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ALLELOPATHIC EFFECTS OF POLYGONUM AVICULARE L.

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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ALLELOPATHIC EFFECTS OF *Polygonum aviculare* L.

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

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ALLELOPATHIC EFFECTS OF Polygonum aviculare L.

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ALLELOPATHIC EFFECTS OF *Polygonum aviculare* L.

CHAPTER I

INTRODUCTION

Polygonum aviculare (prostrate knotweed) was observed to have a strong interference against *Cynodon dactylon* (L.) Pers. (bermudagrass) resulting in drastic changes in vegetational patterning. It shows a great ability to spread rapidly into heavy stands of bermudagrass after a short period of time indicating that an allelopathic mechanism may be involved in addition to competition.

The results of this study are presented in two separate papers prepared according to the instructions for contributors to the Journal of Chemical Ecology. The first paper was conducted to determine whether an allelopathic mechanism is involved, using weedy and cultivated species, and to determine the ways in which the suspected allelopathic agents are released into the environment. The second paper was undertaken to isolate and characterize the allelopathic compounds and determine their biological activities.

These papers have been submitted to the editors of the Journal of Chemical Ecology for consideration for publication.

CHAPTER II

ALLELOPATHIC EFFECTS OF Polygonum aviculare L.

Paper I. Vegetational Patterning

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Abstract - Polygonum aviculare was observed to spread rapidly into heavy stands of Cynodon dactylon (L.) Pers. indicating a strong interference against Cynodon dactylon. Measurements of selected soil minerals and physical factors indicated that competition was probably not the chief cause of that interference. Soil collected under senescent Polygonum was very inhibitory to all test species except Sporobolus pyramidatus (Lam.) Hitch., suggesting the presence of inhibitory compounds. Tops and roots of Polygonum, root exudates, and leachate of the tops inhibited seed germination and seedling growth of most test species. Therefore, allelopathy appeared to be the dominant component of the interference, with competition probably accentuating its effects. Polygonum aviculare was inhibitory to Gossypium barbadense L. and Sorghum bicolor (L.) Moench indicating that allelopathy is an important component of the interference by Polygonum against crop yields.

Key Words - Allelopathy, interference, patterning, biological control, Polygonum, Cynodon, Chenopodium, Sorghum, Gossypium.

INTRODUCTION

Several workers have shown that allelopathy may play an important part in weed-weed interactions (Wilson and Rice, 1968; Rasmussen and Rice, 1971; Newman and Rovira, 1975) and weed-crop interactions (Bell and Koeppe, 1972; Tames et al., 1973; Colton and Einhellig, 1978). Such interactions may lead to the reduction or elimination of associated plants.

Polygonum aviculare (prostrate knotweed) is a serious, principal or common weed in crops in many parts of the world (Holm et al., 1979) resulting in marked reductions in their yields. It is also a pernicious weed in lawns in many countries, including the USA.

The junior author observed a rapid encroachment of prostrate knotweed into bermudagrass (Cynodon dactylon) lawns in Norman, Oklahoma. Bermudagrass dies in the patches of prostrate knotweed (Figure 1) while bermudagrass at the edges of the knotweed patches turns yellow.

The rapid invasion of heavy bermudagrass sod by Polygonum and the existence of Polygonum in pure stands after a short period of time suggests that an allelopathic mechanism may be involved in its interference in addition to competition. Therefore, the present study was conducted primarily to determine whether Polygonum aviculare is allelopathic to bermudagrass, other commonly associated species, and selected crop plants. Another goal was to obtain preliminary data concerning the feasibility of using prostrate knotweed in the biological control of bermudagrass and other weeds in certain crops.

EXPERIMENTATION AND RESULTS

Selection of Test Species. Cynodon dactylon and Sporobolus pyramidatus were chosen as test species because they were found to be associated with Polygonum aviculare. Chenopodium album L. was included because it is a serious weed in cultivated lands and is sometimes associated with P. aviculare (Bhomik and Doll, 1979; Holm et al., 1979). Sorghum (Sorghum bicolor) and cotton (Gossypium barbadense) were chosen as test species because they are important crops in which bermudagrass is often a noxious weed (Jordan, 1977). If Polygonum aviculare is markedly allelopathic to them, it is unlikely that it could be used for biological control of Cynodon dactylon in these crops.

Rate of Invasion of Bermudagrass Sod by Prostrate Knotweed. Stakes were placed around the perimeters of several prostrate knotweed stands in bermudagrass lawns in April, 1980. Measurements were made in November, 1980 to determine the rate of spread. It was found that Polygonum stands increased in diameter at an average rate of 1.5 m per growing season even though they were surrounded by a heavy sod of bermudagrass.

Physical and Mineral Properties of Soil. Selected physical and mineral properties of soil were analyzed to determine whether P. aviculare causes changes in those soil properties which could account for the alteration of vegetational patterns in the field.

Ten soil samples minus litter were collected to a depth of 30 cm within a Polygonum stand and 10 similar samples were taken in the adjacent areas where only

Cynodon dactylon was growing. The samples were air-dried, passed through a sieve with 2 mm openings, and analyzed for pH by the glass electrode method of Piper (1942). Soil texture was determined by mechanical analysis using a modified Bouyoucos hydrometer method (Bouyucos, 1936; Piper, 1942). The remaining soil was ground to pass through a 0.5 mm sieve and analyzed for total nitrogen by the macro-Kjeldahl method of Bremner (1965), and for total phosphorus by the method of Shelton and Harper (1941). Easily extractable potassium, magnesium, calcium and zinc were determined by atomic absorption after extraction of 5 g of each soil sample with 20 ml of a 0.075 N acid mixture consisting of equal volumes of 0.05 N HCl and 0.025 N H_2SO_4 (Perkin-Elmer, 1976). All calculations were based on the oven-dry weight of the soil.

Calcium was the only soil factor tested which differed significantly between the Polygonum stand and the surrounding bermudagrass stand, and it was higher in the Polygonum area (Table 1).

Effects of Field Soils on Test Species. The following experiment was designed to eliminate possible competition due to differential ion uptake, and to determine if allelopathic compounds are released by prostrate knotweed into the soil and remain stable long enough to affect plant growth. Soil minus litter was collected under a Polygonum stand and placed in 10 cm diameter glazed pots. A similar collection was made under Cynodon dactylon and used as a control. Soil collections were made in July during active growth of Polygonum and in March, four months after Polygonum became senescent. Each collection was treated as a separate experiment.

Twenty five seeds of all test species except Sporobolus pyramidatus were planted in their respective pots. Large numbers of S. pyramidatus seeds had to be

planted in all experiments to obtain a sufficient number of seedlings for the tests. Ten test and ten control pots were used per test species in this and all subsequent experiments described below, and all experiments were run under greenhouse conditions except the U-tube experiment. Germination was recorded two weeks after the seeds were planted, at which time the plants were thinned to the three largest per pot and allowed to grow another two weeks. All plants were harvested, separated into roots and tops and compared on the basis of oven-dry weights.

