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BIOCHEMICAL INHIBITION (ALLELOPATHY) EXHIBITED BY ITALIAN RYEGRASS (*LOLIUM MULTIFLORUM* L.)

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Abstract

Italian ryegrass (*Lolium multiflorum* L.) is a common grass species in many temperate parts of the world. Laboratory and green house experiments showed that besides toxic root exudates, water soluble toxins are also present. The presence of toxins in root exudates and in the above ground parts of Italian ryegrass was confirmed during the soil leaching, soil mulching experiments and artificial rain drip bioassays. These toxins are capable of significant inhibition of germination and growth of *Avena*, *Bromus*, *Lactuca* and *Trifolium* tested. The possible role of these toxin producing species in agronomy, vegetational dynamics and range land ecology is discussed.

Introduction

Italian ryegrass (*Lolium multiflorum* L.) is a native of Central and Southern Europe, but is now naturalized in many temperate parts of the world. In Southern California, thousands of acres of burned over brushlands are commonly seeded with this species. The principal objectives are to provide a plant cover effective in lessening soil erosion, to supply forage for game and livestock, and to furnish competition to reduce surival of brush seedlings which may appear in great abundance following fires (Schultz *et al.*, 1955; Biswell, 1952).

The capacity of *Lolium* to furnish competition to the brush seedlings and also to many other plants has attracted the attention of many investigators. It was concluded that *Lolium* when sown in combination with other species, the growth of most other species is usually inhibited. However, most of the evidence given seems inconclusive and it appears as if there is some other mechanism also involved in addition to the competition. On the other hand Cubbon (1925) and Osvald (1949) hinted at a possible allelopathic mechanism. Even McKell *et al* (1969) suspected the existence of such a phenomenon in the ryegrass litter experiment. With this purpose in mind a project was initiated to reveal the actual mechanism underlying the presumed strong interfering nature of ryegrass. Any investigation about an allelopathic mechanism is not logical unless the possibility of competition is thoroughly investigated and evaluated. Therefore, the possible competitive reactions of ryegrass were studied. Some of the experiments performed elsewhere (McKell *et al*, 1969) were modified and repeated, and new experiments designed. The retarding influence of *Lolium* is not fully explained by competition for the physical factors of the environment, and possibly some kind of biochemical interference or allelopathy is involved in this case as well (Naqvi, 1972). In this paper some of the experiments performed on the allelpothic aspect of Italian ryegrass (Naqvi, 1969) are discussed.

Materials and Methods

In an attempt to investigate the possibility of any allelopathic mechanism involved in the suppression of other species by ryegrass, the following points were taken into consideration: (1) the toxins may be released by the decay and decomposition of the straw and other parts of the dead ryegrass plant, (2) toxins may be exuded from the roots and other parts of the living ryegrass plants into the soil, (3) the toxins may be leached from the living or dead straw with rain, fog, dew, etc., (4) there may be a combination of these processes. Each possibility was analysed in a separate and independent experiment.

Soil leaching experiment.—The experiment was designed to investigate the possibility of any inhibitory substances present in soil leachates of living ryegrass. Eight, 9-inch plastic pots were filled with equal volumes of standard greenhouse soil without any extra organic manure. Ten seeds of ryegrass were sown in each pot. An identical set of 8 pots filled with soil was left as control. All the pots were set up on a specially designed wooden platform with a hole under each pot. They were allowed to remain there for two months until the ryegrass seedlings were mature enough to be used. These pots on the wooden platform will be referred to as "upper series". A "lower series" of pots consisted of twenty four, 4-inch plastic pots with two seedlings of *Bromus rigidus* grown in each. These 24 pots of the "lower series" were divided into 6 groups of 4 pots each and

(i) watered twice a week with leachate collected from 4 ryegrass pots of upper series:

(ii) watered as in (i) and also given 50ml of half concentration Hoagland's solution weekly;

(iii) watered twice a week with leachate collected from 4 pots of upper series without any ryegrass growing in them;

(iv) watered as in (iii) and also given 50ml of half concentration Hoagland's solution weekly;

(v) watered with tap water twice a week;

(vi) as in (v) and also given 50ml of half concentration Hogland's solution weekly.

The experiment was allowed to proceed for 4 weeks at the end of which the plants of the "lower series were harvested and dry weight determined. During the entire experimental duration of 3 months, the pots remained in the greenhouse.

Soil leachate bioassay.—Leachate was collected from the *Lolium* pots used in the preceding experiment. was concentrated four times and ten times in a vacuum flask evaporator. A normal concentration was also used in the experiment. A standard sponge bioassay (Muller, 1966) was set up using *Bromus rigidus* as the test species against the 3 concentrations of *Lolium* leachate. Double distilled water was used as control. At the end of the experiment, the radicle length of the seeds of *B. rigidus* were measured as an indication of growth.

