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AND ITS ROLE IN OLD-FIELD SUCCESSION.

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ALLELOPATHY AS EXPRESSED BY Helianthus annuus
AND ITS ROLE IN OLD-FIELD SUCCESSION

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ALLELOPATHY AS EXPRESSED BY Helianthus annuus
AND ITS ROLE IN OLD-FIELD SUCCESSION

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CHAPTER I

INTRODUCTION

Booth (1941) depicted succession in abandoned fields in central Oklahoma and southeastern Kansas as proceeding through four major stages: (1) weed stage, lasting 2 to 3 years, composed of Helianthus annuus,¹ Erigeron canadensis L., Digitaria sanguinalis, Haplopappus ciliatus, Croton glandulosus and others; (2) annual grass, for 9 to 13 years, dominated by Aristida oligantha; (3) perennial bunchgrass, lasting an undetermined length of time, dominated by Andropogon scoparius and (4) the climax prairie dominated by A. scoparius, A. Gerardi, Panicum virgatum, and Sorghastrum nutans.

Rice, Penfound and Rohrbaugh (1960) studied the nitrogen and phosphorous requirements of A. oligantha, A. scoparius and P. virgatum; three species that come into old-fields at different stages of succession. They found

¹Nomenclature follows Waterfall (1966) unless authority is given.

that the order of increasing requirements for nitrogen and phosphorus is the same as the order in which these species invade abandoned fields. However, experimental observations based on greenhouse nutrient studies indicate that differing nutrient requirements cannot account for the replacement of the rapid growing, robust weeds of the first stage by the slow growing, depauperate A. oligantha. Field observations suggest that competition for water, light and space are also not the major cause for the replacement of the weed stage by the second stage, Aristida oligantha.

Smith (1940) stated that the first plant of the weed stage to be lost is Digitaria, followed by Croton, Helianthus and Haplopappus. The last three species become stunted and fewer in number each succeeding year. Abdul-Wahab and Rice (1967) and Ahshapanek (1962) suggested that certain weeds of the first stage may bring about stunting and elimination of themselves and associated species through the production of phytotoxins. Molisch (1937) coined the term allelopathy to account for the deleterious effect that one higher plant has on another higher plant through the production of chemical retardents. However, the current use of the term allelopathy is much broader. It now includes any direct or indirect deleterious effect that one plant has on another plant through the production of chemical compounds that escape into the environment. This broader definition encompasses all methods of inhibition,

(whether due to direct biochemical effects, physiological effects, alteration of associated microbial populations, or an interference in the uptake of essential mineral elements), and all types of plants. The salient point concerning allelopathy is that its effect is dependent upon a chemical compound being placed into the environment by an allelopathic agent. Thus allelopathy is separated from competition which involves the removal or reduction of some factor from the environment that is required by some other plant sharing the habitat.

Many studies indicate that allelopathy may play an important role in the structure and composition of plant communities. Hamilton and Buchholtz (1955) found that several herbaceous species were significantly reduced and others stimulated on plots containing live Agropyron repens (quackgrass) rhizomes when compared with plots from which quackgrass rhizomes has been removed. Grümmer and Beyer (1960) isolated several phenolics, p-hydroxybenzoic acid, vanillic acid and agropyrene, from the roots and rhizomes of Agropyron.

Went (1942) observed that the desert shrub Encelia farinosa Gray does not harbor annuals as do other shrubs growing in the same locality. Went suggested the possible occurrence of allelopathic agents. Gray and Bonner (1948) identified 3-acetyl-6-methoxybenzaldehyde as a phytotoxin present in the leaves of E. farinosa, and Bonner (1950)

illustrated that the germination of seeds of species normally growing under shrubs was inhibited by placing a mulch of Encelia leaves over the substratum. Muller and Muller (1964), Muller (1965), and Muller and del Moral (1966) found that the rapid invasion by some shrub species into certain California grasslands was due at least in part to the production of volatile inhibitors (terpenes) by these shrubs. Bode (1940) showed under field conditions that Artemisia absinthium L. inhibits the growth of some plants in its surroundings through the production of absinthin.

Rice (1964) found that certain species of the first and second stages of abandoned fields in central Oklahoma produce phenolic compounds that inhibit nitrifying and nitrogen-fixing bacteria. This inhibition of nitrogen accumulation aids in explaining the long duration of the second stage of succession in these fields. Keever (1950) working on old-field succession in North Carolina reported that one of the dominant species of the first stage, E. canadensis, loses its dominance after the first year due to phytotoxic products from its own decaying roots.

Cooper and Stoesz (1931) described fairy-ring growth patterns of Helianthus scaberrimus Ell. as having a diameter of 11 m, with the outermost plants sterile and depauperate. After growing H. annuus and wheat in soils from various zones of the ring the authors stated that "these experiments would seem to point toward a chemical

factor in the soil as the cause for the fairy-ring patterns." Clones of H. rigidus (Cass.) Fern. and H. occidentalis Riddell also exhibit the fairy-rings and according to Curtis and Cottam (1950) they are related to the presence of a phytotoxin derived from the underground plant parts of the sunflowers.

