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Within the tall grass prairie community a number of shrubby invaders are encroaching on the established vegetation. One of these invaders is buckbrush, Symphoricarpos orbiculatus. This study was undertaken to determine if allelopathic inhibition was a factor in that invasion. The site selected for the research was an established prairie community on the Ross Natural History Reservation, northwest of Emporia in Lyon County, Kansas.

Over a four year period, observations revealed that one established stand of S. orbiculatus increased an average of 0.636 m/year in diameter. Extracts of the root, foliage and fruit of S. orbiculatus were prepared for use in seed germination, seedling growth and development and chromatographic portions of the study. The major members of the

tall grass prairie (Andropogon gerardi, Andropogon scoparius, Panicum virgatum and Sorghastrum natans) were used as the test species in the germination and development tests.

The effects of these extracts established that an inhibition was taking place. A. scoparius, P. virgatum and S. nutans showed significant decreases in germination and development. The species showing the least susceptibility to the inhibition was A. gerardi. Chromatograms were used to verify the presence of potentially alleopathic phenolic compounds in the S. orbiculatus extracts.

THE ALLELOPATHIC POTENTIAL OF *SYMPHORICARPOS ORBICULATUS* IN THE KANSAS TALL GRASS PRAIRIE

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INTRODUCTION

Rice (1974) suggested that there are two types of interactions between plants causing deleterious effects. These are competition and allelopathy. Competition encompasses the reduction of physical aspects of the environment required for plant growth, such as water, minerals, food and light. Allelopathy results in deletion or reduction of one plant due to the release of chemical inhibitors by another.

Allelopathy has been given much attention in the past decade as a factor influencing old-field succession and vegetational composition (Muller, 1966; Parenti and Rice, 1967; Buell, et al, 1971; del Moral and Cates, 1971). Studies have dealt with the effect of higher plants on higher plants, higher plants on microorganisms and microorganisms on higher plants (Rice, 1974). Researchers have looked at the effects of woody species on the herbaceous species found in the same habitat.

Muller (1966) found that Salvia leucophylla, Artemisia california and Adenostema fasciculata were shrubs that had a toxic effect on the annuals of the southern California grassland area. He also suggested that such biochemical inhibitional effects are most likely to be

manifested in a dry habitat. A study of Rhus glabra and R. aromatica indicated that these two species had an inhibitory effect on the grasses of the native tall grass prairie of Kansas (Croak, 1969). In the case of R. glabra and R. aromatica allelopathy was not suggested as the only reason for their success as shrubby invaders.

Another species that has been encroaching on the native tall grass prairie areas in East Central Kansas is Symphoricarpos orbiculatus Moench. S. orbiculatus, commonly known as buckbrush, is a woody perennial shrub member of the Caprifoliaceae family typically found in rock woodlands, roadsides, fence rows, sandy or rocky hillsides and prairies. Observations have shown that S. orbiculatus occurs in pure stands with none of the grassland vegetation typical of the area occurring within the boundaries of the colonies.

Several factors indicated that an allelopathic situation was occurring. Chou amd Muller (1972) stated that pure stands of any long-lived species is highly suggestive of chemical dominance. Caprifoliaceae was characterized as a particularly inhibitory family by del Moral and Cates (1971) in their study of the vegetation of western Washington. This family was the only dicot family tested that had an inhibitory level comparable to the conifers which have a high concentration of inhibitory terpenes. One species of the Caprifoliaceae tested was Symphoricarpos albus, wolf-berry, which showed a definite allelopathic potential.

Since field observations have shown that Symphoricarpos orbiculatus successfully competed with and took over sizable areas of the native prairie community, it warranted further investigation. Its success as a competitor may be partially due to the fierce competition of its roots for available water, but it was hypothesized that chemical inhibition also was a factor. This study was undertaken as an initial look at S. orbiculatus as an allelopathic invader with specific interest in its effect on the major grasses of the native tall grass prairie.

MATERIALS AND METHODS

Location and Description of Study Area

The site selected for study was an established grassland community on the Ross Natural History Reservation, 17 miles northwest of Emporia in Lyon County, Kansas. The community was located in quadrat A-42 of the Reservation. The study area was a portion of a former farmstead but the plot had never been farmed and was for the purpose of this study a native prairie. The major grasses present in the community were Andropogon gerardi Vitman, Andropogon scoparius Michx, Panicum virgatum L and Sorghastrum nutans (L) Nash. Numerous colonies of S. orbiculatus were present and of varying ages and sizes. All plant material and soil samples were collected from this area from April to October, 1976.

