CHAPTER V

ALLELOPATHY AMONG <u>POA</u> <u>ANNUA</u>, <u>POA</u> <u>PRATENSIS</u>, AND <u>LOLIUM</u> PERENNE SEEDLINGS AND MATURE FIELD TURFS

ABSTRACT

Researchers have implicated allelopathy in the interaction of the cool-season turfgrasses, <u>Poa annua</u> L., <u>Poa pratensis</u> L., and <u>Lolium perenne</u> L. Allelopathy is the effect of one plant on another as a result of the transfer of a chemical agent. The purpose of this study was to determine whether allelopathy is a significant factor in the interaction of these grasses. Allelopathy was investigated by means of a field experiment and a series of laboratory germination tests. This study represented, perhaps, the first controlled allelopathy study conducted under field conditions.

The field apparatus was based on the staircase system, whereby Species A (the receptor) receives water and any chemicals by gravity flow from Species B (the donor). Thirty-seven of the 84 different records taken on the parameters of the grass showed significant effects of allelopathy in one or more of the three receptor species. Allelopathy was significant even though a considerable cation-exchange capacity (10.6 cmol (p⁺) kg⁻¹) was present in the top 5 to 7 cm of the rootzone. Approximately half of the significant effects could be construed as having been beneficial to the receptor, the rest as detrimental. Disease severity in several instances was associated with allelopathy effects.

<u>P. pratensis</u> seed germination in the laboratory was lower in the presence of <u>P. annua</u> seedlings. Inhibition of <u>P. pratensis</u> germination did not occur, however, when aqueous extracts of mature P. annua plants were used.

Seedling root and shoot growth of all three species was affected by the nearby seedlings of the other species and by extracts from mature plants. <u>P</u>. <u>pratensis</u> was the most sensitive of the three species to treatment.

Germination rates of the three species were unaffected by light (versus total darkness). The germination rate of <u>P. pratensis</u> in distilled water was reduced 13% by the addition of suspended soil particles.

Additional Index Words: Annual Bluegrass, Kentucky Bluegrass, Perennial Ryegrass, Turfgrass, Disease.

INTRODUCTION

Speculation has occurred many years about the possible occurrence of allelopathy among turfgrasses. Allelopathy is the effect of one plant on another as a result of the transfer of a chemical agent. Over 40 years ago, Ahlgren and Aamodt (1) investigated the interaction of several turfgrasses and concluded that "harmful root effects" seemed to be occurring. Allelopathy among warm-season grasses has already been documented. <u>Sporobolus pyramidatus</u> has been shown to inhibit the growth of <u>Cynodon dactylon</u> and <u>Buchloe</u> dactyloides by the excretion of secondary metabolites (13).

<u>Poa annua L., Poa pratensis L., and Lolium perenne L.</u> are three cool-season turfgrasses that differ in their ability to dominate a stand. <u>L. perenne</u> has been described as inhibitory to the growth of <u>P. pratensis</u>, in seedling stands and mature swards (7). <u>P. annua</u> may also act detrimentally to the growth of <u>P. pratensis</u> (Chapter I). <u>P. annua</u> is an annual, grassy weed in fine turf, whereas <u>P.</u> pratensis and <u>L. perenne</u> are desirable lawn grasses.

A powerful germination inhibitor, coumarin, is quite common in the tissue of most grasses (9). In fact, coumarin is largely responsible for the pleasant smell of freshly cut grass (9). Coumarin's effect on seed germination is so renowned, that Soviet researchers use RCU's, relative coumarin units, for describing the activity of an inhibitor (8). Therefore, ample evidence existed to suggest the presence of allelopathy in cool-season turfgrasses.

Whittaker (17) has stated that two criteria must be satisfied before the presence of allelopathy can be concluded: (i) an inhibitory chemical must be present in effective concentrations in the soil, and (ii) the measured effect must not be due to competition for light, water, or

nutrients. The latter criterion can usually be satisfied by the use of a staircase testing system, such as the one used by Bell and Koeppe (4). In this type of system, pots of two different species are physically separated on the steps of a staircase. Water and excreted chemicals are permitted to flow from pot to pot via interconnecting hoses and pumps. The staircase and the enclosing greenhouse, however, impose artificial conditions on the plants, especially with regard to light intensity and quality (12). Because of the limitations encountered in greenhouse-based systems, some researchers are beginning to view out-of-doors experiments as a possible solution (W.B. Duke, 1978, personal communications). To our knowledge, no controlled allelopathy test has yet been conducted under field conditions.

