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ALLELOPATHIC EFFECTS OF BUR BUTTERCUP TISSUE ON GERMINATION AND GROWTH OF VARIOUS GRASSES AND FORBS *IN VITRO* AND IN SOIL[1]

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Abstract.— The allelopathic effects of bur buttercup (*Ranunculus testiculatus*) tissue on selected grasses and forbs varied according to the substratum for germination and growth. The *in vitro* effects of an aqueous extract of buttercup tissue on germination and root development of five grasses were strongly inhibitory in all cases. However, in soil the effects of buttercup tissue on germination and growth of seven grasses and two dicotyledonous herbs were small to nonsignificant. Deleterious effects were less severe in fine- as opposed to coarse-textured soils. Under field conditions, the ability of seedlings of the grasses to compete with buttercup varied with the species.

Bur buttercup (*Ranunculus testiculatus* Crantz), a native of southeastern Europe and central Asia (Benson 1948, Davis 1965) was first collected in North America by A. O. Garrett near Salt Lake City, Utah, in 1932. Since that time, the species has spread throughout the Great Basin, the Snake River Plain, and the Columbia Plateau. In much of that area, the species exists in large populations in native vegetation and waste places of the semiarid zone. Small, isolated populations are also known from northeastern Wyoming, northern Arizona, northern California, and interior British Columbia (Buchanan 1969).

The species is a diminutive annual (averaging ca. 5 cm in height) that completes its life cycle during the early spring. In northern Utah germination occurs in late fall, but at more northerly latitudes, germination may be postponed until spring (Buchanan 1969). Despite its small size, the species often forms a dense carpet over literally thousands of acres of dry farm and range land during the period from March to May in the eastern Great Basin.

The invasion of bur buttercup into North America prompted an early interest in its potential as a weed. Two memoranda in the files of the Intermountain Forest and Range Experiment Station (IFRES) of the U.S. Department of Agriculture (Ogden, Utah) report early observations of bur buttercup in Utah and consider its potential as a weed (Clark 1941, and Stewart 1941). The memos, dated 18 July and 3 September 1941, respectively, discuss the plant's history, spread, importance as a weed, and possible control in the West. However, when our study began in 1966, no aspect of the dispersal or ecology of the species had been reported in the scientific literature (exclusive of floras) of North America.

Because of rapid spread of bur buttercup and the dearth of information concerning the species, the Intermountain Forest and Range Experiment Station initiated a program to investigate its life history, ecology, and distribution. Concern over possible deleterious effects of bur buttercup on winter wheat crops and on range grass seedings prompted this study of the competitive and allelopathic effects of bur buttercup on wheat and on grasses used in range revegetation. Germination studies using both glass

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and soil systems were utilized so that results might be extrapolated to field situations.

Methods

In vitro studies in petri dishes compared the effect of a leachate from bur buttercup tissue with both distilled water and mannitol as media for germination and root development of five grasses.

The leachate was prepared by soaking one part of air-dried whole plant tissue at fruiting state in 30 parts distilled water at room temperature for 24 hours. The air-dried tissue was first ground through 40-mesh screen. Following soaking, the leachate was filtered through #2 filter paper and stored at 1 C until used. The leachate had an osmotic concentration (O.C.) of 1.8 atmospheres and a pH of 5.5.

Seeds of crested wheatgrass (*Agropyron desertorum*), tall wheatgrass (*A. elongatum*), common rye (*Secale cereale*), Delmar fall wheat (*Triticum aestivum*), and Gaines fall wheat (*T. aestivum*) were each subjected to 6 treatments as follows:

Treatment No.	Wetting Agent	O.C. of Agent (Atm.)	pH of Agent	Effect Tested
1	Distilled H_2O (buffered)	0.0	7.0	Control
2	Distilled H_2O	0.0	5.5	pH
3	Mannitol in H_2O	1.8	7.0	Osmotic pressure
4	Mannitol in H_2O	1.8	5.5	Osmotic pressure and pH
5	Leachate	1.8	7.0	Leachate (pH adjusted)
6	Leachate	1.8	5.5	Leachate

Two pieces of filter paper were placed in each petri dish and 25 seeds were positioned thereon in five rows of five seeds each. Each treatment was replicated 10 times. Dishes were initially wetted with 6 ml of treatment solution. Thereafter, dishes were rewatered daily with 2 ml of distilled water for the duration of the experiment.