Soil collected in July did not affect seed germination and seedling growth of any of the test species significantly (Table 2). Soil collected in March, however, markedly inhibited seed germination of all test species except Sorghum bicolor. Seedling growth of all species, except Sporobolus pyramidatus, was significantly inhibited by the soil collected in March from Polygonum stands. In some species, the inhibition was primarily due to a retardation in root growth and in others primarily to a retardation in top growth. Both root and top growth of bermudagrass were significantly inhibited, however. These results indicate a phytotoxic effect of soil closely associated with prostrate knotweed and eliminate any competitive mechanism associated with its presence. Several types of experiments were run subsequently to determine the source of the toxins in the soil under Polygonum and these are described below.

Effects of Leaf Leachate. Artificial rain in the form of a fine spray of cistern water was allowed to fall on fresh mature plants of Polygonum. The leachate which dripped from the leaves was collected and used to water test pots each containing a substrate consisting of two parts of soil and one part of sand. Each control pot contained a similar substrate plus 25 seeds of a given test species

but was watered with an equal amount of cistern water that had not been sprayed over Polygonum. Germination percentages were recorded two weeks after planting, after which the plants were thinned to the four largest per pot, allowed to grow for two weeks and compared on the basis of oven-dry weights.

The leachate reduced germination percentages slightly in all test species (Table 3). Chenopodium album was significantly inhibited in growth while Cynodon dactylon was significantly stimulated. The leachate did not affect growth of other test seedlings.

Effects of Decaying Shoots. Based on clipped quadrats in a P. aviculare stand, it was found that mature plants of Polygonum produce an average of 4.1 g of air-dried tops per kilogram of soil to a depth of 10 cm (average depth of rooting of P. aviculare). To test the possible phytotoxicity of Polygonum tops on test species, 25 seeds of each test species were planted in separate glazed pots containing 4.1 g of air-dried Polygonum tops per kilogram of a 2:1 soil-sand mixture. An equal concentration of milled peat moss was added to the soil-sand mixture in the control posts to keep the organic matter content the same. All pots in the experiment received equal amounts of cistern water when necessary. The percentage of seed germination was determined 14 days after planting, after which the seedlings were thinned to the four largest per pot allowed to grow an additional 14 days, then harvested. The biomass of the test species was determined on the basis of oven-dry weights.

Decaying Polygonum tops drastically inhibited germination of cotton and Chenopodium album (Table 4), and germination of other species was also appreciably reduced. Decaying tops of Polygonum significantly reduced seedling growth of all test species.

Effects of Decaying Roots. Field sampling of Polygonum at the end of July when the plant was mature revealed an average of 1.9 g air-dried roots per kilogram of soil to a depth of 10 cm. To test the possible effects of decaying Polygonum roots on the test species 25 seeds of each test species were planted in separate pots containing either 1.9 g of air-dried roots per kilogram of a 2:1 soil-sand mixture, or an equal concentration of milled peat.

Germination percentages were determined two weeks after planting. The plants were thinned to the four largest per pot and were allowed to grow for another two weeks, at which time they were harvested and oven-dry weights were determined.

The decaying roots reduced the germination percentages of Cynodon dactylon and Chenopodium album appreciably (Table 5). There was virtually no effect on germination of other test species. Seedling growth of Gossypium barbadense and Cynodon dactylon was significantly reduced but the other test species were not affected.

Effects of Different Periods of Decomposition of Residues. In order to study the dynamics of the phytoxic compounds which might be liberated from decaying materials of Polygonum in soil, an experiment was designed to determine effects of different periods of decomposition on growth of test species.

Twenty five seeds of each test species were placed in separate pots containing either 6 g of Polygonum (2 g of air-dried roots and 4 g of air-dried tops) per kilogram of a 2:1 soil-sand mixture (tests) or an equal concentration of milled peat moss (controls).

Root biomass of cotton, sorghum and Chenopodium was significantly reduced by decomposing Polygonum residues at the end of 20 days and root biomass

of all species, except sorghum, was still significantly reduced after 40 days (Table 6). It is notable that root biomass of bermudagrass was significantly reduced after 40 days but not after 20 days. Shoot biomass of all test species except sorghum was significantly reduced at the end of 20 days but only shoot biomass of cotton and bermudagrass was still reduced at the end of 40 days. Whole plant biomass of all test species except Sporobolus pyramidatus was significantly reduced after 20 days and this reduction in whole plant biomass persisted for 40 days in all these species except sorghum.

Effects of Root Exudates on Test Species. P. aviculare plants about 5 inches tall were transferred from the field to pots filled with washed quartz sand. The experiment was designed to eliminate competition for light, water and minerals between Polygonum and the test species. Pots containing seeds of the test species and pots of Polygonum were placed on alternate steps of a staircase device (Bell and Koeppe, 1972). A control series consisted of pots of test species alternating with pots of washed sand. Complete nutrient solution (Hoagland and Arnon, 1950) was pumped from a reservoir at the bottom of each series. The nutrient solution dripped from pot to pot down to the bottom reservoir where it was recycled every twelve hours. One week after germination, seedlings of the test species were thinned to the two largest per pot, allowed to grow another four weeks and compared on the basis of oven-dried weights.

The root exudate significantly inhibited root growth of sorghum, top growth of bermudagrass, and root and top growth of Chenopodium album (Table 7). Seedling growth of other test species was not significantly affected.

To eliminate possible effects from algal growth on the sand surface of some pots in the staircase experiment, a U-tube experiment was designed to

determine whether the root exudate of Polygonum aviculare was toxic to the same test species (Tubbs, 1973).

U-tubes made from Pyrex tubes 2.5 cm in diameter and 56 cm long, were painted with aluminum paint to exclude light. These were filled with aerated Hoagland's nutrient solution. Ten test and 10 control tubes were used for each test species. In the test series, test seedlings of uniform shoot and root length were placed in one end of the U-tubes, one seedling per tube, and a Polygonum seedling was placed in the other end of each tube. In the control series, one test seedling was placed in each end of each U-tube. Roots of all seedlings were inserted through a one-hole rubber stopper and held in place with non-wetting cotton. The nutrient solution was aerated for 10 minutes each day. Test seedlings were allowed to grow for three weeks after being placed in the U-tubes, at which time they were harvested and compared on the basis of oven-dry weights. All plants in these experiments were grown on a 14-hr photoperiod (1500 ft-c) at 28°C and a 10-hr dark period at 20°C.

Root exudates of Polygonum significantly reduced seedling growth of Chenopodium album and Cynodon dactylon but no other species (Table 8). The reduction in Cynodon dactylon was primarily against shoot growth. It is noteworthy that these two species were significantly retarded in growth in the staircase experiments also, thus substantiating results of those experiments.