Verification of Whittaker's (17) first criterion of allelopathy has proven to be the more difficult of the two. Many naturally occurring chemicals can be shown to inhibit plant growth. Even amino and nucleic acids in high concentrations can be toxic (14). Although it is easy to extract a toxic substance from a plant, it is difficult or impossible to prove that the compound is excreted in the field and is of ecological significance (12). Furthermore, often more than one chemical is involved in the interaction, or inert chemicals are changed into a toxic form after release into the environment (12). Thus, a more realistic approach to the study of allelopathy would be to first

verify that effects of allelopathy are indeed occurring, before beginning a search for the causal agent.

The purpose of this study was to determine whether allelopathy is a significant factor in the interaction of <u>P</u>. <u>annua, P. pratensis</u>, and <u>L. perenne</u>. A field experiment was utilized to investigate allelopathy under actual turfgrowing conditions. Laboratory methods were employed for investigating allelopathy among turfgrass seedlings.

MATERIALS AND METHODS

Field Experiment

The field experiment was based on the staircase design used by Bell and Koeppe (4) and others. A wooden framework was constructed on a graded site at the Joseph Valentine Turfgrass Research Center, University Park, PA (Fig. 5.1). The base of each chamber was leveled with sand and lined with a double layer of 6-mil plastic. Silica sand, which contained 74% of its mass as 0.1 to 0.5-mm particles, was added. An 8:2:2 mix (volume basis) of sand, Hagerstown silt loam soil (a fine, mixed, mesic, Hapludalf), and reed-sedge peat was applied in a layer atop the sand. The pH of the top mix was 5.8, and the cation-exchange capacity was 10.6 cmol (p⁺) kg⁻¹. The plot area was fumigated with methyl bromide at a rate of 112 kg ha⁻¹ to eliminate potential



Fig. 5.1. Schematic diagram of a portion of the apparatus which was used in the study of allelopathy in field turf.

weeds.

The staircase arrangement imposed some restrictions on the randomization of the treatments. A channel consisted of seven chambers running down the slope (Fig. 5.1). One species was planted in the top two chambers, a second species in the next two chambers, and a third in the bottom three chambers. Six channels contained all combinations of the three species in each of the three positions, top, middle, or bottom. Four additional channels were allocated entirely to a single species, to serve as a check treatment. In all, 18 check chambers were planted to <u>L</u>. <u>perenne</u>, and 11 each to <u>P</u>. <u>annua</u> and <u>P</u>. <u>pratensis</u>. A total of five chambers in two replicates were allocated to each receptor species. The 10 channels were arranged in a completely randomized design.

Water was input to the 10 top chambers through a pressure-regulated, 2.5-cm diameter, manifold pipe via CW36-121 drip tubes at the rate of 1.4 1 hr-1 at 1 m of head. The 10 drip tubes were periodically checked for equal deliveries. Depth of water in each chamber was monitored through 5-cm diameter vertical pipes (Fig. 5.1). The normal maintenance level for pool depth was 10 cm. Sprinkler irrigation was used frequently during establishment and then

1CW36-12 drip tubes are a product of Chapin Watermatics Inc., Watertown, NY. The use of trade names in this publication does not imply endorsement by the Penna. State University of the products named nor criticism of similar ones not mentioned.

afterwards whenever the pool depths dropped.

The three species, <u>P</u>. <u>annua</u> (Oregon origin), <u>P</u>. <u>pratensis</u> (cv. 'Touchdown'), and <u>L</u>. <u>perenne</u> (cv. 'Pennfine'), were seeded at 323 pure-live seeds dm-2.