Allelopathy may also play a role in forest associations. Ljubic (1955) grew many combinations of tree seedlings in crowded pots and found that Quercus robur L. inhibits Fraxinus pennsylvanica and that Ulmus parvifolia antagonizes Q. robur. Davis (1928) isolated juglone (5-hydroxy-naphthoquinone) as the toxic material in the roots and hulls of walnut, the first woody species from which a specific allelopathic agent was identified. Cook (1921) and Massey (1925) had previously reported the allelopathic nature of Juglans nigra and J. cinerea L. The leaves of Ailanthus altissima contain a compound that is allelopathic to pine and birch seedlings. Mergen (1959) states that "the toxic substance may be one of the important factors that limits succession in Ailanthus stands." More extensive summaries concerning allelopathy and the role it plays in plant associations are to be found in the review articles of Rice (1967) and Muller (1966).

Thus, the role of allelopathy in the determination of plant associations appears to be well documented. With this in mind a study was undertaken to ascertain what role

H. annuus plays in aiding the possible self-elimination of the weed stage and its passage into the annual grass stage in old-field succession.

CHAPTER II

MATERIALS

Helianthus annuus plants and seeds as well as the seeds of eight species (Table 2), known to be associated with H. annuus were collected from abandoned fields near Norman, Oklahoma. These species were selected as test plants in determining the possible allelopathic effects of H. annuus.

Aqueous extracts of various organs of H. annuus were used in preliminary tests to determine if this species exhibited allelopathy. To prepare these extracts, 10 g fresh weight of plant material were boiled in 100 ml of distilled water for 10 min, ground in a Waring blender for 10 min, allowed to stand for 20 min, and filtered through Whatman No. 1 filter paper. The filtrate was made up to 100 ml with distilled water, extracted with two half volumes of petroleum ether to remove most of the chlorophyll, and adjusted to pH 5.8.

In July and October of 1967 a series of soil samples, minus litter, was taken within 0.25 m of several sunflower plants; another series of samples was taken at a distance greater than 1 m from the same plants. The soils

were collected with a post-hole digger that yielded a cylinder 8 inches in depth and 3 inches in diameter. By this method the soil cylinders could be placed in 4 inch glazed pots without disturbing the vertical stratification of the soils. Additional soil collections taken at the two specified distances from the sunflowers were each composited, air dried, passed through a 2 mm sieve and used for soil analyses.

CHAPTER III

EXPERIMENTATION AND DISCUSSION

Establishment of Field Patterns

A field near Norman, Oklahoma abandoned for 1½ years after having last been cultivated in spring wheat was selected as a study site. In this and other fields of the same stage definite zones of reduced growth of some associated species were seen around sunflowers. To quantify these observations all the plants within a 0.25 m² quadrat frame, laid around the base of the sunflowers, were clipped and separated as to species. Clippings were repeated 0.5 m and 1 m from the same plants using the same size quadrats. The series of quadrats was placed in a predetermined direction and the last quadrat of each series did not lie within 1 m of adjacent sunflowers. Five quadrat series were clipped every two weeks for 5 months, June through Oct. in 1966 and 1967. Because Bromus japonicus is a winter annual, 75 quadrats were clipped for this species only in late May and early June 1967. The oven-dry weight of Erigeron canadensis and Rudbeckia hirta was significantly lower in the quadrat

around the sunflower than at 1 m away (Table 1). Hap-
lopappus ciliatus and B. japonicus had lower mean oven-
dry weights near the sunflowers than at 1 m away but the
reduction was not statistically significant. Croton
glandulosus expressed a different pattern, because its
mean oven-dry weight increased in the middle quadrat but
decreased in the outermost quadrat. The absence of D.
sanguinalis in these samples agrees with the observations
of Smith (1940), that crabgrass is one of the first weeds
eliminated from the weed stage. Amaranthus retroflexus
did not occur in the sampling or reconnaissance methods
used here, indicating that this species is also lost from
the weed stage very early.

Phytotoxicity of Various Extracts

Experiments were designed to determine if the
vegetative patterns surrounding the sunflowers in the field
were the result of allelopathy. Seedlings of H. annuus
and eight associated species were grown in quartz sand for
14 days with complete nutrient solution (Hoagland and
Arnon, 1950); then transferred to plastic vials containing
a 1:5 ratio of nutrient solution to various H. annuus
extracts, grown for 10 days on a 16 hr photoperiod at 28°C
and a night temperature of 18°C. Control vials contained
a 1:5 ratio of nutrient solution to distilled water grown
under the same conditions. All extracts significantly

Table 1. Results of field clippings of species associated with Helianthus annuus.