At the end of the third-to-seventh day (depending on species), percent germination was recorded. Seeds were considered germinated if a root of 5 mm or longer had emerged from the seed coat. Root length data were taken by randomly selecting two rows (10 seeds) from each petri dish and recording length of all roots.

In addition to *in vitro* studies, the inhibitory effects of bur buttercup were tested on seven grasses and three forbs in potted silty clay soil. This study involved two levels of bur buttercup tissue mixed with soil in undrained pots: average annual field production of bur buttercup (0.7g/dm^2, 1X) and twice average annual field production of bur buttercup (2X). The study was designed for twelve species, three treatments (control and two levels of tissue), and nine replicates per treatment. Only ten species or varieties were used, but two species (Gaines wheat and crested wheatgrass) appear twice in the design, the second time in sand. The design was a 3 × 3 trimultiple latin square for each species (Cochran and Cox 1957). Sixteen seeds were planted in each pot by pressing seeds through a template to insure uniform spacing (four seeds per row in four rows).

The silty clay soil (15 percent sand, 43 percent silt, and 42 percent clay) used in this experiment was taken from the top 15 cm of a pasture on the Benmore Experimental Range not infested with bur buttercup. The soil was air dried and passed through a 2 mm sieve before potting. Soil pH was 7.5. A layer of sand (about 1 cm deep) was placed over the soil to reduce crusting. A sand (90 percent sand, 5 percent silt, and 5 percent clay), pH 8.0, was likewise dried, screened, and potted as a second substratum for Gaines wheat and crested wheatgrass. The sand was taken from a stream channel at the Benmore Range.

Plastic pots were filled with 625 g of soil (or 955 g of sand) and topped with 80 g of sand. The experiment was conducted in a greenhouse at the University of Utah. Species and substrata are listed below.

Species	Substratum
Bur buttercup	Soil
Fairway wheatgrass (*Agropyron cristatum*)	"
Halogeton (*Halogeton glomeratus*)	"
Alfalfa (*Medicago sativa*)	"
Delmar wheat (*Triticum aestivum*)	"

Tall wheatgrass (*A. elongatum*)	"
Crested wheatgrass (*A. desertorum*)	"
Gaines wheat (*T. aestivum*)	"
Common rye (*Secale cereale*)	"
Western wheatgrass (*A. smithii*)	"
Gaines wheat	Sand
Crested wheatgrass	"

The grasses are all often sown in areas infested with buttercup, halogeton is an introduced annual often associated with bur buttercup in the eastern Great Basin, and alfalfa is a perennial frequently seeded in areas supporting dense stands of buttercup.

On 13 January, all pots were watered with distilled water to water-holding capacity (WHC) and thereafter to WHC when the soil became noticeably dry.

Number of seeds germinated was recorded after 5, 7, 10, 13, 15, 17, 25, and 28 days from the initial wetting of the pots. Beginning on day 34 and continuing through day 40, the species were clipped, oven dried, and weighed. All replications and treatments of a species were harvested on the same day.

Another soil system tested the influence of buttercup on yield of each of 9 grasses sown in the same rows with buttercup, in a cold frame at Benmore. On 5 October, 100 seeds of each grass were planted in each of three rows 150 cm long. Rows planted to the same grass were spaced 2 dm apart, while 4 dm separated rows between species. Fifty and 100 bur buttercup seeds were planted with the grass in the second and third rows, respectively. The first row assigned to each grass species was maintained as a control. Grasses tested were: crested wheatgrass, Fairway wheatgrass, Russian wildrye (*Elymus junceus*), pubescent wheatgrass (*Agropyron trichophorum*), intermediate wheatgrass (*A. intermedium*), western wheatgrass, common rye, Delmar wheat, and Gaines wheat.

Rows were watered 5 October, at planting, and again the following day by rain. Thereafter, lids of the cold frame were closed (except for periodic waterings) until early April. All plants were clipped on 27 and 28 April. Each row was clipped in 15 segments, each 1 dm long. Tissue was bagged and taken immediately to the University of Utah laboratory for drying. Samples were dried at 32 C and weighed. To reduce edge effect from the walls of the cold frame, clipping segments 1 and 15 were not used for determination of the mean production per segment.

RESULTS

In vitro studies in petri dishes indicate that a compound(s) inhibitory to germination and growth of the five species or varieties tested is produced by bur buttercup tissue or the products of its decay (Table 1).