A 1:50 aqueous dilution of Soil Gard² was applied at 3.3 L m⁻² for erosion control. A 5-cm deep layer of sterilized oat (<u>Avena sativa</u> L.) straw was also applied. The straw was removed after 1 week.

Approximately 1 year after planting, all of the <u>P</u>. <u>annua</u> plants in the experiment flowered and died. The dead material was removed, and <u>P</u>. <u>annua</u> was immediately replanted as before.

Phosphorus and potassium levels in the seedbed were adjusted according to Penn State soil test recommendations. Starter fertilizer was applied at 98 kg N ha-1 from 10-4.4-8.3 (N-P-K). Maintenance fertilizer was applied at 146 kg N ha-1 from 38-0-0 in 1980, and at 263 kg N ha-1 from 20-1.7-6.6 in 1981. The 20-1.7-6.6, which contained micronutrients (1% Ca, 0.6% Mg, 1% S, 0.03% B, 0.05% Cu, 0.1% Fe, 0.05% Mn, 0.0005% Mo, and 0.05% Zn), was applied in six applications throughout the growing season.

Mowing commenced 13 days after planting. The plots were clipped at a height of 2.5 cm, twice weekly during the growing season, using a reel mower. Clippings were removed so that cross-contamination of clippings, from one channel

2Soil Gard is a product of the Alco Chemical Corp., Phila., PA.

to another, could be minimized.

The plot area was topdressed on two occasions, 2.5 and 9.5 months after planting. A 1-cm layer of sterilized 8:2:2 mix was applied and leveled. Topdressing was used to enhance the uniformity of the surface.

Chloroneb (1,4-dichloro-2,5-dimethoxybenzene) was applied to all plots at a rate of 12 kg ha-1 on 21 July 1980 for control of a Pythium blight epidemic. No other pesticides were applied to the field experiment.

The plots were evaluated for various characters (Table 5.1). Shoot density and leaf-area index (LAI) were determined from plugs taken with a Noer profile sampler. Three plugs were sampled from each plot (chamber). The LAI was determined using the sizing method (Chapter II). The size of every shoot in every plug was visually ranked on an integer scale of 1 to 5, with 5 being largest. Approximately 85 shoots, representing all five size classes of all three species, were later selected for purposes of size calibration. The leaf area of each shoot was measured under a dissecting-type microscope. A 40x magnification was used for shoots of size 1 and 10x for sizes 2 to 5. The total leaf area within a sample equaled the number of shoots in each size category times the corresponding "average" leaf area of the category. LAI equaled the total leaf area over the ground surface area of a plug (988 mm²).

The shoot mass and underground vegetative mass were measured at 16 months after planting. Shoots were severed 1

		Dates	of evaluat	tion
Parameter	Measurement criterion	First	Last	No. of times
		months afte		
Shoot density	dm ⁻²	4	16	3
Leaf-area index	m2m-2	11.5	16	2
Shoot mass	g dm-2	16	16	1
Underground vegetative mass	g dm 3	16	16	1
Above/below ground veg. mass	g g-1	16	16	1
Specific leaf-area	dm2g-1	16	16	1
Relative shoot-size	1 to 5 scale	11.5	16	2
Leaf width. sheath-axis width	m	11.5	16	2
Leaves - shoot-1	no.	11.5	16	2
Clipping dry mass	a	10.5	13.5	2
Moisture content of clippings	a a-1	13.5	13.5	1
Blade texture	1 to 5 scale	4	4	1
Seedhead profusion	0 to 5 scale	11	11	1
Green color (versus vellow)	0 to 9 scale	1.5	11	12
Ground cover		1	16	22
Dollarspot severity	ICT . plot-1	1.5	14.5	6
Pythium blight severity	0 to 9 scale	1.5	2.5	2
Brown natch severity	0 to 9 scale	1.5	2.5	3
Quet soverity	0 to 9 scale	2.5	5.5	5
leafsnot severity	i i i i i i i i i i i i i i i i i i i	3.5	17	12

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Table 5.1 Parameters with which the field allelopathy experiment was evaluated.

