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THE INFLUENCE OF OSMOTIC PRESSURE IN TESTS FOR ALLELOPATHY

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ABSTRACT - The influence of osmotic pressure on the early growth of seedlings used in tests for allelopathy was determined for two bioassay systems. Bromus rigidus (Ripgut Grass) was used to test the bioassay procedures against solutions of sucrose, mannitol, KCl, $NaNo_3$, and multiples of full strength Hoagland's solution. Significant growth reductions were observed with concentrations as low as 75 milliosmoles. Aqueous extracts of Adenostoma fasciculatum (Chamise) foliage and dead Brassica nigra (Black Mustard) leaves produced significant retardation of growth with concentrations of 25 milliosmoles.

INTRODUCTION

Allelopathy or the biochemical suppression of one plant by another has been a subject of increasing ecological interest. Several recent studies (Muller et al., 1968, Wilson and Rice, 1968; McPherson and Muller, 1969; del Moral and Muller, 1970; and Bell and Muller, 1973) have utilized various laboratory bioassay procedures to elucidate an allelopathic mechanism. These bioassay methods generally involve the direct use of plant tissue or the application of water extracts of suspected phytotoxic material. The suppression of some growth or germination response has been cited as evidence in favor of an allelopathic influence. None of the recent papers, however, have considered the effects of the osmotic pressure exerted by these extracts. Interference with water absorption may be the direct effect causing the reduced growth. The present study was undertaken to evaluate the relative influences of osmotic concentration and phytotoxic suppression in tests for antibiosis.

MATERIALS AND METHODS

Two laboratory bioassay methods were employed in this study, the first designated as the sponge bioassay and the second designated as the sand bioassay. The sponge bioassay technique involved the use of 100 x 15 mm petri dishes with seedbeds of 50 x 50 x 3 mm cellulose sponge covered with filter paper. Moistening was accomplished by immersing the sponge in the solution

to be tested and shaking off the excess solution. Test seeds were soaked for 2 hours in the appropriate solution before planting. Following soaking, 10 seeds were planted in two parallel rows on the edge of the seedbed to allow continued contact with solution as the radicle emerged from the caryopsis. Each plate was covered with parafilm to protect against desiccation. Measurement of radicles to the nearest mm followed 48 hr of incubation in the dark at a constant 25°C.

The sand bioassay employed similar plastic petric dishes with 50 g addition of sand as the seedbed. The sand mixture used was standardized as that particle fraction collected between 1.0 mm and 0.1 mm standard soil sieves from plaster sand. Twenty seeds were planted radially and 10 ml of the test solution was pipetted into the sand. Incubation and measurement of radicle growth was accomplished as before.

Field-collected seeds of Bromus rigidus Roth. was used as the assay species. This introduced Mediterranean grass species was chosen for its ease of collection, high germination percentage, and uniform early growth.

Solutions tested were prepared in a graduated series of osmotic pressure and measured on a Fiske Model G62 osmometer. Materials tested include solutions of sucrose, Mannitol, KCl, $NaNO_3$, and multiples of full strength Hoagland's solution. Phytotoxic materials included water extracts of dead Brassica nigra leaves (Bell and Muller, 1973) and living Adenostoma fasciculatum foliage (McPherson and Muller, 1970). Each sample was tested in triplicate and each bioassay was completed in triplicate. The data were analyzed by the technique of the honestly significant differences (Steele and Torrie, 1960).

Table 1. Effect of osmotic concentration (O.C. in milliosmoles) on radicle growth of Bromus rigidus in sponge bioassay (mm after 48 hrs.).

O.C.	Hoagland's	Hoagland's	$NaNO_3$	KCL	Sucrose	Mannitol
0	16.3±2.0	16.3±1.8	17.1±2.3	16.0±2.0	16.2±2.1	16.9±2.4
25	14.5±2.1					
50	12.9±2.1		14.0±2.1	14.2±2.0	11.4±2.2	13.7±2.0
75	11.3±2.3					
100	10.5±1.9		10.4±2.3	12.1±1.5	7.5±2.0	9.5±1.9
125		10.2±1.9				
150		8.7±2.2				
175		7.6±1.6				
200		6.6±1.4	6.5±1.9	7.6±1.1	2.0±1.2	5.1±1.2
300			2.5±1.3	4.4±1.2	1.1±0.0	2.3±0.8
400			1.1±0.0	2.2±0.8	1.0±0.0	1.2±0.0
500			0.0±0.0			0.0±0.0
HSD						
.05	4.6	4.0	3.6	3.0	3.0	3.2

RESULTS

The effect of osmotic concentration on early radicle growth in Bromus rigidus is observed in both the sponge bioassay (Table 1) and the sand bioassay (Table 2). Seedling growth was inversely proportional to osmotic concentration. It is evident that radicle growth was significantly inhibited in osmotic concentrations as low as 75 milliosmoles (approximately 1.7 atm). Suppression of growth was even greater when the large molecule non-electrolytes were used as the osmoticant. An uptake of the ions in the electrolyte tests, followed by a more rapid osmotic uptake of water, most likely resulted in the apparent differences in growth retardation. Greater water uptake also probably contributed to the difference in growth achieved in the two bioassay types. A greater absorptive surface was presented by the sand bioassay since the seeds were planted into the sand medium and surrounded by the test solution.

Table 2. Effect of osmotic concentration (O.C. in milliosmoles) on radicle growth of Bromus rigidus in the sand bioassay (mm after 48 hrs.).