TABLE 1. Effect of six treatments on germination and root development of two grasses and three cereal grains *in vitro*.*

		Treatment					
		Control Buffered	Control Acidified	Mannitol	Mannitol Acidified	Leachate pH Adjustment	Leachate
		pH–Osmotic Concentration					
Species	Growth Time (days)	7.0–0.0	5.5–0.0	7.0–0.0	5.5–1.8	7.0–1.8	5.5–1.8
		Percent Germination					
Crested wheatgrass	7	83^{a}	92^{ab}	90^{ab}	93^{b}	0^{c}	0^{c}
Tall wheatgrass	5	85^{a}	87^{a}	81^{a}	82^{a}	0^{b}	0^{b}
Common rye	3	72^{a}	72^{a}	71^{a}	73^{a}	7^{b}	9^{b}
Delmar fall wheat	4	83^{a}	81^{a}	78^{a}	80^{a}	12^{b}	9^{b}
Gaines fall wheat	3	96	96^{a}	94^{a}	95^{a}	24^{b}	16^{b}
		Root Length (mm)					
Crested wheatgrass	7	29^{ab}	25^{a}	31^{ab}	37^{b}	0^{c}	0^{c}
Tall wheatgrass	5	26^{a}	22^{b}	19^{b}	22^{b}	0^{c}	0^{c}
Common rye	3	75^{a}	63^{a}	84^{a}	80^{a}	8^{b}	11^{b}
Delmar fall wheat	4	62^{a}	57^{a}	66^{a}	67^{a}	9^{b}	7^{b}
Gaines fall wheat	3	92^{ab}	76^{a}	86^{ab}	100^{b}	10^{c}	13^{c}

*Means for a given species followed by the same letter, in superscript, do not differ at the 1 percent probability level (t-test).

Acidic distilled water had no significant effect on germination, but tended to decrease root length of all species below control levels, although observed differences were not usually significant. At the level tested, osmotic concentration also appeared not to influence germination of these species.

The *in vivo* study in which buttercup tissue was applied to soil in pots showed a different response than was obtained in petri dishes (Table 2). In soil, buttercup tissue significantly retarded germination of only one species, crested wheatgrass. This effect was observed only at the 2X concentration of buttercup tissue. In no case was average weight of individual test plants significantly different than that of control plants.

In sand, germination of crested wheatgrass and Gaines fall wheat was significantly reduced by both 1X and 2X concentration of buttercup tissue (Table 2 and Fig. 1). Average weight of surviving plants of crested

TABLE 2. Effect of two levels of bur buttercup tissue on germination and dry matter production of seven grasses and two forbs planted in soil or sand. Treatments are: C, control (no tissue added); 1X, tissue added equivalent to average production of buttercup per unit area in pastures (0.7 g/dm²), and 2X, tissue added equivalent to twice average production of buttercup.

Species	Growth Medium	Treatment	Average Weight per Individual (g)	Days after Planting: 5	10	15	25	28
				Percent Germination				
Halogeton	Soil	C	[1]	3[2]	9	13	12	12
		1X		3	8	15	10	10
		2X		1	6	13	14	14
Western wheatgrass	Soil	C	0.16	0	3	35	65	67
		1X	0.23	0	11	45	61	61
		2X	0.23	0	3	51	71	71
Fairway wheatgrass	Soil	C	0.36	4	21	56	67	67
		1X	0.32	4	36	53	58	58
		2X	0.36	3	42	53	60	60
Tall wheatgrass	Soil	C	0.30	3	44	89	94	94
		1X	0.34	2	62	89	90	90
		2X	0.34	0	57	80	87	87
Crested wheatgrass	Soil	C	0.23	3	38	81	87	89[a]
		1X	0.27	13	58	76	80	80[ab]
		2X	0.28	1	37	62	65	65[b]
Alfalfa	Soil	C	0.36	23	41	69	71	71
		1X	0.32	21	46	62	64	64
		2X	0.36	15	50	71	71	71
Common rye	Soil	C	0.24	8	19	19	19	19
		1X	0.32	17	26	27	27	27
		2X	0.24	12	24	25	25	25
Delmar fall wheat	Soil	C	0.38	3	21	36	37	37
		1X	0.41	6	34	49	49	49
		2X	0.42	14	35	42	42	42
Gaines fall wheat	Soil	C	0.39	15	44	53	53	54
		1X	0.42	27	53	57	57	57
		2X	0.43	25	59	62	62	62
Crested wheatgrass	Sand	C	0.20[a]	45[a]	90[a]	94[a]	94[a]	94[a]
		1X	0.20[a]	3[b]	67[b]	76[b]	76[a]	76[a]
		2X	0.11[b]	0[b]	24[c]	39[c]	41[b]	41[b]
Gaines fall wheat	Sand	C	[3]	94[a]	96[a]	96[a]	96[a]	96[a]
		1X		56[b]	83[b]	85[b]	85[b]	86[b]
		2X		21[c]	56[c]	60[c]	62[c]	62[c]