[†]Infection centers.

to 2 mm above the crown and dried at 60oC for 48 hours. The plug was rinsed free of soil and dried separately. The underground vegetative mass included crowns, 1 to 2 mm of sheath, roots, rhizomes, and thatch. The specific leaf-area (16) of each sample was calculated from the LAI and the shoot mass.

The severities of the following diseases were visually rated on a whole-plot basis: dollarspot (<u>Sclerotinia</u> <u>homoeocarpa</u> Bennett), Pythium blight (<u>Pythium ultimum</u> Trow. or <u>P. aphanidermatum</u> (Edson) Fitzpatrick), brown patch (<u>Rhizoctonia solani</u> Kuhn), rust (<u>Puccinia</u> spp.), and leafspot (<u>Helminthosporium</u> spp.). All epidemics were naturally occurring; artificial inoculum was not introduced.

The data were analyzed using a general-linear-models, analysis-of-variance procedure and Duncan's multiple range test at the 0.05 level (DMRT, 0.05).

The water-flow characteristics of the field apparatus were evaluated at 16 months after planting. All pool depths were first brought to their maintenance level (10 cm). On 19 Oct 1981, 1.000 g of Rhodamine B^3 , diluted in 200 ml of water, was added to the feed pipe of one of the <u>L</u>. <u>perenne</u> check channels. Rhodamine B is a fluorescing, water soluble, moderately non-ionic dye. Fluid samples were taken from the sampling pipes within the <u>L</u>. <u>perenne</u> channel, and adjacent channels, over a 50-day period. The samples were

3Rhodamine B is a product of Allied Chemical Corp., NYC.

briefly centrifuged to remove particulate matter. Rhodamine B concentration was determined using a Turner model III fluorometer and a set of standard dilutions.

Laboratory Experiment

Seed germination and seedling growth were measured in the laboratory using the same basic procedure in each of five trials. Seeds were placed on a 7.5-cm square of blotter paper within a plastic germination (petri) dish. Four replicates of 100 seeds each were used per treatment. Ten ml of fluid, either a test solution or a distilled-water check, were initially added to each dish. Additional distilled water was added over time as needed.

The dishes were placed in a germinator, which was maintained at a 30oC, 8-hour light period, and a 15oC, 16-hour dark period. <u>P. annua</u> and <u>L. perenne</u> were evaluated after 1 week, and <u>P. pratensis</u> after 2 weeks. The number of germinated seeds per dish, and the root and shoot lengths of 12 randomly selected seedlings per dish were recorded. Data were analyzed using Duncan's multiple range test (DMRT, 0.05).

<u>Trial 1</u>: <u>Coincident germination</u>. The purpose of this trial was to determine the effect of neighboring seedlings. A few modifications of the previously described procedure were used. Fifty seeds of a donor species were germinated per dish. Fifty seeds of a receptor species were interspersed within the donor when the latter had a shoot length of about 1 cm (at 5 days for <u>L</u>. <u>perenne</u>, 6 for <u>P</u>. <u>annua</u>, and 8 for <u>P</u>. <u>pratensis</u>). Germination and growth were measured.

<u>Trial 2</u>: <u>Prior germination</u>. Seed was placed in each dish to provide 10,000, 10,000, or 2,500 pure-live seeds of <u>P. annua, P. pratensis</u>, or <u>L. perenne</u>, respectively (4.230, 4.850, or 4.230 g of seed, respectively). After 30 days in the germinator, the plants and blotters were dried at 60°C for 24 hours. All plant material was then removed from the blotters. The receptor species were germinated on the same blotters. Dishes of two of the four replicates were individually wrapped in aluminum foil, to exclude light.

<u>Trial 3</u>: <u>Turf extracts</u>. Grass clippings of all three species were obtained from the field experiment. Ten grams of fresh plant mass were combined with 200 ml of distilled water and homogenized for 5 minutes in a Waring blendor. The slurry was filtered through cheesecloth and was brought to 1 1 with the addition of distilled water. One half of the solution was mixed with 25 g of sterilized 8:2:2 soil in the blendor and filtered through cheesecloth. Some soil particles remained in suspension after filtration. The two solutions, one soil-treated and one untreated, were used for germinating seed as previously described.