O.C.	Hoagland's	Hoagland's	$NaNO_3$	KCL	Sucrose	Mannitol
0	18.5±2.3	18.2±2.3	18.0±2.5	18.1±2.5	18.4±2.1	18.2±2.6
25	17.1±2.0					
50	15.3±2.2		14.9±2.5	15.8±2.4	14.1±2.1	18.0±1.9
75	13.9±2.4					
100	11.3±2.1		12.0±2.4	13.6±2.6	11.7±1.9	12.7±2.1
125		9.6±1.5				
150		7.6±1.4				
175		6.4±1.2				
200		5.1±1.2		9.7±1.8	5.5±1.5	
300			3.9±1.1	6.8±1.4	1.7±0.6	
400				3.4±1.0	1.0±0.0	
500			1.0±0.0			1.1±0.0
1000			0.0±0.0			0.0±0.0
HSD						
.05	4.7	3.4	3.6	3.4	3.0	2.1

Growth retardation was also indicated in the tests with suspected phytotoxic materials (Table 3). When these data are figured as a percentage of control growth (0 O.C) and plotted against a representative osmoticant (Hoagland's solution), the resulting growth inhibitions are significantly greater than those expected by osmotic pressure alone (Fig. 1).

Table 3. Inhibitory effects of phytotoxic extracts of known osmotic concentration (O.C. in milliosmoles) on the radicle growth of Bromus rigidus (mm after 48 hrs.)

	Sponge Bioassay	Sand Bioassay	
O.C.	Brassica nigra	Brassica nigra	Adenostoma fasciculatum
0	16.9±2.1	18.4±1.9	16.6±2.6
2			13.5±2.6
28	9.5±2.1	12.9±2.0	10.7±2.3
60	6.1±1.7	10.7±1.6	
88	4.7±1.3	8.7±1.3	
118	4.2±1.0	7.6±1.2	
HSD			
.05	2.9	3.5	6.4

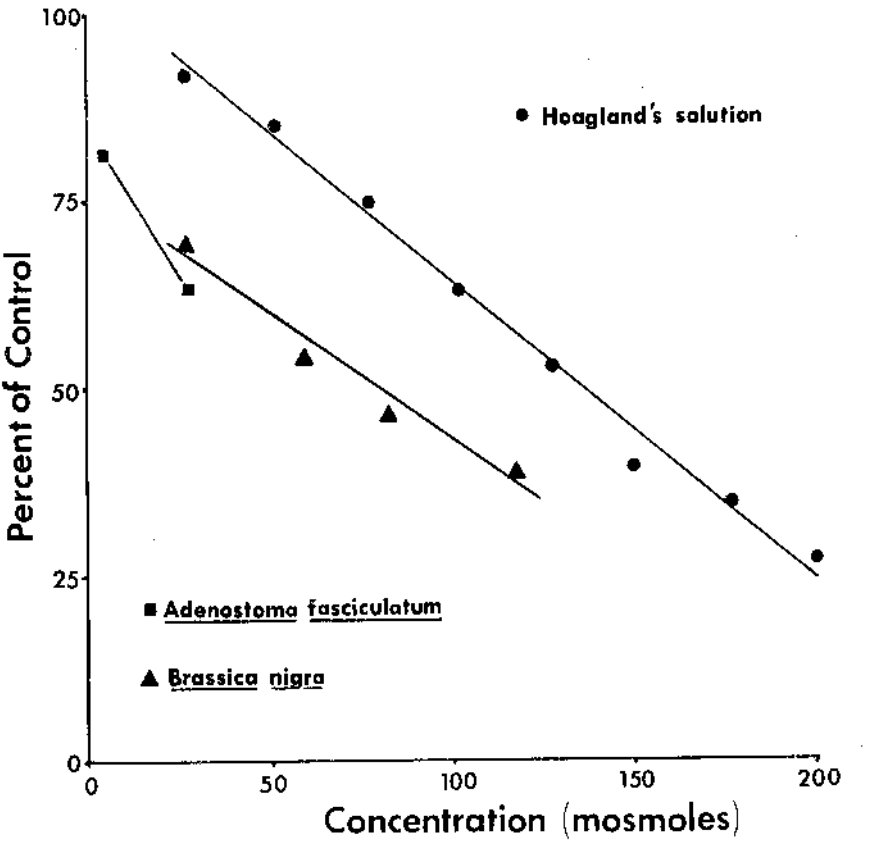

Fig. 1. Osmotic and phytotoxic inhibition of aqueous solution. Radicle growth of Bromus rigidus in concentrations of Hoagland's solution and aqueous extracts of Adenostoma fasciculatum foliage and Brassica nigra dead leaves. All points of equal osmotic concentration significantly different at 0.01 by t test.

DISCUSSION

Growth as a biological activity monitored through laboratory bioassays is subjected to several variables even in the most carefully controlled conditions. Osmotic concentrations in excess of 50 milliosmoles should be interpreted with care. The complex mixture of organic and inorganic compounds leached from plant material (Tukey, 1970) present a rather difficult question of interpretation. Some compounds, such as phenolic acids or simple sugars, may actually be stimulatory. Laboratory bioassays for suscepted phytotoxic compounds cannot stand alone as evidence for an allelopathic mechanism. Careful examination of all contributing mechanisms of interference is needed in order to properly place laboratory bioassay results into a field situation.

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