Species	Number of sunflowers sampled	Mean oven-dry weight in g/0.25 M ²			Fs
		A	Quadrats ¹ B	C	
<i>Erigeron canadensis</i>	100	7.64	10.78	26.97	21.8 ^a
<i>Rudbeckia hirta</i>	100	0.23	0.51	0.63	12.5 ^a
<i>Haplopappus ciliatus</i>	100	5.38	8.06	11.88	1.7
<i>Bromus japonicus</i>	75	1.74	2.96	4.52	2.8
<i>Croton glandulosus</i>	100	5.50	9.27	2.22	2.9

¹Quadrat A includes the sunflower plant.
 Quadrat B extends 0.5 M from quadrat A.
 Quadrat C extends 0.5 M from quadrat B.

^aSignificant difference among quadrats.

reduced the oven-dry weights of all species tested (Table 2), but leaf and young inflorescence extracts appeared to be more allelopathic than extracts of stems and roots. The inhibited plants had mottled and chlorotic leaves, dead primary roots, and an increase in adventitious roots.

-Seed germination for the same nine species was also tested by placing two hundred seeds per test on No. 1 filter paper in glass Petri plates. Prior conditions for germination were met before placing the seeds in the plates, H. annuus required 90 days at 4°C in moist sand (Lane, 1965), C. glandulosus required mechanical scarification, all other seeds germinated at 22°C. Five ml of a 1:5 ratio of nutrient solution to various H. annuus extracts were added to the plates and the seeds allowed to germinate for 8 days in the dark. Control plates received a 1:5 ratio of nutrient solution to distilled water.

All extracts caused a reduction in percentage germination of Haplopappus ciliatus and R. hirta (Table 3). Inflorescence extracts caused a reduction in seed germination of all species except Digitaria sanguinalis with the effect being slight on Amaranthus retroflexus and Aristida oligantha. Germination of A. oligantha was not greatly altered by any of the extracts.

The extract tests indicated that H. annuus does produce phytotoxins that are antagonistic to seed germination and seedling growth of itself and many of its

Table 2. Effects of extracts of different organs on seedling growth.

Species	Exp. No.	Mean oven-dry weight/plant, mg ^c					Fs
		Con- trol	Leaf Ext.	Inflor Ext.	Root Ext.	Stem Ext.	
Helianthus annuus	1	908	452 ^{ab}	325 ^{ab}	567 ^a	598 ^a	39.3
	2	862	401 ^{ab}	302 ^{ab}	548 ^a	587 ^a	26.4
Erigeron canadensis	1	568	209 ^a	135 ^{ab}	228 ^a	253 ^a	56.2
	2	421	179 ^a	158 ^a	206 ^a	241 ^a	79.8
Rudbeckia hirta	1	753	156 ^a	157 ^a	215 ^a	204 ^a	93.9
	2	624	148 ^a	151 ^a	193 ^a	185 ^a	102.7
Digitaria sanguinalis	1	945	294 ^a	300 ^a	438 ^a	455 ^a	59.4
	2	738	158 ^a	146 ^a	367 ^a	360 ^a	21.4
Amaranthus retroflexus	1	489	100 ^a	136 ^{ab}	104 ^a	182 ^{ab}	38.3
	2	531	129 ^a	141 ^{ab}	112 ^a	231 ^{ab}	16.4
Haplopappus ciliatus	1	737	251 ^a	136 ^{ab}	306 ^a	281 ^a	66.1
	2	658	228 ^a	193 ^{ab}	300 ^a	284 ^a	41.1
Bromus japonicus	1	326	92 ^a	92 ^a	146 ^{ab}	114 ^a	190.0
	2	309	98 ^a	103 ^a	139 ^{ab}	126 ^a	106.8
Croton glandulosus	1	703	530 ^a	417 ^{ab}	527 ^a	554 ^a	6.6
	2	659	486 ^a	434 ^{ab}	491 ^a	506 ^a	14.8
Aristida oligantha	1	369	66 ^a	60 ^a	189 ^{ab}	112 ^a	82.0
	2	346	78 ^a	81 ^a	176 ^{ab}	101 ^a	106.6

^aWeight significantly different from that of control.

^bSignificant difference from all other extracts.

^cEach figure represents the mean of 15 plants.