To test whether inhibitory activity of the exudate was related to a particular period during the growth and development of Polygonum, root length of Cynodon dactylon was measured every four days during the 20 day growing period in the U-tube experiment. Inhibition in root growth started at the beginning of the experiment and continued throughout the growing period (Figure 2). The inhibition was significant ($P<0.05$) on all days of measurement except day 12.

DISCUSSION

Interference among plant species can be due to competition or competition plus allelopathy in certain cases (Hull and Muller, 1977; Anderson et al., 1978; Rice, 1979). Such interference often leads to a superiority of a particular species and failure of a second under natural conditions.

Field studies indicated that the rapid spread of Polygonum into a solid stand of bermudagrass is apparently not due to changes in pH, minerals and/or soil texture. However, soil collected form Polygonum stands four months after death of P. aviculare plants drastically reduced seed germination and seedling growth of all test species except Sporobolus pyramidatus. Subsequent experiment demonstrated that toxins are released from the Polygonum plants through leaching of the leaves with rain, exudates of the roots, and decomposition of both root and shoot residues. The toxins remain stable in the soil for moderately long periods of time as indicated by the toxicity of soil in Polygonum stands for at least 4 months after death of the plants. Moreover, half of the test species were significantly inhibited in growth for at least 40 days after addition of prostrate knotweed residues to soil.

It is significant that P. aviculare was inhibitory to bermudagrass in all tests whereas it inhibited Sporobolus pyramidatus in only two tests, and both of these included decaying shoots. Thus, it is obvious that the toxins produced by P. aviculare are much more inhibitory to growth of bermudagrass than to Sporobolus pyramidatus, and this probably explains why the latter continues to grow nicely in association with Polygonum after bermudagrass has been completely eliminated. It is noteworthy that Sporobolus pyramidatus was previously found to be allelopathic to bermudagrass also (Rasmussen and Rice, 1971).

It is clear from the evidence that allelopathy was the basic phenomenon responsible for the patterning of vegetation observed in this study. However, once seed germination and growth of a species are inhibited byh allelopathic compounds, competition undoubtedly accentuates the growth inhibition. It is also clear that allelopathy is an important component of the interference exhibited by P. aviculare against crop yields. In our experiment, however, the allelopathic effects against cotton and sorghum were not as pronounced generally as they were against Chenopodium album and bermudagrass but more than they were against Sporobolus pyramidatus.

Root exudates and leaf leachates of Polygonum were not inhibitory to growth of cotton so it appears that it might be possible to plant Polygonum aviculare between cotton rows to help control bermudagrass, Chenopodium album and perhaps some other weeds. Sorghum roots were inhibited slightly by root exudates of prostrate knotweed in the staircase test but not in the U-tube test, and the leachate of Polygonum had no effects on sorghum. Thus, it might even be possible to plant prostrate knotweed between sorghum rows to control various weeds including bermudagrass and Chenopodium album.

Even though decaying shoots of P. aviculare were inhibitory to sorghum and decaying roots and shoots were inhibitory to cotton, it appears likely that residues of Polygonum could be used as a mulch between cotton and sorghum rows to control growth of bermudagrass, Chenopodium album, and perhaps other weeds. No doubt smaller amounts of residues could be used than were used in our experiments and the mulch could be kept a slight distance away from cotton or sorghum rows. In our experiments the residues were mixed with soil in which crop species were planted.

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Legend for Figures

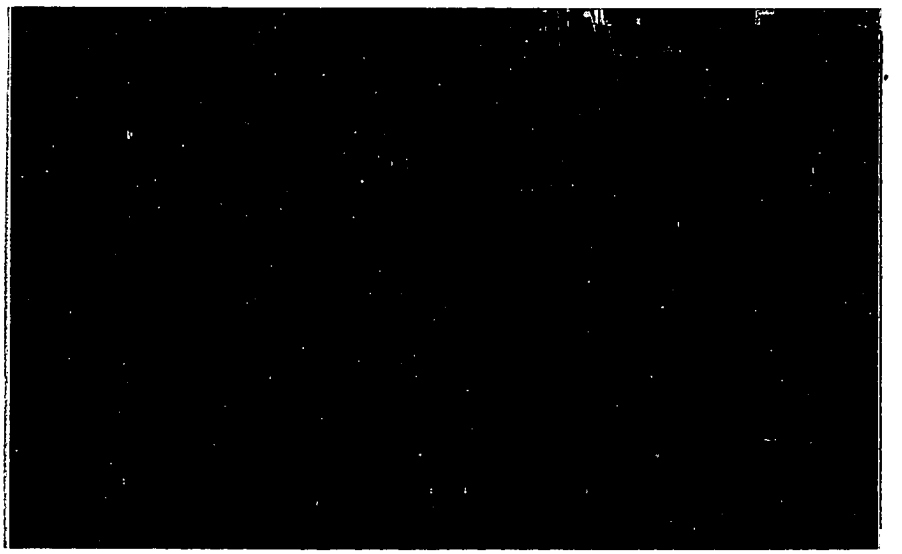
Fig. 1 Invasion of Cynodon dactylon sod by Polygonum aviculare.

(a) Photograph taken in June - A, green Cynodon dactylon; B, Cynodon dactylon turning yellow adjacent to Polygonum aviculare; C, pure stand of Polygonum aviculare; D, Sporobolus pyramidatus associated with Polygonum aviculare.

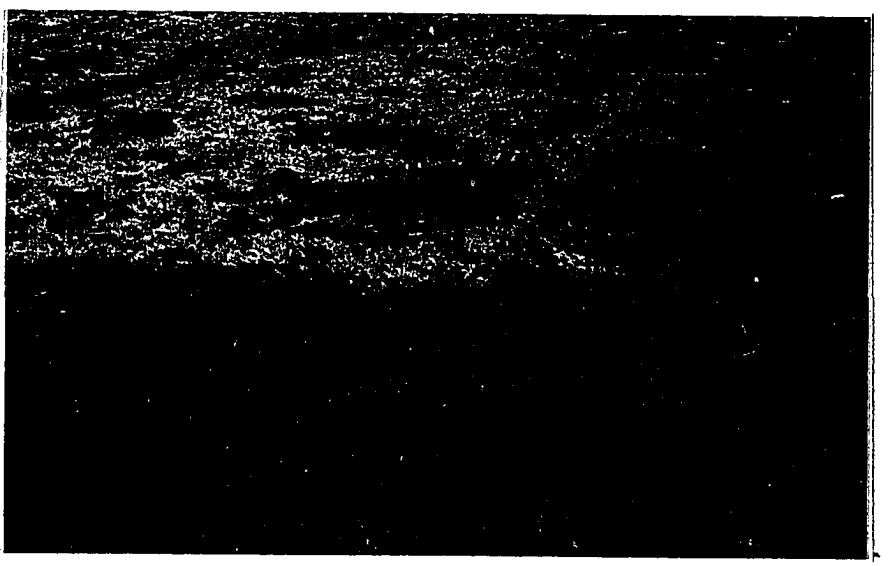
(b) Photograph taken in late November - A, Cynodon dactylon; B, pure stand of Polygonum aviculare.

