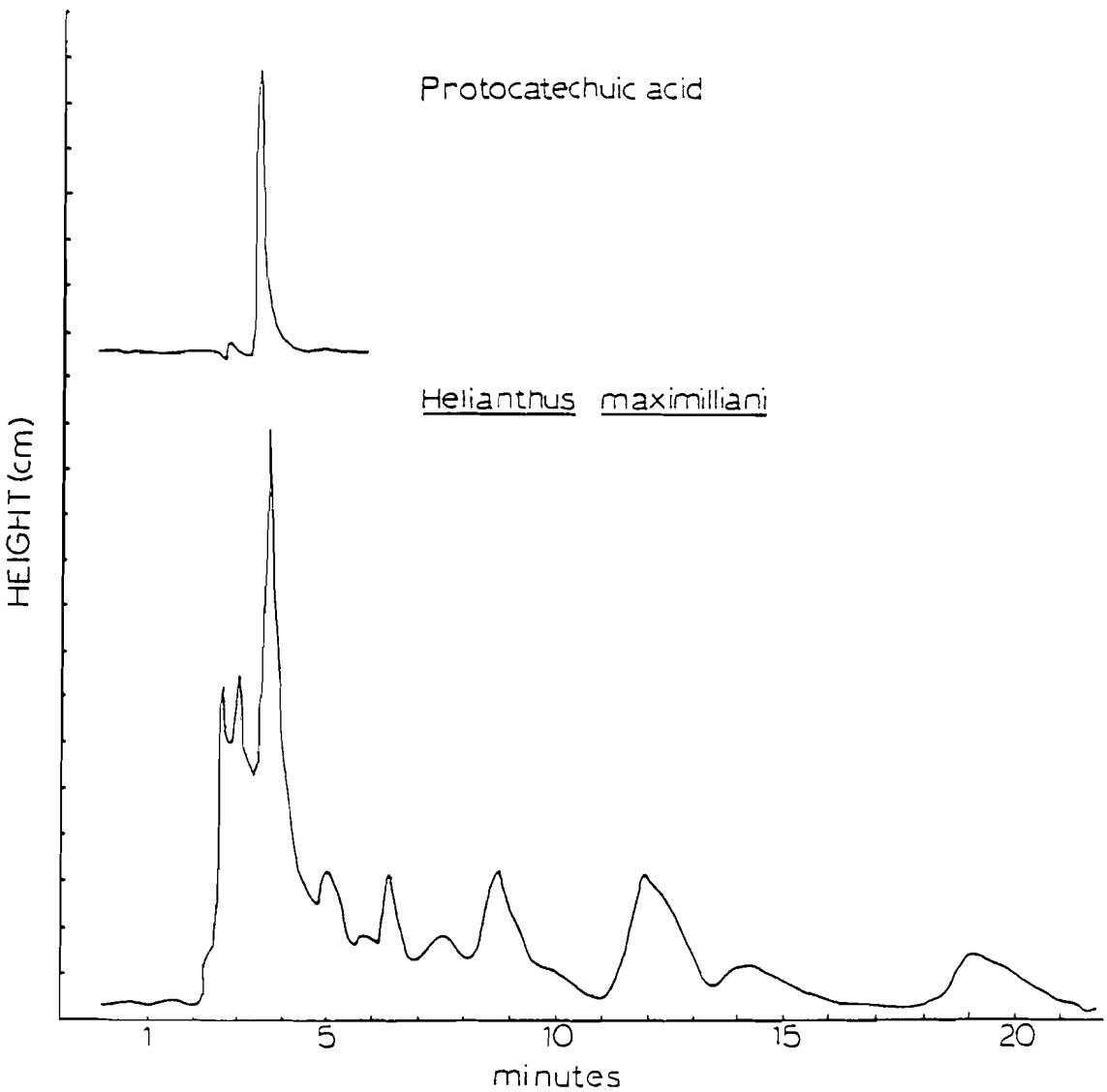