Rate of Movement

In the summer of 1973, metal stakes were set at five points on the boundary of one clone. (Figure 1) After the onset of the growing season in 1977, measurements were made to determine the size of the clone after four growing seasons. Transects were drawn between each of the five points and the plant located at the farthest point on the transect was used for the 1977 measurement. The plant used

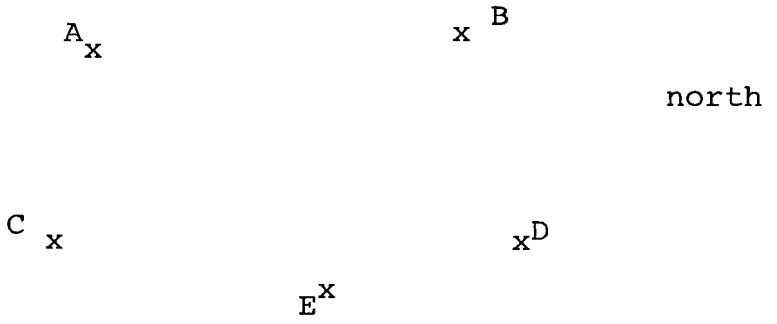

Figure 1. Placement and identification of transects of Symphoricarpos orbiculatus clone in quadrat A-42 on Ross Natural History Reservation, Lyon Co., Kansas.

in the final measurement was an established individual and not one produced from a runner. The increase of each transect was expressed as total growth after four seasons and also as an average growth per season. The weather conditions for the growing seasons also were taken into consideration.

Preparation of Extracts

Aqueous extracts of the foliage, root and fruit of S. orbiculatus were prepared for use in the seed germination and seedling growth and development tests. On 3 September 1976, S. orbiculatus foliage and root material were collected from the study site. The foliage material was separated from any extraneous stem material and the roots were washed in warm water and allowed to air dry to remove the excess soil. The fruit material was collected on 19 October 1976.

The aqueous extracts were prepared by grinding 10 g of the fresh material in 100 ml of distilled water in a Waring Blendor for 10 minutes. The mixture was allowed to set for 20 minutes and then filtered through several layers of cheese-cloth in a Buchner funnel to remove the fibrous material. The volume was returned to 100 ml with distilled water and the extracts were stored under refrigeration (Croak, 1969).

Methanol extracts of the foliage, root and fruit material were prepared for use in the chromatographic study.

These were prepared in the same manner as the aqueous extracts with the exception that 15 g of fresh material to 150 ml of methanol were used.

Seed Germination

Seeds of the following species were obtained and tested for viability before the germination test began: Andropogon gerardi (big bluestem), Andropogon scoparius (little bluestem), Panicum virgatum (switch grass), Sorghastrum nutans (Indian grass) and Plantago ovata (psyllium). The germination test was carried out by placing 75 seeds of one species in a sterile Petri plate containing a germination disc and seven ml of the designated aqueous extract were added. The three aqueous extracts of S. orbiculatus root, foliage and fruit were used as the test groups and distilled water as the control. The Petri plates were incubated in a dark growth chamber at 26 C for the duration of the test. After 24 hours, the number of seeds germinated was recorded at 12 hour intervals for 108 hours. The test was conducted in three replicates with 300 seeds of each species being treated with each extract in the separate segments, so that by the conclusion of the testing 900 seeds were tested in each group.

Seedling Growth and Development

Seedlings of big bluestem, little bluestem, switch grass, Indian grass and Lycopersicum esculentum (tomato)

were grown in white quartz sand supplied with nutrient solution (Hoagland and Arnon, 1950) until they reached two weeks of age. The seedlings were then transferred to individual amber plastic vials containing a 5:1 ratio of aqueous extract to nutrient solution. Ten individuals of each species were placed in the solution of the three extract treatments and the distilled water control.

The seedlings were then placed in a growth chamber with a 14 hour photoperiod at 27 C and 19 C as the night temperature. After 10 days the seedlings were harvested and dried for 48 hours at 38 C. The oven-dry weight for each individual plant was then determined.

Soil Analysis

Four soil samples were taken from within the clones at the study site at the 0" - 6" depth in April 1976. The samples were analyzed as to soil type by the Atterberg method.