In a related experiment, clippings of the three species were composted within separate, clear, plastic bags. The

bags remained out-of-doors for 6 weeks, after which the contents were processed in the manner described for the fresh clippings.

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Extracts were also prepared from the underground vegetative matter of a mature sod. Plugs were taken from a 9-year-old stand of Touchdown, Pennfine, and <u>P. annua</u>. The foliage was removed at the crown, and the plug was trimmed to a depth of 3 cm. The plug was rinsed free of soil and was dried at 60°C for 24 hours. The underground vegetative matter included crowns, roots, rhizomes, and thatch. One gram of dry mass was homogenized in the blendor and brought to 100 ml with distilled water. The same procedures were used for germination and evaluation as described for the fresh clippings.

<u>Trial 4</u>: <u>Clipping leachates</u>. Grass clippings of the three species were obtained from the field experiment. Twenty-five grams of fresh clippings were added to a flask containing 250 ml of distilled water. A separate flask was used for each species. The clippings were leached in the water at ca. 4°C for 3 days. After filtration, the solutions were tested for their effect on seed germination.

<u>Trial 5: Coumarin as a standard inhibitor</u>. Four dilutions of coumarin⁴ in distilled water were prepared for use as treatments: 0.1, 1, 10, and 100 ppm. Distilled

⁴Coumarin was obtained from MC&B Chemical Corp., Norwood, OH.

water was used as a check treatment.

RESULTS AND DISCUSSION

Field Experiment

Both increases and decreases in the measured value of plant parameters occurred in association with allelopathy in the field experiment (Table 5.2). Significant results occurred throughout the 17 months of observation. Of the 84 records taken at various dates (Table 5.1), 37 showed significant effects in one or more of the three receptor species. About half of the effects can be construed as having been beneficial to the receptor. Putnam and Duke (12) have urged that scientists not ignore the certain advantages as well as disadvantages of chemical interactions which may occur in mixed culture.

Disease severity in several instances was associated with allelopathy (Table 5.2). The diseases might have been merely responding to the transfer of pathogen propagules from a donor to a susceptible receptor. But the possibility also exists that chemicals from the donor plants were affecting the growth of disease organisms in the receptor. For example, Dietrich and Valio (6) found that coumarin

		Characteristics of the receptor							
Receptor species	Donor species	Parameter	Months after planting	Measured value					
<u>P. annua</u>	<u>P</u> . <u>pratensis</u>	Leaves shoot ⁻¹ Sheath-axis width Shoots of size 1 Above/below gnd. mass Ground cover Ground cover Dollarspot severity Leafspot severity	11.5 11.5 11.5 16 12 16 2.5 17	<pre>% of check¹ 104 108 46 125 89 103 200 93</pre>					
P. annua	L. perenne	Above/below gnd. mass Ground cover Ground cover Leafspot severity Green color (spring)	16 2.5 16 17 9.5	125 74 104 93 69					
<u>P. pratensis</u>	P. annua	Shoots of sizes 4 & 5 Underground veg. mass Ground cover Leafspot severity Green color (winter) Green color (spring)	11.5 16 3 to 9.5 12.5 16 & 17 7 9.5	67 52 90 300 72 167 125					
P. pratensis	L. perenne	Shoot density Underground veg. mass Leafspot severity Green color (winter) Green color (spring)	4 16 17 7 9.5	77 61 79 178 119					
L. perenne	P. annua	Specific leaf-area Rust severity Leafspot severity Leafspot severity	16 2.5 10.5 & 12.5 17	135 70 50 85					
L. perenne	<u>P. pratensis</u>	Leaves-shoot-1 Sheath-axis width Rel. shoot-size Shoots of size 1 Rust severity Rust severity Leafspot severity Pythium blight severity	11.5 11.5 11.5 2.5 5.5 11 & 12.5 2.5	105 109 50 60 120 142 280					

Table 5.2. Allelopathy effects occurring in the field experiment.