Table 3. Effects of extracts of different organs on percent germination.¹

Species	Germination expressed as percent of control			
	Leaf Ext.	Inflor. Ext.	Root Ext.	Stem Ext.
<i>Helianthus annuus</i>	90	78	98	97
<i>Erigeron canadensis</i>	95	62	109	94
<i>Rudbeckia hirta</i>	50	30	85	66
<i>Digitaria sanguinalis</i>	92	99	100	100
<i>Amaranthus retroflexus</i>	81	89	98	69
<i>Haplopappus ciliatus</i>	62	45	73	71
<i>Bromus japonicus</i>	91	66	95	97
<i>Croton glandulosus</i>	93	82	95	98
<i>Aristida oligantha</i>	100	90	111	100

¹Data represent the means for a single test, three similar tests were performed with comparable results.

associated species. To be ecologically active, however, these phytotoxins must leave the sunflower and enter the substratum. It was postulated that the phytotoxic materials may escape H. annuus by at least three methods: decomposition of sunflower debris, via root exudates, and in leachates from the leaves. There is considerable evidence that the light quantity and quality have an effect on the production of plant phenolics (Taylor, 1965 and Lott, 1960), the class of compounds to which the phytotoxins produced by sunflower belong. Because of this the plants used in testing the above hypothesis were taken from the field or grown in the greenhouse only during the summer.

Effects of Decaying Sunflower Leaves on Germination and Seedling Growth

To test the allelopathic effects of decaying sunflower leaves, 25 test seeds were placed in 4 inch glazed pots containing 1 g air dried sunflower leaf in the test pots or 1 g air dried peat moss in control pots per 454 g of a mixture consisting of 2/3 soil to 1/3 sand. The amount of sunflower leaves used is a realistic figure based on quadrat sampling for the amount of air dried sunflower leaves present per 454 g of soil to the depth of plowing (top 6 2/3 inches) in a stand of sunflowers. The plants were grown under greenhouse conditions with equal volumes of distilled water. After germination was completed the pots were thinned to the 5 largest plants per

pot, the plants were grown for an additional 2 weeks and then compared on an oven-dry weight basis (Table 4).

Decaying sunflower leaves had an inhibitory effect on the germination of H. annuus, E. canadensis, A. retroflexus, and H. ciliatus (Table 4). There was no effect on percentage germination of R. hirta and the three grasses tested, B. japonicus, D. sanguinalis, and A. oligantha. The mean oven-dry weights of the seedlings of all species except H. ciliatus were significantly reduced, but the seedlings of A. oligantha were stimulated in growth by decaying sunflower leaves.

Effects of Root Exudate on Seedling Growth

The effects of H. annuus root exudate on the test species were investigated using a set-up modified from Martin and Rademacher (1960). Test species were grown in quartz sand for 14 days with nutrient solution. The sunflowers for these tests were brought into the greenhouse from the field when approximately 2-3 feet high and also planted into quartz sand in 4 inch glazed pots. The experimental design was arranged to eliminate competition for light, minerals and water between the sunflowers and the test species. Pots containing the 14 day old test seedlings and pots containing sunflowers were placed on alternate steps of a modified staircase and connected with glass tubing sprayed with aluminum paint to keep down the growth of algae. A control sequence consisting of pots of

Table 4. Effects of decaying sunflower leaves on growth of seedlings and germination.

Species	Exp. No.	Mean dry weight of seedlings, mg		Fs	Germination ^b
		Control	Test		
Helianthus	1	44	22 ^a	45.4	52
annuus	2	36	21 ^a	10.7	40
Erigeron	1	54	19 ^a	20.0	87
canadensis	2	32	16 ^a	9.4	71
Rudbeckia	1	17	3 ^a	36.0	95
hirta	2	12	2 ^a	19.3	81
Digitaria	1	126	16 ^a	67.8	106
sanguinalis	2	97	11 ^a	84.4	97
Amaranthus	1	78	12 ^a	23.7	56
retroflexus	2	91	16 ^a	41.6	32
Haplopappus	1	13	8 ^a	7.0	71
ciliatus	2	26	10 ^a	12.6	64
Bromus	1	47	17 ^a	114.0	97
japonicus	2	39	15 ^a	71.8	94
Aristida	1	15	21	3.7	97
oligantha	2	19	23	2.2	102

^aDry weight significantly different from control.

^bExpressed as percent of the control.

test species only was placed on the staircase adjacent to the test series. Complete nutrient solution was pumped from a reservoir at the bottom of each series to a reservoir at the top of each series where it dripped from pot to pot down the staircase for recirculation 4 hrs daily. This method excludes all mutual effects except the interaction of root exudates. The term exudate has been used in a very broad sense here to refer to any substance which gets into the substrate directly from the roots of the inhibitory plants, which results from the breakdown of cells which are sloughed off the roots, or which results from the action of micro-organisms on substances that get out of the roots in any way.

Growth of H. annuus, E. canadensis, R. hirta, D. sanguinalis, and A. retroflexus was significantly reduced by root exudate of sunflower after 14 days on the staircase (Table 5). The exudate did not significantly reduce the oven-dry weights of Haplopappus ciliatus, B. japonicus, Croton glandulosus, or A. oligantha.