Soil extracts for the separation of inhibitory compounds from the soil under the S. orbiculatus clones were prepared by mixing 75 g of dry soil in 125 ml of methanol and boiling gently for 8 hours. The extract produced was filtered through Whatman 1 and reduced in volume to 10 ml in vacuo (Croak, 1969). Five ml of each extract was spotted on Whatman 3MM chromatography paper and developed in a n-butanol:acetic acid:water solvent (63:10:27 v/v) (Parenti, 1967). The developed chromatograms were examined with

ultraviolet light and then treated with ferric chloride-potassium ferricyanide indicator.

Chromatographic Study

The methanol extracts of S. orbiculatus foliage, root, and fruit were used to determine if phenolic compounds were present in the plant. The methanol extracts were spotted on Whatman 3MM paper and developed in a n-butanol: acetic acid:water solvent (63:10:27 v/v). A few known inhibitors were co-chromatographed at the same time. The developed chromatograms were viewed under ultraviolet light and then treated with ferric chloride-potassium ferricyanide, a phenol indicator.

RESULTS

Rate of Movement

After four growing seasons the clone measurement had an average increase of 2.546 m. This would indicate an average increase of 0.636 m/year (Table I). The average rainfall during these growing seasons from 1973 to 1977 had been below normal so that one might assume this movement to be more rapid in years with a normal or heavy rainfall.

Seed Germination

The three extracts of *S. orbiculatus* inhibited the germination of all species with the exception of the root extract effect on *A. gerardi* which showed an increase over the control (Table II). The fruit extract reduced the germination of *A. scoparius*, *P. virgatum* and *P. ovata* significantly when tested at the $p = .05$ level. The foliage extract also significantly reduced the germination of all species except *A. gerardi*. The root extract had the least effect on the species tested, only showing a significant effect on *A. scoparium* and *P. ovata*. The total germination of *S. nutans* was only 3 to 9% for all treatments at the end of 108 hours, so the results were not statistically analyzed.

At the end of the 108 hours test period many of the Petri plates had fungal growth associated with the extracts but not with the control. The fruit extract had the

TABLE I. Transect lengths in meters of Symphoricarpos orbiculatus clone on Ross Natural History Reservation.

Transect	1973	1977	Total growth
A-D	8.85	11.77	2.92
A-E	6.24	8.70	2.46
B-C	7.70	9.28	1.58
B-E	5.68	8.32	2.64
C-D	8.40	11.53	3.13
		average	2.546

of seed germination.

Species	*	Number of seeds germinated at time, hr 24	36	48	60	72	84	96	108	total % germination
Andropogon gerardi	A	10	55	157	252	313	364	418	450	50.0
	B	5	21	82	143	211	257	307	345	38.3
	C	5	26	64	135	206	253	290	326	36.2
	D	8	28	94	181	241	299	348	373	41.2
Andropogon scoparius	A	0	6	35	79	128	160	217	254	28.2[a]
	B	1	4	10	33	50	78	95	130	14.4[a]
	C	0	1	10	35	72	107	154	183	20.3[a]
	D	4	14	42	128	206	266	318	369	41.0
Panicum virgatum	A	115	221	404	559	663	691	702	712	79.1
	B	24	73	161	236	362	450	496	528	58.7[a]
	C	9	19	54	145	204	250	316	372	41.3[a]
	D	150	320	508	665	685	724	728	734	81.6
Sorghastrum nutans	A	0	0	2	9	11	15	23	24	2.7
	B	1	2	4	7	8	14	22	29	3.2
	C	0	0	0	4	8	8	16	25	2.8
	D	3	3	5	20	24	34	42	55	9.2
Plantago ovata	A	48	227	386	477	520	547	563	586	65.1[a]
	B	23	68	117	177	207	224	246	261	29.4[a]
	C	52	89	124	181	204	223	239	257	28.6[a]
	D	260	548	664	708	725	733	739	746	82.9[a]

* A - Root extract
B - Foliage extract
C - Fruit extract
D - Control, distilled water

[a] - Significantly different from control at p = .05

greatest amount of fungal association. Also observed was an irregularity of the shoot emerging from the seed before the root emerged. This irregularity was present in those seeds treated with the three plant extracts but not in the control situation.