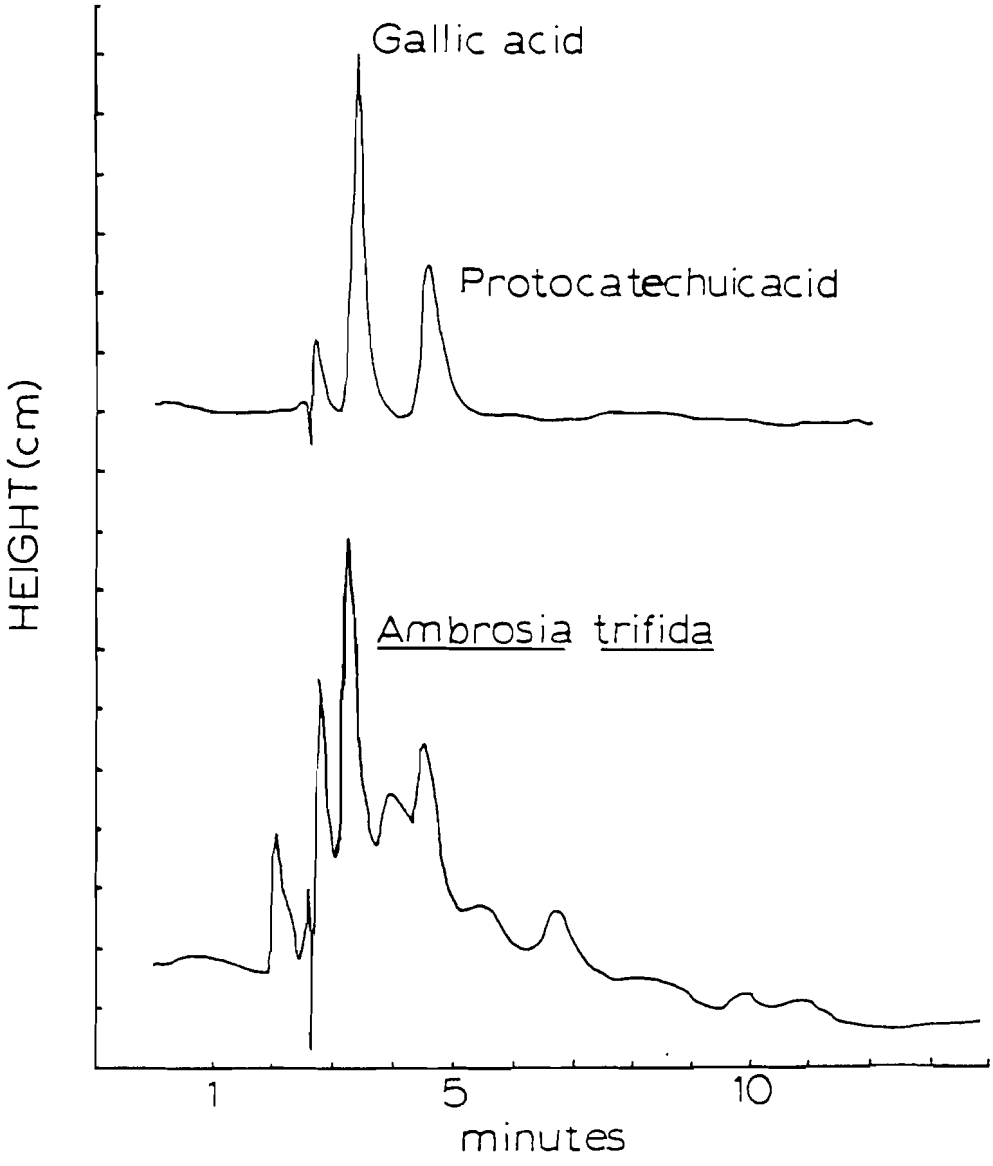