[1]Plants not harvested.
[2]Sets differing among themselves at the 5 percent probability level are followed by superscripts. Means followed by the same letter do not differ significantly.
[3]No data.

wheatgrass was also significantly reduced by the 2X treatment on sand (Table 2).

Unfortunately bur buttercup seeds failed to germinate in these greenhouse trials. The seeds apparently require some cold treatment for germination, since seed from the same lot did germinate in the cold frame experiment reported in Table 3.

Buttercup appeared to significantly inhibit growth of seedlings of fairway and western wheatgrass in the cold frame (Table 3). The results offer no evidence as to whether inhibition is caused by simple competition, allelochemic effects, or both.

The cereal grains (common rye and the fall wheats) were far more effective than perennial grass seedlings in suppressing buttercup growth in the cold frame (Table 4). The cereals germinated within two weeks of planting and attained heights of 15 cm or more prior to the onset of severe cold. Thus buttercup seedlings growing with them were heavily shaded throughout the experimental period. Buttercup plants growing with these species never produced seed.

Buttercup production was highest in rows seeded to fairway and western wheatgrasses and Russian wildrye. Fairway and crested wheatgrass differed markedly in respect to the amount of competition they offered buttercup. Although fairway yielded far more growth than crested wheatgrass, buttercup yields were over twice as great in rows sown to fairway as opposed to crested wheatgrass.

Discussion and Conclusions

Klikoff (1964) has postulated that the effect of tissue of one species on germination and growth of others could possibly be reduced or eliminated in soil by adsorption of reactive materials on colloidal surfaces, by microbial degradation of the material, by leaching, and/or dilution of concentration by diffusion. In this study, the leaching ef-

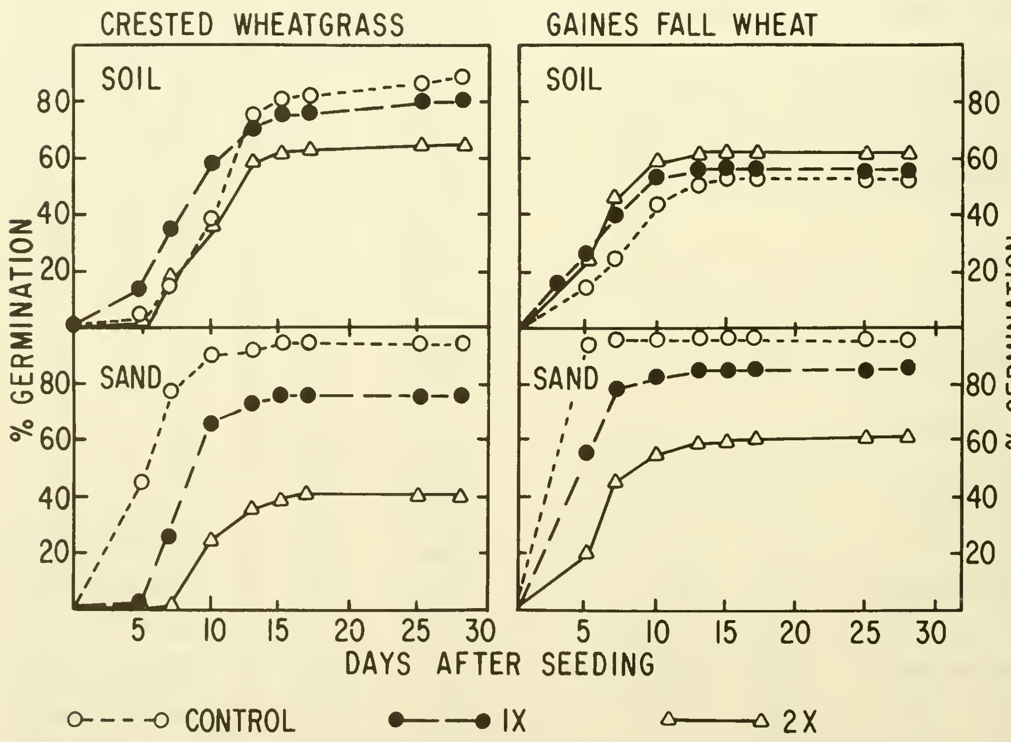

Fig. 1. Effect of three levels of bur buttercup tissue on germination of crested wheatgrass and Gaines fall wheat on Benmore soil and sand. See text for details.