Fig. 2 Timing of release of inhibitor(s) from roots of Polygonum aviculare as indicated by growth in length of Cynodon dactylon roots.

a



b



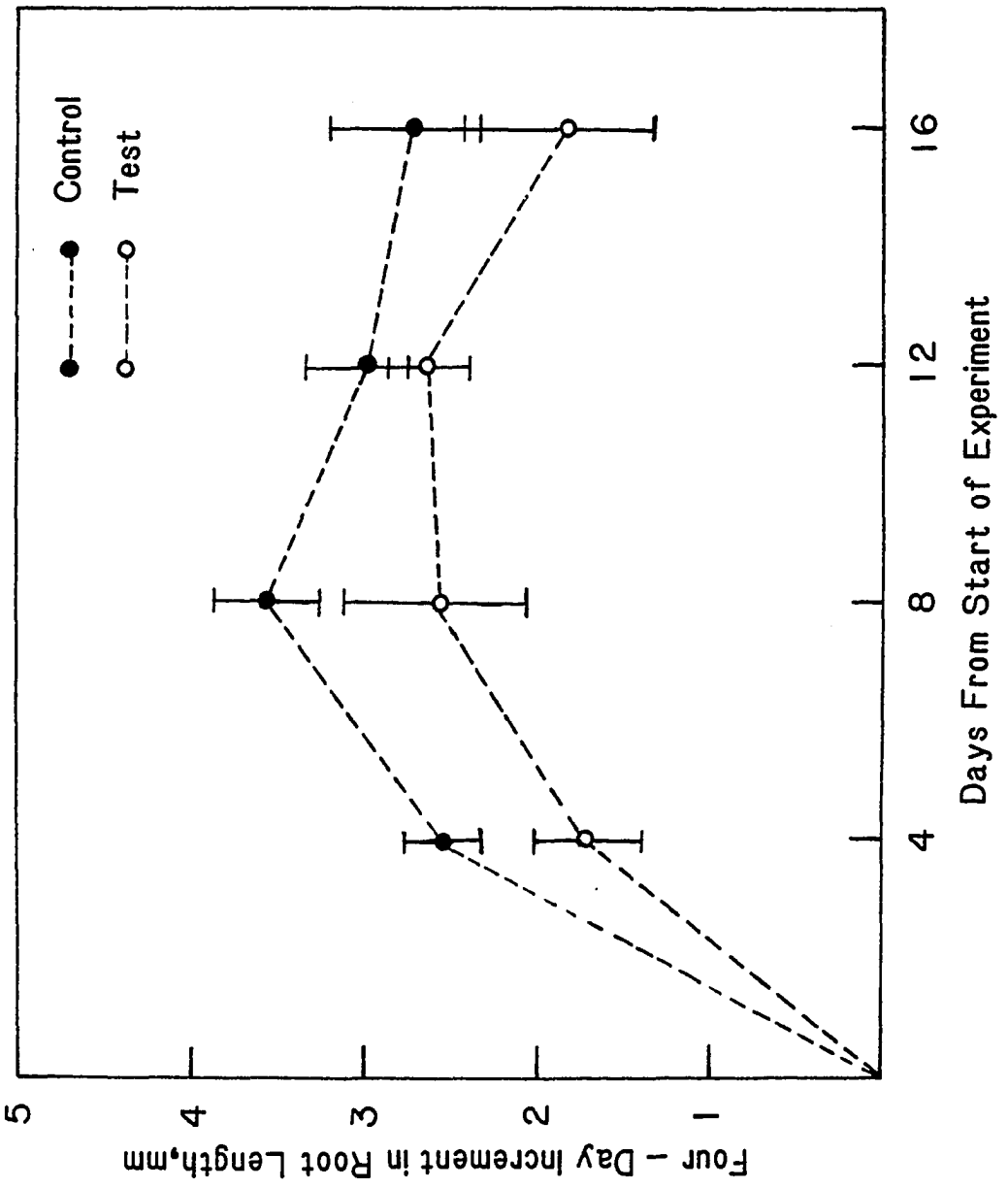

Control
Test
Four – Day Increment in Root Length,mm
5
4
3
2
1
4
8
12
16
Days From Start of Experiment

Table 1. Comparison of Soil Factors Within a Polygonum Stand and Outside it.

Test[a]	Inside Polygonum Stand	Outside Polygonum Stand
pH	7.58	7.72
Percent Sand	80.03 ± 1.25	78.14 ± 1.12
Percent Silt	6.53 ± 1.00	6.09 ± 1.00
Percent Clay	14.44 ± 0.96	15.76 ± 0.39
Percent Total N	0.57 ± 0.04	0.58 ± 0.03
P, ppm	14.10 ± 0.29	14.30 ± 1.58
K, ppm	129.40 ± 0.85	128.34 ± 1.58
Mg, ppm	370.32 ± 48.26	328.00 ± 14.24
Ca, ppm	521.80 ± 30.33[b]	474.20 ± 12.41
Zn, ppm	8.32 ± 2.10	7.00 ± 1.13

[a]Each value is average of 10 replicates.

[b]Difference significant at 0.05 level.

Table 2. *Effects of Field Soils from and adjacent to* Polygonum *Stands on Germination and Seedling Growth of Selected Species*

Test Species	Date Soil Taken	Mean dry weight with standard error, mg[a]						Germination
		Control			Test			% of Control
		Root	Shoot	Whole Plant	Root	Shoot	Whole Plant	
Gossypium barbadense	July 30	313 ± 44	832 ± 36	1145 ± 52	324 ± 43	772 ± 97	1096 ± 69	91
	Mar. 20	95 ± 17	263 ± 18	358 ± 19	67 ± 17	217 ± 26[b]	284 ± 28[c]	54
Sorghum bicolor	July 30	573 ± 43	497 ± 32	1070 ± 71	597 ± 42	540 ± 37	1137 ± 56	96
	Mar. 20	257 ± 12	287 ± 18	544 ± 22	147 ± 37[b]	219 ± 16	366 ± 50[c]	96
Chenopodium album	July 30	279 ± 19	404 ± 49	683 ± 70	234 ± 27	240 ± 22	474 ± 45	92
	Mar. 20	107 ± 20	532 ± 40	639 ± 40	44 ± 5[c]	490 ± 76	534 ± 82[b]	66
Sporobolus pyramidatus	July 30	167 ± 18	202 ± 20	369 ± 21	204 ± 22	173 ± 9	377 ± 23	---
	Mar. 20	16 ± 4	39 ± 6	55 ± 14	10 ± 1	36 ± 6	46 ± 8	---
Cynodon dactylon	July 20	149 ± 21	293 ± 47	442 ± 58	141 ± 23	324 ± 40	465 ± 34	97
	Mar. 20	47 ± 5	116 ± 6	163 ± 12	12 ± 1[d]	36 ± 5[b]	48 ± 5[c]	53

[a]Each value is average of 30 replicates.