Protocatechuic acid
Helianthus maximilliani
HEIGHT (cm)
1
5
10
15
20
minutes

Figure 14. HPLC Chromatogram of giant ragweed (Ambrosia trifida) extract, identifying Gallic acid with a standard retention time of 3 minutes and Protocatechuic acid at 4.3 minutes. The method had a range = 0.02, and a mobile phase of 25% methanol : 75% deionized water : 0.25% phosphoric acid.


Gallic acid
Protocatechuic acid
Ambrosia trifida
HEIGHT (cm)
1
5
10
minutes

peak in the Maximillian sunflower extract has a height of 13 cm and an absorbance of 0.0832. The calibration curve indicated a concentration of 8.4×10^{-5}.

The retention times of both gallic acid at 3.0 minutes and protocatechuic acid at 4.3 minutes with a range of 0.02 responded to the large peaks in the giant ragweed extract (Figure 14). The mobile phase consisted of 25 % methanol : 75% water : 0.25% phosphoric acid.

The calibration curves were calculated for protocatechuic and gallic acids at a range of 0.02 (appendix). The corresponding peaks in the giant ragweed extract has a height of 15.6 cm and an absorbance of 0.012 for gallic acid and a height of 7.3 cm and absorbance of 0.00584 for protocatechuic acid. The calibration curve indicates that the gallic acid extract peak has a concentration of about 0.93×10^{-5} and the protocatechuic peak has a concentration of about 1.68×10^{-5}.

IV. DISCUSSION

A. Field Studies

1. Species Area Curve

The species composition for each site showed that the disturbed site (#1) had a higher species list for 30 quadrats than the intermediate (#2) and climax sites (#3).

This can best be explained by the concept discussed by Robert May on stable environments. As a community comes closer to climax, it is closer to equilibrium. The species are fewer and sustainable, whereas a disturbed site has more species fighting for space and nutrients. They compete for a shorter amount of time in a boom and bust cycle until the site becomes depleted and density-dependent. A site loses species that cannot compete in the long run. The climax community excludes *r*-selected species until another disturbance occurs and allows for colonization opportunity.

2. Spatial Patterning

Uniformity is not a natural occurrence in plant communities. The giant ragweed showed uniformity on the constantly-disturbed site. This results from frequent flooding of the area that brings in new seeds and nutrient runoff from adjoining agricultural land. Giant ragweed has a tap root to obtain nutrients and water and allows for closer contact with neighbors. The Maximillian sunflower exhibited clumping in the intermediate site (#2) because of its perennial root system and seed dispersal. This clumping could result from autotoxicity and seed rain. This outcome agrees with the work done by Dr. Carl

Prophet at Emporia State University, in which he found Maximillian sunflower to be clumped in old fields at the Ross Natural History Reservation. The plants at site #2 in this study were located around the perimeter of the old field, moving towards the center. This shows the progression of individuals from the ditch that surrounds the area. Neither the Maximillian sunflower nor the giant ragweed were found in abundance at the climax site (#3). Site #3 was dominated by big bluestem as is characteristic in the tallgrass prairie. The climax community of grasses excluded the more *r*-selected species from the area.

3. Interspecific Association

Both giant ragweed and Maximillian sunflower showed a positive association with cheat grass, although the Maximillian sunflower association was a stronger association. When this was tested in July, the cheat grass had set seed and was at the end of its growing season. This suggests the niche space was being shared for only a short time before the fall-flowering species were at their maximum growth. It should also be noted Solidago missouriensis was found at all three sites. It did show a strong negative association with the Maximillian sunflower in site #2. The variance ratios for

site #1 showed there was no overall association of the species in the disturbance community. Site #2 showed a possible negative association of the species for the intermediate community. The third site had many positive associations that biased most tests, indicating a close-knit community.

B. Laboratory Studies

1. Germination

The germination studies on Solidago were not pursued because there was a lack of germination in the laboratory control. The same problem occurred with giant ragweed. Scarification with sulfuric acid was tried, but without success. Some seeds were accidentally frozen and did show excellent germination results; a longer and colder stratification process was probably needed for the tough seed coat of giant ragweed. Because of a shortage of giant ragweed seeds, this test was not pursued further.

There was a tremendous amount of seed germination variability in the controls of native seeds compared to total germination of agronomic seeds. This explains why most allelopathic studies are done on seeds with a 100% germination in their controls. In future studies, to show field and laboratory associations, there needs to be more

time spent on establishing a good control measure of germination.

The results of the germination percentage tests showed no significant difference with an analysis of variance on the Maximillian sunflower germination percent, but there was a trend toward autotoxicity. This could be the influence that is affecting the distribution of Maximillian sunflower which exhibits clumped spatial patterning. The cheat grass was not significantly inhibited by the giant ragweed extract, but trends of inhibition in the germination tests were seen that do not support the positive association found in the quadrat surveys. This does indicate that the niche space could be shared by the plants with different flowering times. The Maximillian sunflower extract significantly inhibited the cheat grass, which does not support the positive association found in the quadrat surveys. Again, the flowering times differ for the two plants, which could indicate that niche space is being shared. The cheat grass has set seed by July, whereas the Maximillian sunflower does not set seed until fall. As the cheat grass dies back in summer, the Maximillian sunflower becomes more dominant on the site for fall flowering.