"The measured value is expressed as a percent of the value of the check treatment. The "check" was a treatment in which the donor and receptor species were the same. All differences appearing in this table are significant (DMRT, 0.05).

suppresses the growth of <u>Pythium</u> spp., and seven other fungi. The ability of a plant to influence the disease severity of neighboring species might be considered a form of allelopathy and a potentially important adaptation for survival.

Experiments dating back to 1911 have shown that allelopathy can often be demonstrated in pure-sand culture, yet the effects usually disappear when soil colloids are introduced (15). Many secondary metabolites, including coumarin, are ionic and are readily adsorbed to clay and organic matter (2). Significant results occurred in this study (Table 5.2), however, even though a considerable cation-exchange capacity was present in the upper 5 to 7 cm of the rootzone.

The movement of chemicals through the field experiment was simulated with Rhodamine B dye (Fig. 5.2). Twenty-four hours after introducing the dye into the feed pipe, 2.5-cm of sprinkler irrigation was applied to the entire area. A fraction of the dye moved rapidly through the system to the outlet. This surge of dye may have resulted from some surface run-off of the irrigation water. A second, slower surge of dye, resulting from sub-surface flow, moved through the system at a rate of approximately 1.2 m week-1. The dye moved approximately 5 m down the channel per m³ of water input into the uppermost chamber. Negligible transfer of dye occurred between adjacent channels.

The movement of dye through the system is analogous to



Fig. 5.2. Movement of Rhodamine B dye down one of the channels in the field, allelopathy experiment.

the movement of a plant toxin with similar chemical characteristics. Thus, the capability existed in the field experiment for the transfer of chemicals from a donor to a receptor, within the time frame involved in this study.

Laboratory Experiment

<u>Coincident germination</u>. The germination rate of <u>P</u>. <u>pratensis</u> was lower when in the presence of <u>P</u>. <u>annua</u> seedlings (Table 5.3). Germination of <u>P</u>. <u>annua</u> and <u>L</u>. <u>perenne</u> was unimpeded by the presence of other species. Similar findings occurred in a field trial, wherein binary mixtures of <u>P</u>. <u>annua</u>, <u>P</u>. <u>pratensis</u>, and <u>L</u>. <u>perenne</u> were sown (Chapter I). Apparently, germinating seeds of <u>P</u>. <u>annua</u> excrete a chemical which selectively affects the germination of <u>P</u>. <u>pratensis</u>.

The shoot and root growth of all three species reacted to the coincident germination of other species. Increases, as well as decreases, in length were found.

<u>Prior germination</u>. The germination of <u>P</u>. <u>pratensis</u> was once again affected by the germination of <u>P</u>. <u>annua</u> (Table 5.4). In this case, the chemical from <u>P</u>. <u>annua</u> evidently remained in the blotter paper until it was later absorbed by <u>P</u>. <u>pratensis</u>. The effect occurred, however, only in the light treatment.

The germination rates in the light and dark treatments were equal. Some references in the literature have

Table 5.3. Germination of <u>P</u>. <u>annua</u>, <u>P</u>. <u>pratensis</u>, and <u>L</u>. <u>perenne</u> in the laboratory as affected by the coincident germination of another species.

Treatment							Sp	ecies	und	er e	valu	ati	on					
	P	A.	P	P	L	P		PA	P	P	L	P	PI	1	P	P	L	5
	%	ge	rmi	nat	tion			shoot	le	ngth	mn	-		root	le	ngth	-	
PA	92	aŦ	68	с	95	a	10	cde	10	de	20	a	19	d	8	g	25	ь
PP	93	a	83	b	93	a	8	ef	12	c	21	a	15	ef	16	e	22	с
LP	95	a	82	b	93	a	7	f	9	ef	16	b	14	ef	5	h	28	a

[†]PA = <u>P. annua</u>, PP = <u>P. pratensis</u>, LP = <u>L. perenne</u>. The "species under evaluation" was germinated approximately 1 week after the "treatment" species. The check treatment was where the "treatment" and the "species under evaluation" were the same species.

#Means followed by the same letter are not significantly different (DMRT, 0.05). Comparisons among species are permissible. . .