Seedling Growth and Development

The fruit and root extracts of S. orbiculatus significantly decreased the growth and development of all species except A. gerardi. Both extracts decreased the growth of A. gerardi but not to a significant extent in the dry-weight determination (Table III). The foliage extract had a significant effect on all seedlings but those of A. gerardi and S. nutans. All three extracts caused a decrease in the dry-weight of all species tested even though all were not statistically significant.

At the end of the 10 day test period all the seedlings treated with the plant extracts were observed to be stunted with the leaf shoot dry. A fungal growth, which was not present in the control solution, was evident in all three extract solutions in the vials when harvested.

Soil Analysis

The Atterberg soil analysis determined that the soil the S. orbiculatus colonies were established in, was a clay classification in each case. In the clay soils, the micelles will persist longer, allowing water soluble

Table III. Seedling growth and development in extract solution, expressed as mean ± S.E. dry weight in mg after 10 days in solution.

Species	Extracts			
	Root	Foliage	Fruit	Control
Andropogon gerardi	8.7±1.64	9.6±2.22	9.4±2.59	10.7±3.02
Andropogon scoparius	4.6±1.26 *	3.8±1.23 *	5.7±2.00	7.6±2.32
Panicum virgatum	4.0±0.94 *	4.5±0.71 *	4.6±1.65 *	6.2±0.92
Sorghastrum nutans	5.0±1.33 *	6.5±1.43	5.2±1.62 *	8.1±2.38
Lycopersicum esculentum	5.5±3.02 *	5.8±2.20 *	5.2±1.87 *	35.3±9.10

* - Significantly different from control at p = .05

compounds to also be present in the soil for longer periods of time. The chromatograms of the soil extracts revealed only a faint presence of phenolic compounds when viewed under ultraviolet light and after treatment with the ferric chloride-potassium ferricyanide indicator (Table IV). A comparison to known compounds also developed showed a similarity of the soil extracts to coumaric acid, hydroxy-coumarin, hydro-coumarin, feruin, salicyclic acid and synaptic acid.

Chromatographic Study

When the developed chromatograms were reviewed, they showed a definite presence of phenolic compounds in each of the three methanol extracts. The foliage and fruit extracts exhibited the best separations. The foliage and fruit extracts exhibited the best separations. The foliage extract had separated into three bands visible under ultraviolet light and after treatment with the phenolic indicator. Four distinct bands were present in the fruit extract. The root extract had the poorest separation with only three faint bands visible. (Table V).

Table IV. Results of soil extract chromatography in n-butanol:acetic acid:water (63:10:27 v/v).

Sample	Rf	Color in UV light
Clone #1	.89	lt. blue
Clone #2	.90	lt. blue
Clone #3	.87	lt. blue
Clone #4	.90	lt. blue
Coumaric acid	.89	lt. blue
Hydro-coumarin	.92	white-lt. blue
Hydroxy-coumarin	.95	lt. blue
Feruin	.86	lt. blue
Salicylic acid	.91	lt. blue
Synaptic acid	.82	lt. blue

Table V. Chromatography results of Symphoricarpos orbiculatus extracts in n-butanol:acetic acid:water solvent (63:10:27 v/v).

Extract		Rf	Color under UV
Root	1	.16	yellow
	2	.47	white
	3	.89	green
Foliage	1	.27	-----
	2	.41	blue
	3	.88	orange
Fruit	1	.15	blue
	2	.20	red/purple
	3	.27	bt. yellow
	4	.91	orange

DISCUSSION

The results of the measurement of the rate of growth indicated that the established stand of S. orbiculatus increased at a substantial rate each year. The age of the center of this clone was unknown but was estimated to be over 10 years old. During the growing seasons involved, the rainfall of the area was below the average expected. This decreased amount of water available also may have decreased the rate of growth in the S. orbiculatus stand.

In the seed germination and seedling growth and development tests, the results showed that allelopathy was a factor in the relationship between the grasses of the native tall grass prairie and S. orbiculatus. Even though A. gerardi was not inhibited to a significant level by the test extracts, it showed a slight decrease in its growth and development and seed germination, except in the case of the root extract. In this case, the A. gerardi treated with the root extract exceeded the germination of the control. Robert L. Parenti, Emporia State University (personal communication), has suggested that the stimulatory effect of a plant extract on seed germination may be a contributing factor in species dominance. The S. nutans did not germinate to a sufficient extent to be analyzed in the seed germination test but the

overall trend showed a decrease in the germination in the test groups as compared with the control.