Effects of Leaf Leachate on Germination and Seedling Growth

Many organic materials are known to be leached from leaves (Morgan and Tukey, 1964) and to be allelopathic to associated plants (Grümmer, 1961). To investigate the allelopathic nature of H. annuus leaf leachate a fine mist of cistern water was sprayed over mature sunflower plants.

Table 5. Effects of sunflower root exudate on seedling growth.

Species	Exp. No.	Mean dry weight, mg		Fs
		Control	Test	
Helianthus	1	44	32 ^a	5.1
annuus	2	58	40 ^a	12.6
Erigeron	1	53	36 ^a	8.6
canadensis	2	59	45 ^a	6.7
Rudbeckia	1	36	18 ^a	23.6
hirta	2	33	14 ^a	18.7
Digitaria	1	53	39 ^a	12.6
sanguinalis	2	43	25 ^a	21.9
Amaranthus	1	71	23 ^a	64.3
retroflexus	2	64	15 ^a	41.8
Haplopappus	1	63	53	1.6
ciliatus	2	48	42	1.2
Bromus	1	63	61	1.5
japonicus	2	54	52	1.1
Croton	1	34	32	0
glandulosus	2	47	46	0
Aristida	1	99	95	0
oligantha	2	96	91	0

^aDry weight significantly different from control.

The leachate collected in this manner was used to water pots containing a mixture of 2/3 soil and 1/3 sand and 25 seeds each of one of the test species. Control pots received equal volumes of cistern water that had not passed over the sunflower leaves. After germination was completed the pots were thinned to the 5 largest plants per pot, the plants were grown for an additional two weeks and compared on an oven-dry weight basis (Table 6).

H. annuus leaf leachate reduced the percentage germination of H. annuus and A. retroflexus, but the germination of most other species was not greatly affected (Table 6). The leachate significantly reduced the oven-dry weight of seedlings of E. canadensis, R. hirta, D. sanguinalis, A. retroflexus, and Haplopappus ciliatus, but did not affect Helianthus annuus, B. japonicus, C. glandulosus, or Aristida oligantha (Table 6). The leaf leachate experiments were first attempted using quartz sand as the substrate for the test species, but under these conditions the leaf leachate was not phytotoxic. When a mixture of 2/3 soil to 1/3 sand was used as the substrate, however, the reported results were obtained. Gray and Bonner (1948), working with Encelia and Grümmer (1961), with Artemisia absinthium found that the phytotoxic effects of leaf leachates were greater in sand than in good soil. According to Evenari (1961), this was because the inactivation by colloid binding and biological destruction of the

Table 6. Effects of leaf leachate on germination and seedling growth.

Species	Exp. No.	Mean dry weight of seedlings, mg		Fs	Germination ^b
		Control	Test		
Helianthus	1	47	41	3.7	78
annuus	2	48	37	3.8	63
Erigeron	1	137	75 ^a	20.0	103
canadensis	2	147	50 ^a	32.7	89
Rudbeckia	1	73	23 ^a	26.4	106
hirta	2	60	30 ^a	9.8	91
Digitaria	1	37	8 ^a	30.7	95
sanguinalis	2	26	15 ^a	14.9	91
Amaranthus	1	228	80 ^a	46.9	75
retroflexus	2	206	72 ^a	84.2	68
Haplopappus	1	16	6 ^a	51.5	127
ciliatus	2	29	17 ^a	34.7	92
Bromus	1	73	72	0	102
japonicus	2	60	51	0	97
Croton	1	73	67	0	92
glandulosus	2	82	80	0	81
Aristida	1	15	12	0	104
oligantha	2	22	24	0	101

^aDry weight significantly different from the control.

^bExpressed as percent of the control.

phytotoxins is much less pronounced in sand. The data presented here suggest that the colloidal material of the soil may play a role in accumulating these phytotoxins to a toxic level.

Effects of Field Soils on Germination and Seedling Growth

To determine if the phytotoxins of H. annuus are stable in the soil under field conditions, soil minus litter was removed from beneath sunflowers and placed in 4 inch glazed pots. Soil 1 m away from the same sunflowers was removed in a similar way and used as control soil in this experiment. The soil collections were made in July, 1967 during active growth of H. annuus and Oct., 1967 after the accumulation of H. annuus debris; the two collections were treated as separate experiments. Seeds of the test species were placed in their respective pots and allowed to germinate. After germination the 5 largest plants in each pot were grown for 2 more weeks and compared on an oven-dry weight basis (Table 7). The July soils from beneath H. annuus significantly reduced the oven-dry weights of H. annuus, E. canadensis, R. hirta, D. sanguinalis and A. retroflexus (Table 7). Dry weights of these same species plus the seedlings of H. ciliatus and B. japonicus were significantly reduced in the soils of the Oct. collection. Croton glandulosus and A. oligantha were not inhibited by the July or the Oct. soil collections.

Table 7. Effects of field soils previously in contact with sunflower roots on germination and growth.