Treatmen							Sp	ectes	un	der e	valu	ation							
Illumination	Species	F	A		PP		LP	1000	PA		PP		LP		PA		PP		LP
			*	germ	inati	on -		shoot length mm					root length				mm		
Light	PA PP LP Check	95 89 96 94	a [‡] a-d a a	65 81 74 83	f b-e def b-e	89 74 95 93	a-d def a ab	5 6 7 10	hi ghi fgh fg	8 15 11 12	fgh de efg ef	5 21 20 16	hi abc bcd de	12 9 13 19	hi ij hi def	9 19 16 16	ij d-g e-h fgh	15 20 21 28	ghi c-f cde b
Dark	PA PP LP Check	87 93 94 96	a-d ab ab a	84 78 81 83	a-e cde b-e b-e	94 86 91 95	a a-d abc a	0 12 6 16	i ef ghi de	9 9 6 23	fgh fgh ghi abc	25 25 19 22	a ab cd abc	10 10 13 15	1j 1j hi gh	17 15 24 8	d-h fgh c j	24 21 24 36	c cd c a

Table 5.4. Germination of <u>P. annua</u>, <u>P. pratensis</u>, and <u>L. perenne</u> in the laboratory as affected by the residue remaining on blotter paper from the prior germination of seeds.

 $^{\dagger}PA = \underline{P}$. annua, $PP = \underline{P}$. pratensis, $LP = \underline{L}$. perenne. The "species under evaluation" was germinated on blotter paper on which ca. 4.5 g of seed of the "treatment" species had been germinated and removed. Seeds in the "check" treatment were germinated on an untreated blotter. The "light" treatment received 8 hours of illumination per day; the "dark" treatment received none.

[‡]Means followed by the same letter are not significantly different (DMRT, 0.05). Comparisons among species are permissible.

suggested that the germination rate of turfgrass species is generally poorer in darkness than light (3). However, the latter was not supported by the results of this study (Table 5.4). Shoot length was generally greater in the dark treatment than in the light. The response of root length was mixed.

In the dark treatment, the growth of <u>P</u>. <u>annua</u> shoots was completely inhibited by the prior growth of <u>P</u>. <u>annua</u> seedlings. Self-inhibition was not as prominent in the light treatment or with the other species. Coumarin has been shown to have a light-sensitive effect on the growth of some seedlings (11).

<u>Turf extracts.</u> <u>P. pratensis germination rate was not</u> affected by the extracts of <u>P. annua</u> clippings, composted clippings, or underground vegetative matter (Table 5.5). Therefore, inhibition of <u>P. pratensis</u> germination by <u>P</u>. <u>annua</u> probably occurs only when <u>P. annua</u> is in the seedling stage (see Tables 5.3, 5.4). Germination of <u>P. annua</u> and <u>L</u>. <u>perenne</u> was unaffected by the extracts. The light and dark treatments had similar effects on growth, so only the results of the light treatment are presented (Table 5.5).

The germination rate of <u>P</u>. <u>pratensis</u> was reduced by the presence of soil particles in the water. <u>P</u>. <u>trivialis</u>, in a study by Chippindale (5), responded to the presence of soil in the same manner. The germination rate of <u>P</u>. <u>trivialis</u> in water was 78%, and in water plus soil, 25%. In another study, the emergence of <u>P</u>. <u>pratensis</u> in the field was 30%