In each case where there was a significant decrease in the seed germination of the test groups there also was a time lag involved. The extracts delayed the length of time required for germination. This delay in germination may allow the inhibitory plant to establish itself since the grasses are delayed in their establishment of new seedlings. Another observation that may have an effect on seedling establishment was one of root versus shoot emergence. During the germination testing of the plant extracts it was noted that on many occasions the shoot would emerge from the seed first rather than the root as is the general case. This irregularity in emergence was only rarely noted in association with the control groups. This reversal in emergence could have an effect on the total germination and establishment of new seedlings.

During both the germination and seedling experimentation, a fungal growth became associated with the plant extracts but not with the control groups. This fungal association may be present in the natural situations but this research was not designed to explore that possibility. The fungal growth may have been a factor in the decrease of the total germination but in most cases the fungal growths were not visibly present until after 72 hr. This was after a delay and reduction in germination had already been noted.

The action of these microorganisms may be such that the fungal interaction may enhance the deleterious effect of the extracts. Robert L. Parenti (personal communication) related that pioneer research in this aspect of allelopathy has been conducted by Z. A. Patrick, University of Toronto. Patrick has shown that allelopathic substances and plant pathogenic soil organisms were co-factors in causing poor plant growth. This poor growth was generally a result of root degeneration where both inhibitory compounds and microorganisms were present in the soil.

The soil chromatograms revealed a faint presence of inhibitory compounds present in the soil under the existing clones. The two most probable compounds when compared against those used in the tests were comaric acid and salicyclic acid. These two compounds' similarity to the soil extracts provided a clue to the nature of the phenols present. The small amounts of inhibitory compounds in the soil may have been due to the time of year the samples were taken. In 1975, Lodhi did a series of tests on the biological activity of soils containing three known inhibitory compounds throughout the year. Of the three months he tested, April did not show a high level of activity in the upper soil zone. This could be the case with those inhibitory compounds released by S. orbiculatus.

In the chromatographic examination of the plant extracts, it was determined that there are phenolic compounds present in the plant. The isolations revealed that within

the foliage and fruit extracts there were three and four bands, respectively, present. The root extract showed three very faint bands. This follows the trend of the germination and seedling tests, which showed the root extract to have the least amount of biological activity of the three.

With the foliage and fruit extracts showing the greatest allelopathic potential, one must speculate on the introduction of inhibitory substances into the environment. Water soluble toxins can be released through the leaching of both living and dead leaves (Rice, 1974). These toxins would be released as the rain fell on mature plants or on the foliage after it became incorporated into the litter. Another release of plant toxins was through the decay of plant material. As the material decayed its components are released into the environment. This could most logically be associated with the decay of the fleshy portion of the fruit to release the seed for growth. The exact mechanism of release for the S. orbiculatus inhibitory compounds is an area yet to be explored.

SUMMARY

This study reveals that Symphoricarpos orbiculatus has a role as an allelopathic inhibitor in a native tall grass prairie. It affects the seed germination and seedling growth and development of the grass species Andropogon scoparius, Sorghastrum nutans and Panicum virgatum. The plant extract chromatograms confirms the presence of phenolic compounds in the foliage, root and fruit of S. orbiculatus.

This was an initial study and does not seek to answer all questions raised about S. orbiculatus as an inhibitor and competitor. Allelopathy was a factor in its relationship to native grasses but this may not be the only factor involved. Further research into the specific compounds involved in S. orbiculatus inhibition, its relationship with physical factors, the leaching and decay mechanisms and its role throughout the total growing season may reveal the entire picture of Symphoricarpos orbiculatus as an allelopathic inhibitor in the tall grass prairie.

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APPENDIX

I. Known inhibitory compounds used as standards for comparison in soil extract chromatography.

Acolinic acid
Caffeic acid
Catechol
Chlorogenic acid
Coumaric acid
p-coumaric acid
Esculin
Feruin
Gallic acid
Gallotannic acid
Hydro-coumarin
Hydroxy-coumarin
Isochlorgenic acid
Quercitin
Quinic Acid
Salicylic
Sulfosalicylic acid
Synaptic acid
Vanillic acid

II. Known inhibitory compounds used as standards for comparison in S. orbiculatus extract chromatography.

Chlorogenic acid
Coumarin
Esculin
Gallic acid
Gallotannic acid
Naphthol
Naringin
Orcinol
Phthalic acid
Pyrocatechnic
Pyrocatechol
Pyrogallol
Salicylic acid
Vanillic acid