[b]Dry weights significantly different from control at 0.05 level

[c]Dry weights significantly different from control at 0.01 level.

[d]Dry weights significantly different from control at 0.001 level.

Table 3. Effects of Polygonum Leaf Leachate on Seed Germination and Seedling Growth of Selected Species.

Species	Mean dry weight with standard error, mg[a]		Germination
	Control	Test	Percent of Control
Gossypium barbadense	1200 ± 67	1270 ± 67	81
Sorghum bicolor	1260 ± 101	1254 ± 40	96
Chenopodium album	467 ± 10	417 ± 36[c]	90
Sporobolus pyramidatus	76 ± 10	64 ± 8	--
Cynodon dactylon	87 ± 17	109 ± 17[b]	93

[a]Each value is average of 40 replicates.

[b]Dry weights significantly different from control at 0.05 level.

[c]Dry weights significantly different from control at 0.01 level.

Table 4. Effects of Decaying Polygonum Shoots on Seed Germination and Seedling Growth of Selected Species.

Species	Mean dry weight with standard error, mg[a]		Germination
	Control	Test	Percent of Control
Gossypium barbadense	890 ± 23	563 ± 53[c]	25
Sorghum bicolor	804 ± 47	696 ± 37[c]	61
Chenopodium album	190 ± 12	51 ± 19[c]	19
Sporobolus pyramidatus	30 ± 7	16 ± 2[c]	--
Cynodon dactylon	31 ± 9	24 ± 2[b]	78

[a]Each value is average of 40 replicates.

[b]Dry weights significantly different from control at 0.05 level.

[c]Dry weights significantly different from control at 0.01 level.

Table 5. Effects of Decaying Polygonum Roots on Germination and Seedling Growth of Selected Species.

Species	Mean dry weight with standard error, mg[a]		Germination
	Control	Test	Percent of Control
Gossypium barbadense	723 ± 20	611 ± 26[c]	96
Sorghum bicolor	404 ± 50	430 ± 42	94
Chenopodium album	147 ± 34	126 ± 15	81
Sporobolus pyramidatus	23 ± 4	15 ± 2	--
Cynodon dactylon	32 ± 4	24 ± 2[b]	73

[a]Each value is average of 40 replicates.

[b]Dry weights significantly different from control at 0.05 level.

[c]Dry weights significantly different from control at 0.01 level.

Table 6. Effects of Different Periods of Decomposition of Polygonum Plants on Growth of Selected Species.

Species	Treatment	Mean dry weight with standard error, mg[a]					
		After 20 days			After 40 days		
		Root	Shoot	Whole plant	Root	Shoot	Whole plant
Gossypium barbadense	Control	115 ± 7	410 ± 16	525 ± 16	1371 ± 43	350 ± 16	1721 ± 52
	Test	102 ± 7[b]	309 ± 13[d]	411 ± 11[d]	1003 ± 29[d]	233 ± 8[d]	1236 ± 122[d]
Sorghum bicolor	Control	423 ± 36	282 ± 44	705 ± 42	820 ± 71	1324 ± 138	2144 ± 151
	Test	182 ± 14[d]	232 ± 27	414 ± 56[c]	846 ± 71	1094 ± 117	1940 ± 127
Chenopodium album	Control	31 ± 5	60 ± 6	91 ± 2	636 ± 56	1013 ± 179	1649 ± 212
	Test	15 ± 3[b]	31 ± 6[b]	46 ± 9[b]	336 ± 40[d]	748 ± 79	1084 ± 107[b]
Sporobolus pyramidatus	Control	8 ± 1	18 ± 1	26 ± 1	115 ± 8	225 ± 22	340 ± 26
	Test	8 ± 1	13 ± 2[a]	21 ± 2	241 ± 34	111 ± 16	352 ± 23
Cynodon dactylon	Control	9 ± 2	11 ± 1	20 ± 2	95 ± 12	225 ± 29	320 ± 23
	Test	7 ± 1	8 ± 1[b]	15 ± 1[b]	41 ± 5[c]	128 ± 26[b]	169 ± 2[c]

[a]Each value is average of 30 replicates.

[b]Dry weights significantly different from control at 0.05 level

[c]Dry weights significantly different from control at 0.01 level

[d]Dry weights significantly different from control at 0.001 level.

Table 7. Effects of Root Exudates of Polygonum on Growth of Selected Species.

Species	Treatment	Mean dry weight with standard error, mg[a]			Weight as
		Root	Shoot	Whole plant	Percent of Control
Gossypium barbadense	Control	228 ± 32	1317 ± 245	1545 ± 381	
	Test	196 ± 26	1643 ± 125	1839 ± 132	113
Sorghum bicolor	Control	911 ± 78	3137 ± 356	4048 ± 404	
	Test	651 ± 82[b]	2997 ± 437	3648 ± 497	90
Chenopodium album	Control	66 ± 8	280 ± 16	352 ± 21	
	Test	35 ± 5[c]	171 ± 22[c]	206 ± 25[d]	59
Sporobolus pyramidatus	Control	49 ± 7	50 ± 5	99 ± 1	
	Test	37 ± 2	37 ± 1	74 ± 18	75
Cynodon dactylon	Control	24 ± 2	64 ± 12	88 ± 13	
	Test	20 ± 2	43 ± 4[c]	63 ± 5	72

[a]Each value is average of at least 16 replicates.

[b]Dry weights significantly different from control at 0.05 level.

[c]Dry weights significantly different from control at 0.01 level.

[d]Dry weights significantly different from control at 0.001 level.

Table 8. Effects of Root Exudates of Polygonum on Growth of Selected Species in U-Tube Experiments.

Species	Treatment	Mean dry weight with standard error, mg[a]			Weight as
		Root	Shoot	Whole Plant	Percent of Control
Gossypium barbadense	Control	140 ± 30	1000 ± 18	1140 ± 10	
	Test	120 ± 26	1112 ± 22	1232 ± 11	108
Sorghum bicolor	Control	305 ± 27	860 ± 99	1165 ± 112	
	Test	274 ± 43	840 ± 261	1114 ± 299	96
Chenopodium album	Control	26 ± 1	176 ± 6	202 ± 18	
	Test	24 ± 1	148 ± 2	172 ± 6[b]	85
Sporobolus pyramidatus	Control	12 ± 1	41 ± 4	53 ± 5	
	Test	10 ± 1	32 ± 3	42 ± 4	79
Cynodon dactylon	Control	18 ± 1	117 ± 7	135 ± 8	
	Test	18 ± 2	75 ± 13[c]	93 ± 10[b]	69

[a]Each value is average of 10 replicates in test series and 20 replicates in control series.