Relative toxicity of ryegrass parts.—After having shown that there was some leaching of toxic material from the living roots of *Lolium*, attention was then concentrated upon the possibility of the presence of toxic material in grass. Roots, stalks with leaves, and spikes of mature plants of *Lolium* were completely cleaned, dried, and ground. Four grams of each portion of the plant were soaked for two hours in 100ml double distilled water and filtered. Ten seeds of *B. rigidus* were planted on sponges with four replicates for each extract as well as for control, in the "standard sponge bioassay." At the end of 48 hours the radicle growth of *Bromus* was measured.

Ryegrass toxicity against other species.—The experiment was designed to determine the relative effects of *Lolium* extract on the growth and germination of its own seedlings and the seedlings of the test species used in the field and greenhouse experiments (Naqvi, 1972), using the standard sponge bioassay techniques. Ten grams of dry *Lolium* straw were crushed and soaked in 250ml of double distilled water for two hours and then filtered. This extract was used for test purposes in all the treatments. Double distilled water was used for control. Four replicates of the control and test were made, using 20 seeds per replicate. All the seeds were pre-soaked for two hours in double distilled water and *Lolium* extracts respectively. They were allowed to grow in the growth chamber for 72 hours at 26°C. Radicle growth measurements were made at the end of the experiment.

Artificial rain bioassay.—To simulate natural conditions in the field, the experiment was designed in which 200 grams of dry *Lolium* straw were loosely crushed by hand and put in a large funnel attached to a flask. Five-hundred ml of distilled water was then sprayed on this straw in order to soak it thoroughly, and the drained water from the funnel was collected. This spraying process losted about an hour. The only apparent difference between this—spray and the rain was the use of distilled water, and this was intentionally done to avoid adding additional factors. This "artificial rain drip" was filtered, one portion of which was concentrated four times in a vaccuum flask evaoporator. A "standard sponge" bioassay was set up, using these two concentrations of *Lolium* drip against *Bromus* and *Lactuca* seeds. Double distilled water was used as control.

Mulching experiment.—To study the residual effects of *Lolium* on the growth of other seedlings when added as a mulch under controlled conditions, *Bromus mollis* was selected as the test species since it showed relatively high susceptibility in earlier experiments. Equal volumes of washed beach sand were put in 4-inch plastic pots to which 4 grams of crushed *Lolium* straw was added. In another treatment the straw was soaked for four hours, dried and four grams added to each pot. In another set 4 gram. vermiculite soaked in concentrated filtrate left from the straw in water was added to each pot. For control, 4 grams of clean vermiculite was added to each pot. Eight seeds of *Bromus mollis* were planted in each of the sixteen pots. The seedlings were later thinned to four seedlings per pot. All the pots were put an a turn table in the greenhouse to assure equal lighting. Hoagland's solution was added every two weeks and the soil was kept moist. The plants were grown for two months offer which they were harvested and dry weight dertermined.

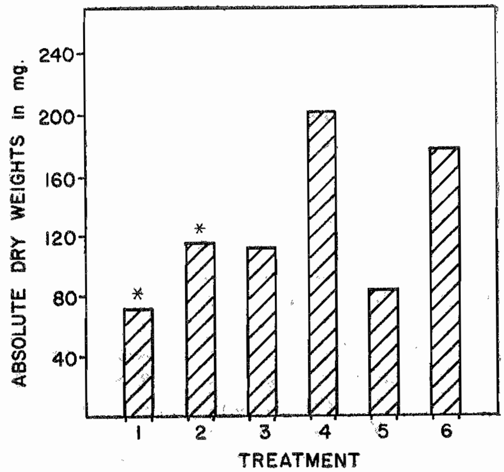

Fig. 1. Results of the soil leaching experiment. Each bar represents the total dry weight of 4 replicates, each with two *Bromus rigidus* seedlings in it. The numbers represent the treatment of the lower series of pots with the following:

1. ryegrass leachate.
2. ryegrass leachate plus Hoagland's Solution.
3. soil leachate.
4. soil leachate plus Hoaglan'd Solution.
5. tap water only.
6. tap water plus Hoagland's Solution.

*Difference statistically significant.

Results

Soil leaching experiment and the leachate bioassay.—Dry weights of the plants which were watered with leachate from ryegrass were significantly reduced compared to other treatments (Fig. 1). This difference is maintained even under high nutrient conditions, suggesting that high nutrients could not totally mask the effects of the toxins. A significant leaching of toxins from ryegrass was observed. The seeds showed a significant inhibition of growth where they were exposed to concentrated leachate from ryegrass (Fig. 2). The reduction in the radicle growth of *Bromus rigidus* with the leachate concentrated ten times was significantly different from control at 0.01 level. The result of this bioassay and the preceding experiment indicate that the leachate from the rhizosphere of living ryegrass roots has growth and germination inhibitors in it and if given enough time these inhibitors could accumulate in the soil where they might be capable of suppressing the growth of other species.