Species	Date soil taken	Mean dry weights of seedlings, mg		Fs	Germination ^c
		Control ^b	Test		
Helianthus annuus	July	24	15 ^a	15.3	63
	Oct.	28	18 ^a	36.5	48
Erigeron canadensis	July	19	2 ^a	44.2	29
	Oct.	24	3 ^a	95.8	41
Rudbeckia hirta	July	14	6 ^a	12.6	78
	Oct.	9	5 ^a	5.8	62
Digitaria sanguinalis	July	28	16 ^a	12.9	94
	Oct.	24	12 ^a	9.0	86
Amaranthus retroflexus	July	50	12 ^a	46.7	83
	Oct.	50	8 ^a	9.9	64
Haplopappus ciliatus	July	12	9	-3.5	64
	Oct.	16	11 ^a	6.8	72
Bromus japonicus	July	11	10	0	79
	Oct.	34	16 ^a	18.5	66
Croton glandulosus	July	28	27	0	89
	Oct.	26	24	0	76
Aristida oligantha	July	9	10	1.5	91
	Oct.	9	12 ^a	14.4	96

^aWeight significantly different from that of the control.

^bControl soils were from same field as test soils but not from around sunflower plants.

^cExpressed as percent of the control.

Both soil collections reduced the germination percentage appreciably of all species except: D. sanguinalis, C. glandulosus and A. oligantha.

Thus, the soil collection experiments indicated a greater phytotoxicity in the soils after accumulation of debris than during the active growth of the sunflowers. Erigeron canadensis and R. hirta exist as winter annuals in these abandoned fields, and during the late winter months zones of inhibition around dead sunflower stalks are expressed through these two species. The greater phytotoxicity of the soils after accumulation of debris may help explain these zones of inhibition, as well as give insight into the carry-over of allelopathy from season to season.

Soil reaction by the glass electrode procedure (Piper, 1942), Walkley and Black values for organic carbon modified from Piper (1942), total phosphorus after Shelton and Harper (1941), and total nitrogen determination by the macro-Kjeldahl method modified from Noggle and Wynd (1941), were compared for field soils taken 0.25 m and 1 m from the sunflower plants. The soil analyses for mineral and physical values indicated no differences that seem capable of causing the vegetation patterns that develop around the sunflower plants (Table 8).

Identification of Phytotoxins from Sunflower Extracts

The procedures used to isolate and identify the phytotoxins from H. annuus were chiefly those of Rice (1965).

Table 8. Comparison of mineral and physical properties of soils around Helianthus annuus.^a

Distance of soil sample from the sunflower plants	pH	Organic carbon ^b %	Total Nitrogen %	Total Phosphorus %
Within 0.25 m	6.20	0.91	0.057	0.004
Greater than 1 m	6.22	1.00	0.056	0.005

^aValues represent the results of triplicate tests run on composited soils from each distance.

^bWalkley and Black organic carbon values.

Aqueous extracts of H. annuus were acidified to pH 2.5 with 1 N HCl and extracted with two half volumes of diethyl ether. The ether fraction was evaporated by air and the residue taken up in 3 ml of 95% ethanol. The ether fraction was chromatographed on Whatman 3 MM paper with n-butanol-acetic acid-water (63:10:27, v/v), BAW. The chromatograms were inspected with short (2537 Å) and long (3360 Å) ultra-violet light. Two light blue bands fluoresced with long and short U.V. light, and the bands turned yellow green under U.V. light after exposure to NH₃. These bands were cut from the paper and eluted with 90% ethanol. The eluates were reduced to dryness in vacuo, taken up in 3 ml of methanol, and chromatographed on Whatman 3 MM paper in four different solvent systems: BAW; 6% aqueous acetic acid, 6%AA; isopropanol-ammonia-water (200:10:20, v/v), IAW; and n-butanol-formic acid-water (82:1.5:16.5, v/v), BFW. The R_f's in the various solvent systems, color in U.V. light with and without exposure to NH₃, various reagent tests (Rice, 1965) and spectral analysis indicated that the fluorescent bands of H. annuus extracts were chlorogenic and isochlorogenic acids (Table 9).