[b]Dry weights significantly different from control at 0.05 level.

[c]Dry weights significantly different from control at 0.01 level.

CHAPTER III

ALLELOPATHIC EFFECTS OF Polygonum aviculare L.

Paper II. Isolation, Characterization and Biological Activities of Phytotoxins

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Abstract-In earlier work, we found that Polygonum aviculare had pronounced allelopathic effects against several test species. Four inhibitors were isolated from living Polygonum plants, three of which were glucosides. Four different inhibitors were isolated from Polygonum residues and soil under Polygonum stands, and none of these occurred in soil from Cynodon dactylon (L.) Pers. stands. Three of these were glycosides containing both fructose and cellobiose as the sugars. Color reactions of all the inhibitors indicated that they are phenolic in nature. All the inhibitors reduced seed germination and/or seedling growth of Chenopodium album L. Moreover some of them inhibited growth of different strains of Rhizobium and Azotobacter.

Key Words - Allelopathy, inhibitors, phenols, glycosides, Chenopodium album, Rhizobium, Azotobacter, Cynodon dactylon.

INTRODUCTION

Numerous kinds of secondary chemical compounds have been isolated from plants, and their inhibitory action upon plants, animals, and microorganisms has been documented (Naqvi and Muller, 1972; Lodhi, 1976; Harborne, 1977; Horsley, 1977; Newman and Miller, 1977; Rice et al., 1981). The ecological significance of such compounds lies in their ability to affect species composition, rate of succession, plant productivity and microbial populations in soil (Rice, 1965a, 1956b; Muller, 1966; Wilson and Rice, 1968; Chou and Lin, 1976).

In an earlier paper (Al Saadawi and Rice 198-) we demonstrated that much of the interference (competition plus allelopathy) of Polygonum aviculare against several test species was due to allelopathy. Moreover, the ways in which the chemical inhibitors are released from Polygonum into the environment were determined. The nature and characteristics of the inhibitory compounds responsible for inhibition of test species were not described, however. Therefore the goals of the present project were to isolate and characterize the phytotoxic compounds from Polygonum and determine their biological activities.

MATERIALS AND METHODS

Isolation and Characterization of Inhibitors. Since the inhibitory activity of Polygonum was associated with both roots and tops (Al Saadawi and Rice, 198-), 10g of air dried roots and 10g of air dried tops were boiled separately in distilled water for 10 minutes, ground in a Waring Blendor for 10 minutes and allowed to stand for 30 minutes. The extracts were filtered through cheese cloth and then centrifuged at 27000g for 10 minutes. The filtrate was acidified to pH 2 with N HCl and extracted three times with two half volumes of diethyl ether. The ether and water fractions were evaporated to dryness under vacuum at 40° C and were made up to 10 ml with absolute ethanol and 10 ml of 50% aqueous methanol respectively.

One milliliter samples of the ether and water extracts were streaked separately on acid washed Whatman #3MM chromatographic paper, and the papers were developed by the descending technique in butanol-acetic acid-water (63:10:27 V/V), BAW. The developed papers were examined with short (2537A) and long (3360A) UV light with and without NH_3. All the fluorescent bands were cut out and eluted with 50% aqueous ethanol. The eluates were streaked on Whatman #3 MM paper and developed in 6% aqueous acetic acid, 6% AA. The fluorescent bands were cut out and eluted again with 50% aqueous ethanol. This method was used to enhance the purification of the isolated compounds.

The eluted compounds were spotted on acid washed Whatman #1 paper and the papers were developed in four different solvent systems: 6% AA, BAW, isopropanol-ammonia-water (200:10:20 V/V), IAW, and isopropanol-n butanol-water (70:10:20 V/V), IBW. The Rf's in these solvent systems, colors under UV light, and

reactions of the different compounds with diazotized p-nitraniline, diazotized sulfanilic acid, and ferric chloride-potassium ferricyanide were determined. Absorption spectra of the eluted compounds were determined in appropriate solvents.

All the compounds were hydrolyzed by refluxing N HCl for 30 minutes to determine whether these compounds were glycosides. The hydrolysate was extracted with two half-volumes of diethyl ether. The ether fraction was evaporated to dryness and taken up in absolute ethanol. The water fraction was evaporated three times in vacuo to eliminate the HCl and taken up in 50% aqueous methanol.

The ether fractions were chromatographed in the same solvent systems as the original compounds and flurescence colors were determined under short and long UV light. The water fractions were chromatographed with BAW in the first dimension followed by lBW in the second direction. The papers were dipped in a benzidine reagent (Smith, 1960, p 250) to locate the sugar spots.

Since our previous studies (Al Saadawi and Rice, 198-) revealed that soils taken from Polygonum stands were very inhibitory to test species, an experiment was designed to isolate and characterize the inhibitory compounds in soil in which Polygonum had grown and in senescent materials from Polygonum plants. Residue was collected at several places in Polygonum stands on March 20, 1981 and was ground to pass a sieve with 1 mm openings. Ten grams of the ground material were extracted in 200ml of acetone for 24 hr in a Soxhlet extractor (Rice and Pancholy, 1974), and the extract was reduced to 20 ml in a flash evaporator.

Soil samples were collected on March 20, 1981, from the top 10 cm at several places in areas occupied by Polygonum. Similar collections were made in an adjacent area where only Cynodon dactylon was present. The soil, from each

vegetation type was mixed thoroughly and air dried. Ten grams of the air-dried soil were then extracted in a Soxhlet Apparatus as described above. The same procedures were used in the isolation and characterization of suspected inhibitors in the senescent materials and soil as described previously.

Effects of Isolated Compounds Against Chenopodium album. The fluorescent bands resulting from chromatographic procedures in the previously described experiments, were eluted with 50% aqueous ethanol, evaporated to dryness and taken up in 12 ml of phosphate buffer (pH 5.5). The solution of each compound was placed in two 5 cm Petri dishes containing washed quartz sand and thirty seeds of Chenopodium album, one of the species used in the previous study (Alsaadawi and Rice, 198-). A paper developed in BAW without application of the extract was subjected to the same procedure and used as a control. The petri dishes were kept in the growth chamber on a 14 hr photoperiod (1000 ft-c) at 29°C and a 10 hr dark period at 21°C. Germination, hypocotyl, and epicotyl length were recorded 7 days after planting.