3. HPLC

The technology of HPLC was an effective means to determine total phenolics in the plant material extracts. The Maximillian sunflower had concentrations of phenolic acids five times higher than giant ragweed at the same range, flow, and mobile phase settings. Based on the literature, a higher amount of these acids shows a better likelihood of allelopathy. Two phenolic acids were identified in the extracts. Gallic acid was identified in the Maximillian sunflower extract and protocatechuic with gallic acid was found in the giant ragweed extract. Both of these phenols were indicated in the literature as being allelochemicals with the ability to inhibit germination.

C. Conclusions

The determination of allelopathic potential for the test species was greater for the Maximillian sunflower than the giant ragweed because of the higher concentration of potential allelochemicals and increased associations in the field. Ragweed is an excellent competitor in early successional areas with increased amounts of seed and competitive strategies. The climax site showed a tightly associated community of *k*-selected plants that are commonly seen on the tall grass prairie. This community

type is more stable because the plants are sustained as density dependent species. Communities at this level in succession have an elevated amount of competition to exclude more *r*-selected plants. This research in allelopathy could lead one to speculate that, as plants proceed in succession, they become more and more capable of allelopathic competition. Other things that can affect allelopathy include environmental stresses (which were not pursued by this study). Often in dry climates, this type of competition is used to obtain more nutrients, because faster growth resulting from water availability is not an option. Allelopathy could be more prevalent when nutrients or water are not readily available for the plant community to help evenly disperse the species present.

Allelopathy should not be excluded from competition studies in plant communities. The response of allelochemicals might not be prevalent in times of plenty but should be accounted for in part of competitive strategies. Plants produce many different chemicals that affect their environment; allelopathy might be one more use for those secondary compounds to help a plant get ahead.

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APPENDIX

Calibration curves

Protocatechuic mol. wt. = 154.12 g/mole
.245g PC x 1molePC/154.12g PC = 0.00158967
Molar Cx = Moles solute/L solution
0.001589Mol/L / 0.250L = 0.00636 mol/L

Gallic mol. wt. = 170.12 g/mole
.255g G x 1 moleG/170.12gG = 0.001498942 mol G
Cx
0.001498Mol/L / 0.250L = 0.00599 mol/L

Cx Vx = C2 V2 standards were diluted in 0.025 liters (V2)
Stock --> Standard (Vx)

		L stock	PC conc.	Gallic conc.
ml of stock	0.001	1E-06	2.5E-07	2.4E-07
	0.01	1E-05	2.5E-06	2.4E-06
	0.05	5E-05	1.3E-05	1.2E-05
	0.1	0.0001	2.5E-05	2.4E-05
	0.2	0.0002	5.1E-05	4.8E-05
	0.3	0.0003	7.6E-05	7.2E-05
	0.4	0.0004	0.0001	9.6E-05

Calibration curves
(Range*Height)/25cm = ABS

Range = 0.16
Max sunflower analysis
Protocatechuic

Conc	ht, cm	Abs
2.5E-05	2.8	0.01792
5E-05	7	0.0448
0.0001	15.2	0.09728
		calc abs
		0.0181
		0.04453
		0.09737

Regression Output:

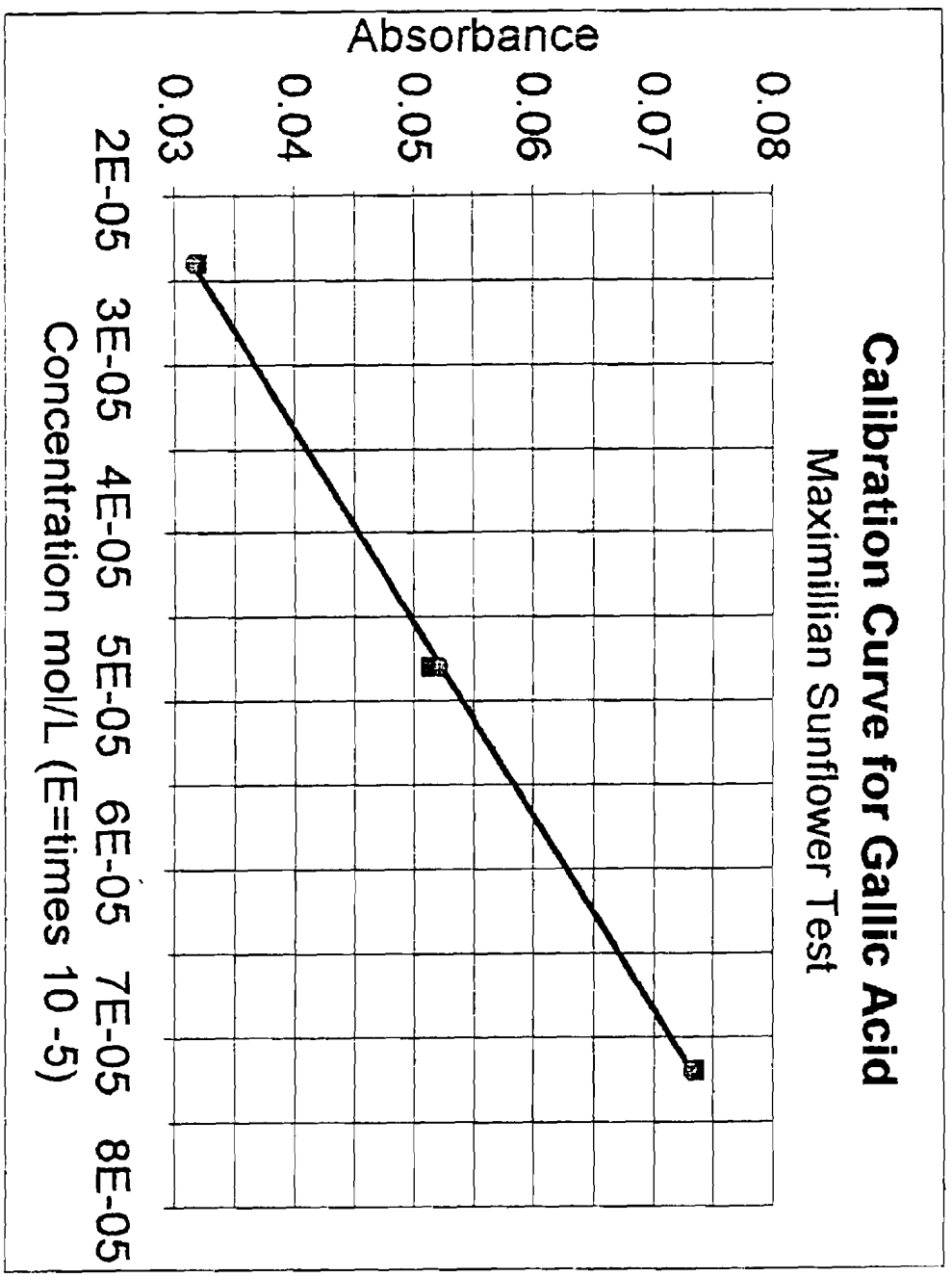
Constant		-0.00832
Std Err of Y Est		0.00034
R Squared		0.99996
No. of Observations		3
Degrees of Freedom		1
X Coefficient(s)	1056.91	
Std Err of Coef.	6.33436	

Gallic

conc	ht, cm	Abs
2.4E-05	5	0.032
4.8E-05	8	0.0512
7.2E-05	11.5	0.0736
		calc abs
		0.03147
		0.05227
		0.07307

Regression Output:

Constant		0.01067
Std Err of Y Est		0.00131
R Squared		0.99803
No. of Observations		3
Degrees of Freedom		1
X Coefficient(s)	866.667	
Std Err of Coef.	38.49	


Calibration Curve for Gallic Acid
Maximillian Sunflower Test
Absorbance
0.08
0.07
0.06
0.05
0.04
0.03
2E-05
3E-05
4E-05
5E-05
6E-05
7E-05
8E-05
Concentration mol/L (E=times 10 -5)

Calibration curves
(Range*Height)/25cm = ABS
Range = 0.02
G. Ragweed analysis

Protocatechuic

conc	ht, cm	Abs
2.5E-07	0.09	7.2E-05
1.3E-05	5.8	0.00464
2.5E-05	11.3	0.00904

calc abs
0.00012
0.00455
0.00908

Regression Output:

Constant		2.5E-05
Std Err of Y Est		0.00011
R Squared		0.99972
No. of Observations		3
Degrees of Freedom		1
X Coefficient(s)	356.603	
Std Err of Coef.	5.93691	