The suspected chlorogenic and isochlorogenic acids were eluted from the chromatograms with 95% ethanol and their absorption spectra determined with a Beckman Model DB-G spectrophotometer before and immediately after the addition of 2 drops of 2N NaOH to the spectrophotometer

Table 9. Chromatography of phytotoxins from Helianthus annuus

Compound	R _f 's on Whatman 3 MM ^a				Fluorescence ^c		Reagent colors ^{b,c}			
	BAW	6%AA	IAW	BFW	Long and short U.V. -NH ₃	Long and short U.V. +NH ₃	p-Nit	Sulfan. acid	FeCl ₃ K ₃ Fe(CN) ₆	Hoepfner reaction
Chlorogenic acid	0.58	0.60, 0.75	0.01	0.42	l.bl.	yel.gr.	brn.	tan	blue	red
Phytotoxin 1 ^d	0.60	0.59, 0.73	0.01	0.42	l.bl.	yel.gr.	brn.	tan	blue	red
Isochlorogenic acid	0.80	0.16, 0.31	0.02	0.73	l.bl.	yel.gr.	brn.	tan	blue	red
Phytotoxin 2 ^d	0.79	0.15, 0.31	0.03	0.73	l.bl.	yel.gr.	brn.	tan	blue	red
Scopolin	0.81	0.44	0.29	--	blue	br.blue	--	--	--	--
Phytotoxin 3 ^e	0.81	0.44	0.29	--	blue	br.blue	--	--	--	--
α-Naphthol	0.88	0.62	0.87	--	bl. pur. ^f	bl. pur. ^f	tan	yel.tan	blue	--
Phytotoxin 4 ^e	0.92	0.66	0.89	--	bl. pur. ^f	bl. pur. ^f	brn.	tan	blue	--

^aSee text for solvent systems. R_f's are averages of many runs.

^bDiazotized p-nitraniline (Bray et al. 1950), diazotized sulfanilic acid (Smith 1960, p. 296), ferric chloride-potassium ferricyanide (Smith 1960, p. 324), and Hoepfner reaction (Hoepfner 1932).

^cl, light; bl, blue; yel, yellow; gr, green; brn, brown; br, bright; pur, purple.

^dPresent in extracts of leaves, young inflorescences, roots and stems.

^ePresent in leaf leachate.

^fVisible only under short U.V. light.

cuvettes. The spectra for known chlorogenic and isochlorogenic acid in 95% ethanol were similarly determined. The absorption maximum for both suspected chlorogenic and known chlorogenic acid in 95% ethanol was 330 m μ , both had an absorption maximum of 369 m μ in 95% ethanol plus NaOH. The absorption maximum for suspected isochlorogenic and known isochlorogenic acid in 95% ethanol was 329 m μ , and both had an absorption maximum of 377 m μ in 95% ethanol plus NaOH.

As a further check on the validity of the two suspected compounds, they were eluted from chromatograms and cochromatographed with known compounds. The cochromatograms were developed in two dimensions with BAW and 6%AA, both suspected chlorogenic acid and suspected isochlorogenic acid coincided with their respective knowns in both directions.

The biological activity of suspected chlorogenic and isochlorogenic acids was determined and compared with known chlorogenic and isochlorogenic acids (2 mg/ml concentration) using an A. retroflexus seed germination bioassay, (Amaranthus retroflexus is an excellent bioassay seed due to its high percentage of germination, relative ease in determining visible germination and the fact that it is one of the species of the first stage of succession). Petri plates containing 100 seeds on filter paper received 2.5 ml of aqueous suspected or known chlorogenic or

isochlorogenic acid, control plates received 2.5 ml of distilled water. Germination results expressed as percent of control germination, were as follows: suspected chlorogenic acid, 34; known chlorogenic acid, 27; suspected isochlorogenic acid, 68; and known isochlorogenic acid, 65. The concentrations of the suspected and known compounds are not equal and for this reason the bioassays do not give relative values. The known chlorogenic and isochlorogenic acids are also toxic to 12 day old seedlings of A. retroflexus in concentrations as low as 0.83×10^{-7} M, and to B. japonicus seedlings at 0.83×10^{-3} M. These same compounds were not found to be toxic to A. oligantha at a concentration of 0.83×10^{-3} M (Olmsted, 1967). Thus it appears that the phytotoxic effects exhibited by various extracts of H. annuus can be attributed to the high concentrations of chlorogenic and isochlorogenic acids present. Chlorogenic and isochlorogenic acids have previously been reported from H. annuus extracts (Rice 1965, Zane 1963, and Sondheimer 1960).

Identification of Phytotoxins from Sunflower Leaf Leachate

Leaf leachate collections consisting of 1.5 liters were evaporated to dryness in vacuo, the residue was taken up in 10 ml of absolute methanol, and 3 ml of the alcoholic preparation were streaked on Whatman No. 1 paper and developed in IAW. The major streaks were eluted off

the paper with 5% methanol, evaporated almost to dryness and made up to 25 ml with distilled water. The biological activity of the eluates was determined by the Amaranthus retroflexus germination bioassay. The phytotoxic bands were eluted off similar chromatograms and run in three solvent systems, IAW, 6%AA and BAW. The R_f 's in the different solvent systems, colors in U.V. light, reagent tests (Rice, 1965), spectral analyses and cochromatograms with known compounds indicated that the most phytotoxic compounds of the leaf leachate from H. annuus are scopolin and a suspected α -naphthol derivative (Table 9).