Effects of Isolated Compounds on Nitrogen-Fixing Bacteria. Rhizobium leguminosarum, American Type Culture, (ATC) strain 10314; R. meliloti, ATC strain 4400; R. japonicum, ATC strain 10324; R. lupini, ATC strain 10319; Azotobacter vinelandii, ATC strain 9104 and A. chroococcum, ATC strain 9043, were used to test the effects of isolated compounds on nitrogen fixing organisms. A yeast extract-mannitol medium (Society of American Bacteriologists, 1957, p. 113) was used for all strains of Rhizobium and a soil extract-manitol medium (Society of American Bacteriologists, 1957, p. 109) was used for Azotobacter strains.

The eluted fluorescent bands from living and senescent Polygonum plants were evaporated to dryness and dissolved in 10 ml of an appropriate solvent. Each compound was tested for antibacterial activity using the diffusion technique on solid media (Rice, 1965b). A sterilized sensitivity disc was saturated with a given dissolved compound. The solvent was allowed to evaporate and the disc was placed in a Petri plate seeded with two tenths of a milliliter of a 48 hr liquid inoculum of Rhizobium or Azotobacter. Control discs were saturated with the same solvent as the treated discs. All plates were incubated at 30°C and zones of inhibition were measured three days after inoculation.

RESULTS

Isolation and Characterization of Inhibitors. Four inhibitors were isolated from living Polygonum plants (Table 1). The isolated inhibitors were associated with both roots and tops and were found in appreciable amounts in the water fraction. Color reagents revealed that the isolated inhibitors were apparently phenolic in nature.

After hydrolysis, the water fractions of inhibitors 1, 3 and 4 yielded glucose as indicated by the Rf values and the benzidine sugar test (Table 2). The ether fraction of each compound gave three spots or more indicating that the original molecules were rather complex phenolic glucosides. No success was achieved in identifying the various aglycones resulting from hydrolysis. Absorption spectra of the original molecules (Table 2) did not help in identifying these compounds.

Four inhibitors were isolated from Polygonum residues and soil under Polygonum stands. These inhibitors were completely different from those isolated from living Polygonum plants but the same inhibitors occurred in the residues and soil under Polygonum stands (Table 3). All the inhibitors gave color reactions characteristic of phenols and all had different absorption spectra.

Chromatograms of water fractions of the hydrolysates revealed that all the inhibitors except A yielded fructose and cellobiose (Table 4). No distinct spots were found on the chromatograms of the ether fraction indicating that acid hydrolysis apparently destroyed the aglycones. Thus we were not successful in identifying any of the inhibitors from the residues or soil under Polygonum. All four were apparently phenolic compounds, with three of them being phenolic glycosides. None of these inhibitors occurred in soil from the stands of Cynodon dactylon.

Effects of Isolated Compounds Against Chenopodium album. All the inhibitors isolated from living Polygonum plants significantly inhibited the hypocotyl and epicotyl growth of Chenopodium album (Table 5). Seed germination was appreciably reduced by inhibitors 1 and 4 and drastically reduced by inhibitors 2 and 3.

Compounds isolated from senescent Polygonum and soil under Polygonum significantly inhibited hypocotyl and epicotyl growth of Chenopodium album, except C (Table 6). Compund D had little impact on seed germination whereas A and B appreciably reduced seed germination. Inhibitor C drastically reduced seed germination even though it did not have significant effects on epicotyl and hypocotyl growth.

Effects of Isolated Compounds on Growth of Nitrogen-Fixing Bacteria. Strain 4400 was the only test strain of Rhizobium inhibited by any of the compounds isolated from living Polygonum plants and it was inhibited by all compounds (Table 7). Compound #2 was most inhibitory to this strain.

Strain 9043 was the only test strain of Azotobacter inhibited by any of the compounds isolated from living Polygonum plants and only compound #3 affected its growth.

All compounds isolated from senescent Polygonum and soil under Polygonum stands were inhibitory to Rhizobium strains 10314 and 4400 (Table 8). Growth of Rhizobium strain 10324 was inhibited only by Compound D, and Rhizobium strain 10319 was not inhibited by any of the isolated compounds.

Compound D inhibited growth of Azotobacter strain 9104, but no other isolated compounds inhibited the test strains of Azotobacter.

DISCUSSION

Results described in our previous paper (AlSaadawi and Rice, 198-) indicated that the phytotoxicity of Polygonum was associated with both the roots and tops. The current study revealed that the same active compounds were present in both roots and tops.

The results also demonstrated that allelopathic compounds isolated from living Polygonum plants were different from those present in senescent Polygonum. This may be attributed to one or a combination of events. For example: (1) certain pathways may only operate at specific stages of development, (2) an inhibitory compound may accumulate due to a change in the turnover rate of the compound whereby it is synthesized more rapidly than it is utilized, (3) certain structural components (macromolecules) may be degraded at certain stages of development whereby small molecular weight toxic compounds accumulate. The events sited above may be a consequence of: (1) metabolic changes associated with the normal course of plant development, (2) metabolic changes brought about by environmental stress such as drought, light, mineral deficiencies etc. Other studies have shown that phenolic compounds in plants and in soil can change markedly under different environmental stresses (Wang et al., 1967; Koeppe et al., 1969; Rice, 1974).

Our results demonstrated conclusively that the allelopathic compounds present in the soil under Polygonum definitely come from senescing Polygonum plants. None of the inhibitors was found in adjacent soil under Cynodon dactylon. These results were particularly striking from an ecological standpoint since the inhibitory compounds remained stable for more than three months after senescence of Polygonum in the field. No attempt was made to isolate the allelopathic compounds after that time.

Inhibitory compounds in living Polygonum may also play an important role in inhibiting the associated species since these compounds can be released into the environment through leaching of the tops during rain, root exudation and decomposition of the materials left in the field after mowing (AlSaadawi amd Rice 198_). Several other scientists have reported that a variety of chemical compounds are released from different plant species in similar ways (Rovira, 1969; Tubbs, 1973; Rice, 1974; Horsley, 1977; Young, 1979).

We did not test for synergistic effects of the eight inhibitors which were isolated, but it is certainly possible that such effects may occur and result in much greater total inhibition than the cumulative effects of individual inhibitors (Wilson and Rice 1968; Rasmussen and Einhellig, 1977; Einhellig and Rasmussen 1978).

Our results indicated that all eight phytotoxins isolated inhibited seed germination and/or seedling growth of a plant species commonly associated with Polygonum aviculare in the field. Moreover, several of them inhibited growth of some of the test strains of Rhizobium and Azotobacter. Thus, Polygonum aviculare can exert a direct allelopathic effect against associated higher plants and indirect allelopathic effects against them through a reduction in the rate of both asymbiotic and symbiotic nitrogen fixation (Rice, 1979).

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Table 1. Inhibitory Compounds from Living Polygonum Plants.