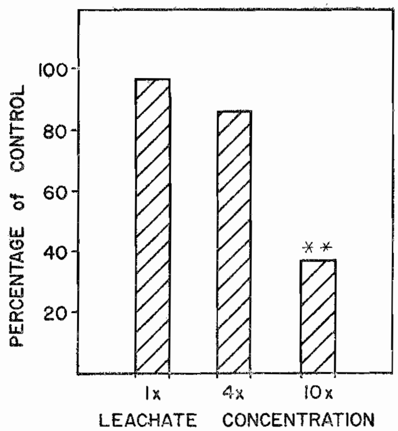

Fig. 2. Result of soil leachate bioassay. All the 3 test conditions are expressed as percent of control, when the seeds were pre-soaked and grown in double distilled water. IX, 4X, 10X represent the concentration of the leachate. Value are based on the average radical growth of *Bromus rigidus* seeds in 3 replicates, each with 10 seeds in it.

**Significant at 0.01 level of probability.

Relative toxicity of ryegrass parts.—Mean radicle growth of *Bromus rigidus* against different test conditions indicate that two of the three parts of *Lolium* showed inhibition of growth (Table 1). However, different parts showed different degrees of inhibition. The reduction in radicle growth of *Bromus* seeds was significant at the 0.01 level when soaked with extract from the above ground parts of ryegrass. Extract of dead roots did not suppress the growth of *Bromus* significantly. In subsequent experimentation, therefore, only the above ground parts of *Lolium* were used for extraction purposes.

TABLE 1. Relative toxicity of ryegrass parts against *Bromus rigidus* seeds.

Condition	Mean	S D	% of control
Control	24.0	2.1	—
Roots etc.	21.0	2.2	91.0
Shoots & leaves	*11.70	0.7	48.0
Spikes	6.5	0.6	27.0

Each value is based on the mean radicle growth of 4 replicates, each having 10 *Bromus* seeds growing in it.

*Significant at 0.01 level of probability.

TABLE 2. Ryegrass toxicity against germination and early growth of ryegrass and other species in bioassays.

		Radical Growth (mm)			Percentage Seed Germination		
Species	condition	Mean	S D	% of control	Mean	S D	% of control
Bromus mollis	control	9.8	0.57	*27.50	27.5	2.9	72.7
	test	2.7	1.88		220.0	7.0	
Trifolium	control	35.9	5.20	23.9	46.2	11.0	72.9
	test	8.6	1.78		33.7	7.5	
B. rigidus	control	39.6	2.65	60.3	95.0	0.0	100.0
	test	23.9	3.30		95.0	5.8	
Avena	control	27.0	0.35	67.7	77.5	9.5	100.0
	test	18.3	0.74		77.5	9.5	
Lactuca	control	19.8	0.75	*35.80	90.0	0.0	22.2
	test	7.1	1.20		20.0	7.1	
Lolium	control	21.1	0.65	*46.60	95.0	0.0	92.1
	test	9.9	1.32		87.5	5.0	

Each value represents the mean of 4 replicates, each with 20 seeds.

*Significant at 0.05 level of probability.

Ryegrass toxicity against other species.—Seeds of *Bromus rigidus*, *Avena* and *Lolium* showed good germination (Table 2). Seeds of *Lactuca* were severaly affected by ryegrass extract and only 22% of these germinated seeds survived under test conditions. The germination of *Bromus mollis* and *Trifolium* were about 75% and seems to have been most severely inhibited by ryegrass extract. *Avena* was the least affected species.

The data shows that the toxic nature of ryegrass is species specific. Moreover, some species are affected in germination, while others are only retarded in growth. Seeds of *Lactuca* and *Bromus rigidus* are the most desirable test species for bioassay since they germinate and grow well, yet are susceptible to toxins. Seeds of *Poa* are the least suitable for bioassay because of their very slow germination and therefore were not included in the table. The inhibition of *Lolium* seedlings by their own toxins would explains the auto-toxicity of ryegrass under field conditions.

Artificial rain bioassay.—Table-3 indicates a tremendous suppression of growth of the two species even under normal concentration of the leachate. In 4 concentration, there was a complete inhibition of the growth of *Lactuca* seeds, while *Bromus* showed a significant reduction in germination at the 0.01 level of probability. It would appear that the toxins are present in the straw and that they

TABLE 3. Artificial rain-drip bioassay affecting growth of radicle in plants.