The suspected scopolin was eluted from the chromatograms with 5% methanol and its absorption spectrum determined with a Beckman Model DB-G spectrophotometer. The absorption maximum for both suspected scopolin and known scopolin in absolute methanol was 326 m μ . The absorption maximum for both the suspected and known compounds in distilled water was 340 m μ .

The activity of suspected scopolin, concentration not known, and known scopolin (1 mg/ml concentration) was determined by the A. retroflexus germination bioassay. Expressed as percent of the control the percentage germination was: known scopolin 12 and suspected scopolin 22.

The suspected α -naphthol derivative was eluted from the chromatograms with 70% ethanol and its absorption spectrum determined with a Beckman Model DB-G spectrophotometer before and immediately after the addition of 1 drop

of 2N NaOH to the spectrophotometer cuvettes. The absorption maximum for the suspected derivative of α -naphthol and known α -naphthol in 95% ethanol were 205 m μ and 211 m μ respectively. In 95% ethanol plus NaOH the absorption maximum for the suspected derivative and known α -naphthol were 207 m μ and 215 m μ respectively.

The activity of the suspected derivative of α -naphthol, of unknown concentration, and a 2 mg/ml concentration of the known α -naphthol was determined by the A. retroflexus germination bioassay. Expressed as percent of the control the percent germination was: known α -naphthol 23 and suspected α -naphthol derivative 51.

Chlorogenic and isochlorogenic acids were not detected in the leaf leachates or in root exudates of H. annuus. Apparently, even though chlorogenic and isochlorogenic acids are present in high concentrations in the extracts of H. annuus they do not escape from the sunflower in the leaf leachate or root exudate. The fact that B. japonicus was previously shown by Olmsted (1967) to be inhibited by chlorogenic and isochlorogenic acids and that this species was only inhibited in the present experiments by extracts, soil with decomposing material added, and field soils collected after the accumulation of debris leads one to the conclusion that the decomposition of sunflower debris may be the only method by which these compounds can escape into the substratum.

Field soils from around H. annuus were extracted according to Wang, Yang and Chuang (1967). Both the ether and aqueous extracts were streaked on Whatman 3 MM paper and developed in BAW. The papers were examined under U.V. light, the visible bands were cut from the paper and eluted with 70% methanol. The eluates were evaporated almost to dryness in vacuo and taken up in 10 ml of distilled water. These aqueous eluates were then tested for biological activity by the A. retroflexus germination bioassay, and the majority of bands were found to be very toxic to this species. Although I have not succeeded in identifying any of the phytotoxins in the soil extracts, I can state that none of the phytotoxins present in the plant extracts or leaf leachate were detected in the soil extracts. There may exist a relationship between the phytotoxins isolated from the plant extracts and leaf leachate and those present in the root exudate and soil extracts, but that relationship is not evident at this time. Certainly greater emphasis on studies concerning the degradation of these compounds is needed.

The fact that none of the phytotoxins of the plant extracts were found in the leaf leachate, root exudate or soil extracts indicates that studies of allelopathy based on plant extracts alone would be highly unsatisfactory. The presence of different phytotoxic substances in the extracts, leachates and exudates indicates that there is

probably an additive effect with each group of toxins perhaps affecting different kinds of processes, and thus accentuating the allelopathic effects of sunflower.

CHAPTER IV

CONCLUSIONS

The first stage of succession in abandoned fields in central Oklahoma and southeastern Kansas is the weed stage in which Helianthus annuus is an important dominant. The causative factors involved in the short-lived existence of this stage, 2 to 3 years, and its passage into the annual grass stage consisting of one dominant, Aristida oligantha have long been sought. The present study was undertaken to ascertain the allelopathic effects of H. annuus on species with which it is associated in abandoned fields. Field studies indicated that certain associated species do exhibit reduced growth around the sunflower plants. Extracts of various organs of sunflower plants, decaying leaves, root exudate, leaf leachate, and soils collected from around sunflowers inhibited the seed germination and seedling growth of H. annuus and many of the associated species, with the exception of Aristida oligantha and Croton glandulosus. Strong correlations were evident between the species that expressed inhibition in the field and those inhibited through various laboratory tests. Chlorogenic acid and isochlorogenic acid, present

in all extracts of the various sunflower organs, and a suspected α -naphthol derivative and scopolin from the leaf leachate were the major phytotoxins isolated from the sunflower plants. The fact that the phytotoxins of the extracts, leaf leachate, root exudate and soil extracts are not the same compounds indicates that there is probably an additive effect with each group of toxins, thus accentuating the allelopathic effects of sunflower. The present results which indicate that H. annuus is allelopathic to many of the early weeds, including its own seedlings, but not to Aristida oligantha corresponds with the findings of Abdul-Wahab and Rice (1967) and Olmsted (1967) and may help in explaining the short-lived existence of the weed stage and its replacement by Aristida oligantha in old-field succession of this region.

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