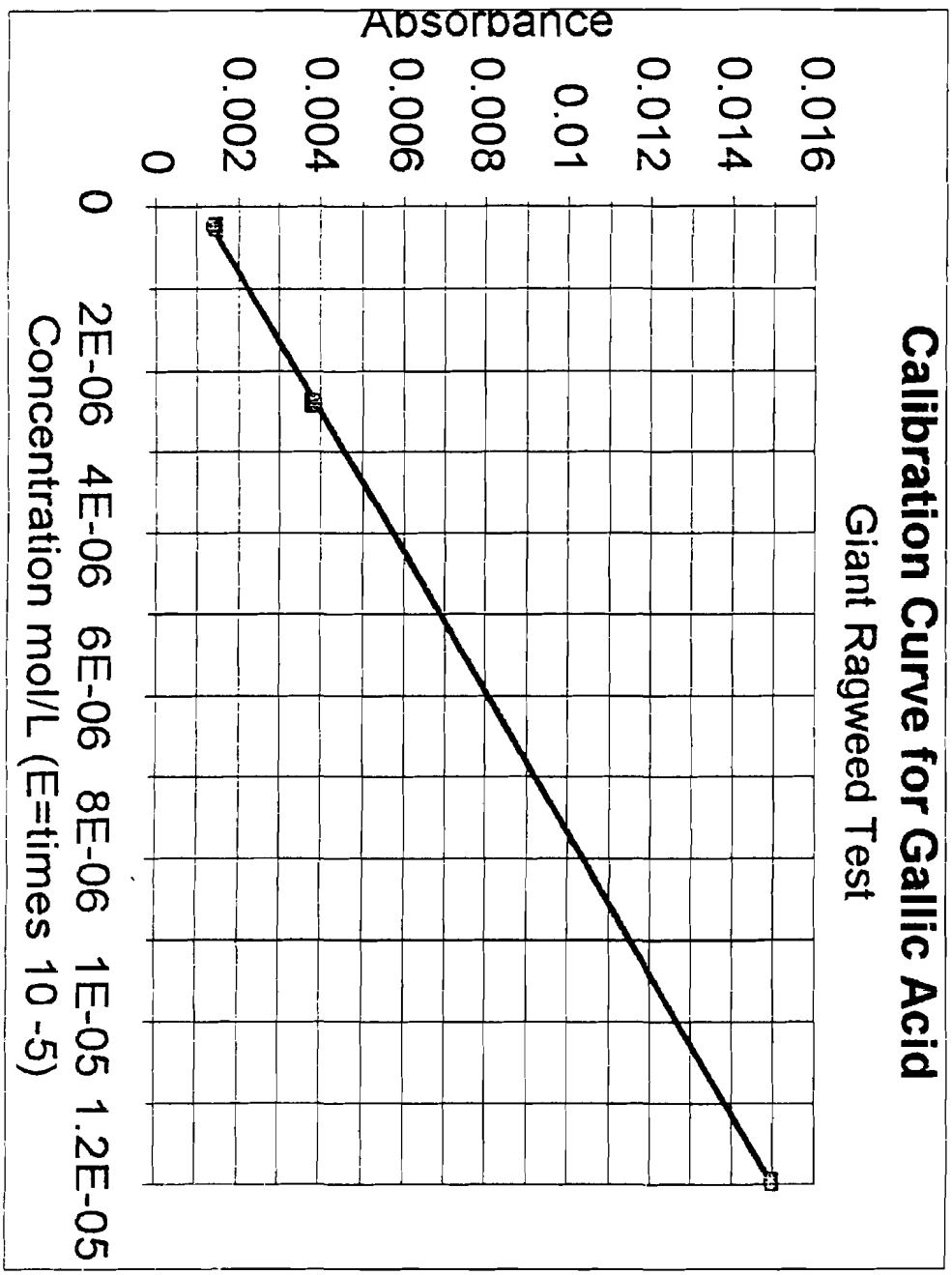
Gallic

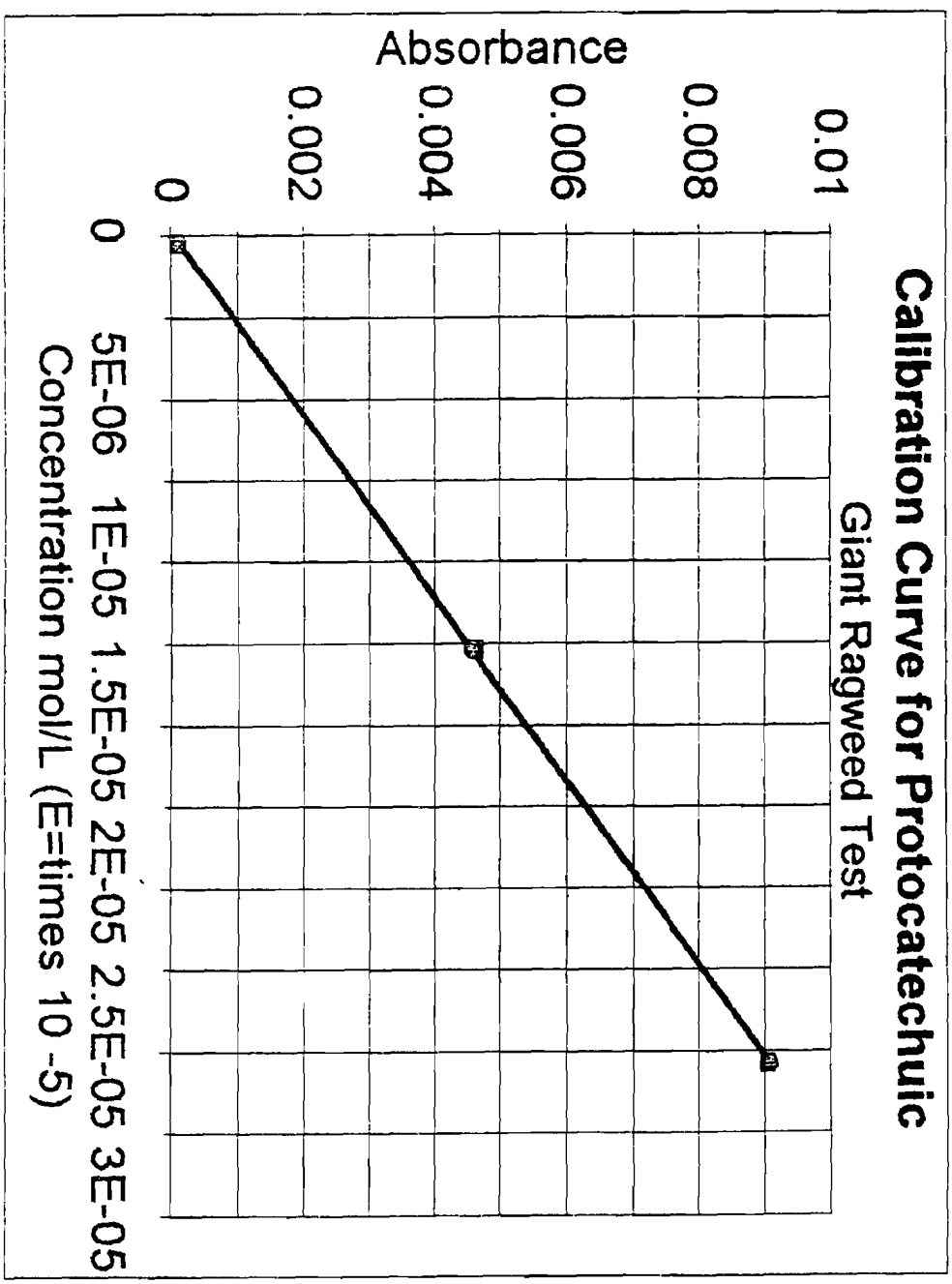
conc	ht, cm	Abs
2.4E-07	1.8	0.00144
2.4E-06	4.7	0.00376
1.2E-05	18.7	0.01496

calc abs
0.00136
0.00386
0.01494

Regression Output:

Constant		0.00108
Std Err of Y Est		0.00013
R Squared		0.99985
No. of Observations		3
Degrees of Freedom		1
X Coefficient(s)	1156.4	
Std Err of Coef.	14.1569	


Calibration Curve for Gallic Acid
Giant Ragweed Test
Absorbance
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
2E-06
4E-06
6E-06
8E-06
1E-05
1.2E-05
Concentration mol/L (E=times 10 -5)


Calibration Curve for Protocatechuic
Giant Ragweed Test
Absorbance
0.01
0.008
0.006
0.004
0.002
0
0
5E-06
1E-05
1.5E-05
2E-05
2.5E-05
3E-05
Concentration mol/L (E=times 10 -5)

2x2 Contingency Table

		Species B: Present	Species B: Absent	
Species A	Present	a	b	m
	Absent	c	d	n
		r	s	N

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Karrie Rathbone
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7-29-96
Date

Determining Allelopathic Potential For Two Weed Species In The Flint Hill
Title of Thesis

Doug Cooper
Signature of Graduate Office Staff Member

July 30, 1996
Date Received