	Control		CONCENTRATIONS					
			1X			4X		
Test species	Mean (mm)	SD	Mean (mm)	SD	% of control	Mean (mm)	SD	% of control
Bromus	19.1	1.2	9.9	1.1	*51.0	6.2	0.7	**32.0
Lectuca	14.2	1.4	3.0	0.5	**21.0	0.0	0.0	0.0

Each value is the mean radicle growth in mm of 3 replicates with 10 seeds each.

*Significant at 0.05 level of probability.

**Significant at 0.01 level of probability.

are highly soluble in water. Under natural conditions, these toxins are washed out of the straw by rain, fog, or dew into the soil where they inhibit growth and germination of species.

Mulching experiment. Plants in the control, series where they were grown in vermiculite and sand only, showed the maximum dry weight followed by plants in other treatments,. This inhibition of growth is due to the toxic nature of the compounds present in ryegrass straw. Evidently one washing did not take out all the toxins of the straw. Moreover, there may also be some water insoluble compounds which could not be washed out of the straw (Table 4).

TABLE 4. Effect of mulching with *Lolium* on *Bromus mollis* seeds.

	Control	Treatments		
	sand and vermiculite only	sand and unwashed straw	sand and washed straw	sand—vermiculite and leachate
Mean	0.736	*0.534	**0.535	**0.491
—SD	0.05	0.04	0.03	0.04
% of control	—	72.5	72.5	66.7

Each value is the mean dry weight in grams of 4 replicates, each with 4 plants.

*Significant at 0.029 level of probability.

**Significant at 0.014 level of probability.

Discussion

The mechanism of interaction of ryegrass with other species, especially the ones tested in this investigation, revealed some very interesting facts. These facts, alongwith some others already established (Naqvi, 1972) can be integrated as follows:

Ryegrass has certain characteristics because of which it is at an advantage over most of the other species when they happen to grow at the same place. Among them, the most important ones are the fast rate of growth, high degree of seedling vigour, and a very extensive fibrous root system. It is obvious that any plant having such characteristics will fare better so far as competition is concerned. However, studies (McKell *et al*, 1969; Naqvi, 1972) have shown that competition alone could not explain the phenomenon and it was suggested that there is some other mechanism involved in this kind of interference which is partially or totally responsible for the observed suppression.

Further experiments concerning the presence of water soluble toxins in the soil leachate where ryegrass was growing, and also in the water extracts of dead plants, explains the growth inhibitory nature of ryegrass to a large extent. It was seen that these aqueous extracts and the soil leachates were capable of inhibiting the germination and growth of the test species. The validity of these bioassays, which were performed under closely controlled conditions, and also the facts gathered from experiments on the residual toxicity of soil, inhibitory nature of artificial rain-drip and the mulching experiment can scarcely be questioned. These findings reveal a double origin of these toxins responsible for the inhibition phenomenon. One source of such toxins is through leaching from the living plants into the soil where they accumulate and build up toxicity for the young seedlings of other species. The other source is through leaching and the decay and decomposition of dead *Lolium* straw. Through such mechanism, the toxins are released in the soil, where they inhibit the germination and growth of other species. Once these toxins re accumulated in a physiologically significant quantity, they are able to reduce the germination percentage and or retard the growth of the suceptible species in the vicinity.

One of the most common problems encountered during our allelopathic investigation is the separation of competition for the physical factors of the environment from biochemical influence. Since the end effects are the same in the two cases (*i.e.* the reduction in growth and sometimes germination of the victim plants), the situation becomes very complex unless the experiments are carefully designed. The best way to overcome this problem is to eliminate the possibility of competition first and then proceed with the experimentation on allelopathy. This point was taken care of earlier (Naqvi, 1972). Another point to consider is the species specificity of these toxins. Not all species are equally susceptible to these inhibitors. For example *Lactuca*, *Poa*, *Trifolium* and to some extent *Bromus mollis*, were quite susceptible to the inhibitors while other species, especially *Avena* were not so. This phenomenon is most probably due to the inherent physiological and morphological characteristics of the species involved.

Regarding the importance of this phenomenon in nature, the authors strongly support the suggestions made by McKel *et al* (1969) that the practice of using ryegrass in mixture with other species for pasture purposes should be avoided. The practice of using ryegrass for sowing on burned over brushlands is valid for two reasons.

If we look at the picture from the range management point view, the practice is very beneficial because this plant does have certain advantageous characteristics. However, if we look at the stituation from the ecological point of view, the practice is deleterious to the native vegetation of the area. Our own observations in the Santa Ynez mountains of California and studies performed elsewhere (Biswell, 1954; Schultz *et al*, 1962) have shown that wherever ryegrass is sown, there is a significant reduction in the regeneration of most of the chaparral species. This means that if the practice is continued extensively and for a long period of time it might disrupt the dynamics of the native vegetation and cause its elimination in due course.

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