Compound	Rf's on Whatman # 1[a]				Fluorescence[b]		Reagent Colors		
	BAW	IBW	6% AA	IAW	Long UV	Short UV	p-Nit.	Sulfan. acid	$FeCl_3$ $K_3Fe(CN)_6$
# 1	0.75	0.79	0.37	0.13	abs	abs	bn	yellow	bl
# 2	0.82	0.80	0.43	0.50	bl	bl	bn	none	bl
# 3	0.21	0.29	0.35	0.04	abs	abs	bn	none	bl
# 4	0.64	0.43	0.47	0.17	pink	pink	bn	none	bl

[a]See text for solvent system. Rf's are average of three runs.

[b]Abbreviations: bl,blue; abs,absorption; bn,brown.

[c]Diazotized p-nitraniline (Bray et al., 1950), diazotized sulfanilic acid (Bray et al., 1950), ferric chloride-potassium ferricyanide (Smith 1960, p. 324).

Table 2. Absorption Spectra of Phytotoxins from Living Polygonum Plants and Sugar Moieties after Hydrolysis.

Compound	Rf's on Whatman # 1[a]		Benzidine	Absorption spectra[b]	
	BAW	IBW	sugar test	µm	Solvent
# 1	0.23	0.27	brown	235,348	Distilled water
# 2	none	none	none	248	Ethanol
# 3	0.22	0.27	brown	165,250	Distilled water
# 4	0.23	0.27	brown	315	Acetone
Known glucose	0.23	0.27	brown	—	———

[a]See text for solvent systems.

[b]Before hydrolysis.

Table 3. Inhibitory Compounds from Polygonum Residues and Soil under Polygonum Stands.

Compound	Rf's on Whatman # 1[a]				Fluorescence[b]				Reagent color[c]		
	BAW	IBW	6%AA	IAW	Long UV		Short UV		p-Nit	Sulfan. acid	$FeCl_3$-$K_3Fe(CN)_6$
					$-NH_3$	$+NH_3$	$-NH_3$	$+NH_3$			
A	0.89	0.85	0.63	0.84	v. bl	v bl	bl	bl	tan bn	yel.	bl
B	0.84	0.83	0.47	0.48	l bl	l bl	l bl	l bl	d bn	none	bl
C	0.87	0.72	0.40	0.63	br yel	yel	yel	yel gr	none	yel	bl
D	0.81	0.77	0.08	0.35	br bl	br bl	br bl	br bl	tan bn	yel	bl

[a]See text for solvent system. Rf's are average of three runs.

[b]Abbreviations: v,violet; bl,blue; l,light; br,bright; yel,yellow; gr,green; bn,brown; d dark.

[c]Diazotized p-nitraniline (Bray et al., 1950), diazotized sulfanilic acid (Bray et al., 1950), ferric chloride-potassium ferricyanide (Smith, 1960, p. 324).

Table 4. Absorption Spectra of Phytotoxins from Polygonum Residues and Soil under Polygonum Stands and Sugar Moieties after Hydrolysis.

Compound	Sugar Moieties	Rf's on Whatman # 1[a]		Benzidine sugar test	Absorption Spectra[b]	
		BAW	IBW		µm	Solvent
A		none	none	none	311	Acetone
B	a	0.23	0.47	gold-brown	235	Butanol
	b	0.13	0.23	brown		
C	a	0.23	0.47	gold-brown	238	Butanol
	b	0.13	0.23	brown		
D	a	0.22	0.45	gold-brown	312	Acetone
	b	0.12	0.23	brown		
Known Fructose		0.25	0.45	gold-brown	---	-------
Known Cellobiose		0.14	0.25	brown	---	-------

[a]See text for solvent systems.

[b]Before hydrolysis.

Table 5. Effects of Compounds from Living Polygonum on Seed Germination and Seedling Growth of Chenopodium album.

Compound	Mean length (mm)[a]				Germination
	Hypocotyl	% of Control	Epicotyl	% of Control	% of Control
Control	12.6±1.9	100	8.2±0.6	100	100
# 1	6.0±1.2[b]	47.8	3.7±0.5[b]	45.3	71.3
# 2	6.8±1.0[b]	53.6	4.6±0.4[b]	56.5	47.6
# 3	5.3±1.8[b]	42.3	4.8±0.5[b]	57.8	52.4
# 4	5.1±1.4[b]	40.2	4.2±0.5[b]	51.4	76.2

[a]Average of at least 25 seedlings.

[b]Mean lengths significantly different from control at 0.01 level or better.

Table 6. Effects of Inhibitory Compounds from Polygonum and Soil under Polygonum Stands on Seed Germination and Seedling Growth of Chenopodium album.

Compound	Mean length (mm)[a]				Germination
	Hypocotyl	% of Control	Epicotyl	% of Control	% of Control
Control	13.7±1.9	100	16.2±1.0	100	100
A	9.4±3.0[b]	68.9	9.5±3.0[d]	58.7	82.5
B	5.5±1.1[d]	40.1	8.6±1.4[d]	53.2	75.0
C	12.4±2.0	90.2	12.9±1.6	79.5	40.0
D	10.0±1.1[b]	72.6	11.0±1.1[c]	68.2	90.0

[a]Average of at least 25 seedlings.

[b]Mean lengths significantly different from control at 0.05 level.

[c]Mean lengths significantly different from control at 0.01 level.

[d]Mean lengths significantly different from control at 0.001 level.

Table 7. Effects of Compounds Isolated from Living Polygonum Plants on Growth Of Nitrogen Fixing Bacteria.[a]

Test organism[b]	Compound			
	#1	# 2	# 3	# 4
R 10314	0.00[c]	0.00	0.00	0.00
R 4400	1.33	2.00	1.66	1.33
R 10319	0.00	0.00	0.00	0.00
R 10324	0.00	0.00	0.00	0.00
A 9104	0.00	0.00	0.00	0.00
A 9043	0.00	0.00	1.00	0.00

[a]Controls had no inhibition.

[b]Symbols: R, Rhizobium; A, Azotobacter. Numbers by these are ATC strain designations.

[c]Each figure is mean radius (mm) of inhibited zone of three trials.

Table 8. Effects of Compounds Isolated from Polygonum Residues and Soil under Polygonum Stands on Growth of Nitrogen Fixing Bacteria.[a]

Test organism[b]	Compound			
	A	B	C	D
R 10314	0.66[c]	1.00	0.33	0.50
R 4400	2.66	2.00	1.16	1.33
R 10319	0.00	0.00	0.00	0.00
R 10324	0.00	0.00	0.00	0.80
A 9104	0.00	0.00	0.00	1.50
A 9043	0.00	0.00	0.00	0.00

[a]Controls had no inhibition.

[b]Symbols: R, Rhizobium; A, Azotobacter. Numbers by these are ATC strain designations.

[c]Each figure is mean radius (mm) of inhibited zone of three trials.