Table 3. Average dry weight of grasses (g/dm of row) for three densities of associated bur buttercup plants.

Species	None (Control)	Seeding Density of Buttercup 50 Seeds per 1.5 m Row	100 Seeds per 1.5 m Row	F-Value
Crested wheatgrass	1.94	2.02	2.40	.30
Fairway wheatgrass	7.52	3.36	4.26	3.99*
Intermediate wheatgrass	8.24	6.99	7.60	.31
Pubescent wheatgrass	9.21	9.74	8.76	.14
Western wheatgrass	1.47	.50	.14	14.16*
Russian wildrye	1.63	1.06	1.31	.54
Common rye	11.07	6.81	10.68	9.53*
Delmar fall wheat	6.68	4.74	8.13	2.25
Gaines fall wheat	9.67	4.76	10.70	4.80*

*Significant at the 5 percent probability level (analysis of variance).

fect was minimized by the use of undrained pots, but the diffusion of material into the greater volume of pots as opposed to petri dishes could partially account for the reduced effect of buttercup tissue on germination and growth of test species in sand or soil. Differential effect of the tissue in sand and soil also implies that adsorption and/or microbial decomposition are operative. Greater adsorption would be expected in silty clay soil than in sand which would have considerably less surface area. Furthermore, decomposition may well be more rapid in this soil, since sands tend to support smaller microbial populations than do heavier textured soils (Clark 1957).

In view of the effect of soil texture on action of an allelopathic agent as observed in this study, it is of interest that del Moral and Cates(1971:1033) conclude that the capacity of soil to detoxify allelopathic compounds is unpredictable. Their results are perhaps related to the fact that they used a moistened sponge rather than uncontaminated soil as a control. A comparison of the germination response of our species on filter paper and in soil (Tables 1 and 2) demonstrates that germination of several species was reduced in soil (as opposed to filter paper) even without the addition of allelopathic material. There is thus a clear need to perform additional experiments before soil texture is dismissed as a significant variable affecting the action of allelopathic compounds.

The pot trials (Table 2) demonstrate that buttercup tissue in quantities that might be expected under field conditions is capable of reducing germination and retarding growth of at least some species under natural conditions. These tendencies may well be accumulative under conditions in which comparable amounts of tissue are added to the system each growing season. The cold frame studies also demonstrate that buttercup seedlings severely suppress the growth of some grasses.

Fairway and western wheatgrass, two species having growth significantly inhibited when competing with buttercup in the cold frame trials, were both slow germinators. Rapid germination of buttercup may give this species a competitive advantage for water, nutrients, and space. Thus, the suppression of these species by buttercup may be related to slow development and growth habit rather than to allelopathic action.

The differing performance of buttercup

Table 4. Total top growth (grams dry wt) of bur buttercup seeded at two rates with various grasses.

Competing Species	Seeding Density of Buttercup 50 Seeds/ 1.5 m Row	100 Seeds/ 1.5 m Row
None	50	80
Crested wheatgrass	17	26
Fairway wheatgrass	43	77
Intermediate wheatgrass	27	34
Pubescent wheatgrass	25	40
Western wheatgrass	60	52
Russian wildrye	31	73
Common rye	0.2	0.3
Delmar fall wheat	0.1	0.1
Gaines fall wheat	0.1	0.01

when grown with fairway and crested wheatgrass, two very closely related species, is remarkable. Although crested wheatgrass produced only about one-half as much biomass as fairway (Table 3), buttercup production was threefold greater when grown with the latter species. Such disparity in performance of buttercup merits further study.

Although bur buttercup has been shown to display allelopathic effects against several grasses, the effects may be of limited importance under most field conditions. Any chemical effects which the species might exert are expected to be most pronounced against slow-developing species growing on coarse-textured soils. Since maximum abundance of bur buttercup occurs on fine-textured soil (Buchanan 1969), adsorption and decomposition of toxic compounds would be expected to be rapid. Certainly no obvious suppression of associated species is seen in the field. Rapid development in late winter and early spring probably contributes more to competitive ability of buttercup than does allelopathy.

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