Treatment [†]		Species under evaluation										
Extract	Spectes	PP	PA	PP	LP I	PA	PP	LP				
		germin.	shoot	length mm		root	root length mm					
Fresh clippings	PA	83 a‡	11 f	16 d	20 bc 1	7 gh	11 k1	30 cd				
	PP	55 e	11 f	17 cd	23 b 10	6 ght	13 1-1	33 b				
	LP	73 cd	10 f	16 d	24 b 10	6 h1	11 k1	33 b				
Composted clippings	PA	82 ab	22 b	15 de	35 a 1	5 hij	18 gh	23 e				
	PP	69 cd	17 cd	13 ef	21 b 1	5 9-1	16 hi	22 ef				
	LP	74 bc	21 b	12 ef	32 a 1	5 h1j	18 gh	24 e				
Check		83 a	10 f	12 ef	16 d 19	9 fg	16 h1	28 d				
Fresh clippings & soil	PA	76 bc	7 g	14 de	20 bc 1	3 1-1	12 .1k1	30 cd				
1000 C.C.	PP	67 d	10 F	15 de	24 b 14	4 h-k	11 kl	33 bc				
	LP	76 bc	7 g	13 ef	24 b 14	4 h-k	10 1	37 a				
Thatch, roots, etc.	PA	76 bc	11 f	14 de	21 6 10	6 h1	12 1k1	32 bc				
1. 1949 TABLES & LEWIS TO THE CONTRACTOR	PP	73 cd	12 ef	12 ef	22 b 1	7 oh	10 1	29 cd				
	LP	79 ab	8 g ·	15 de	23 b 14	4 h-k	11 k1	35 ab				
Check & soil		70 cd	12 ef	19 bc	23 b 23	2 ef	13 1-1	32 bc				

Table 5.5. Germination of <u>P</u>. annua, <u>P</u>. pratensis, and <u>L</u>. perenne in the laboratory as affected by aqueous extracts of fresh clippings, composted clippings, and thatch.

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tPA = P. annua, PP = P. pratensis, LP = L. perenne. The "species under evaluation" was subjected to an aqueous extract of the "treatment species." Seeds in the "check" treatment were germinated using distilled water.

Heans followed by the same letter are not significantly different (DMRT, 0.05). Comparisons among species are permissible. less than the germination rate under soil-free laboratory conditions (Chapter I). Perhaps the low field survival rates of certain <u>Poa</u> spp. might be attributable, in part, to the mere presence of soil.

Fresh P. annua and L. perenne clipping extracts inhibited the germination of P. pratensis, however, the effects were negated by the addition of soil (Table 5.5). P. annua shoot growth, on the other hand, was inhibited by fresh P. annua clipping extracts only when soil was present. These findings indicate that soil is an important factor in determining the effect of allelopathy.

In several instances, composted clippings were less detrimental to shoot and root growth than fresh clippings. Underground vegetative matter had about the same effect as foliar matter on the growth of seedling shoots and roots.

<u>Clipping leachates</u>. Only one detrimental effect occurred as a result of aqueous leachates of clippings. The germination rate of <u>P</u>. <u>pratensis</u> was 7Z lower and the root length was 19Z shorter when grown in the presence of <u>L</u>. <u>perenne</u> leachates. Hence, rainwashing of <u>L</u>. <u>perenne</u> clippings in the field may release chemicals which can potentially affect the germination of <u>P</u>. <u>pratensis</u> seeds.

<u>Coumarin</u> as a standard inhibitor. The relative insensitivity of <u>P</u>. annua and <u>L</u>. perenne germination to unknown inhibitors in the previous trials was reflected in their relative insensitivity to a known inhibitor, coumarin. <u>P. annua</u> and <u>L. perenne</u> required 100 ppm of coumarin to effect a significant decrease in germination rate, whereas <u>P. pratensis</u> needed only 0.1 ppm. The root and shoot growth of all three species was significantly decreased by a 0.1 ppm concentration. A coumarin concentration of 100 ppm limited all shoot and root elongation to 1 mm.

Implications of This Research

This study has shown that field experimentation can be successfully employed in the study of allelopathy. In fact, certain parameters, such as disease severity, can be readily examined in the field, whereas they may be totally overlooked in greenhouse studies. We, therefore, recommend the use of field experiments, whenever possible, in the study of allelopathy.

Allelopathy seemed to be an important contributing factor in the interaction of <u>P</u>. <u>annua</u>, <u>P</u>. <u>pratensis</u>, and <u>L</u>. <u>perenne</u>. The effects of allelopathy, which may be beneficial or detrimental to the receptor, began at seed germination and continued throughout the growth of the mature stand. Some of the effects of allelopathy in this study were quite large in magnitude, such as a 3x increase in disease, or a total inhibition of shoot growth. On the other hand, many effects were relative subtle, especially when compared to the impact of competition (Chapter IV). It is possible, though, for even a subtle form of allelopathy to act cumulatively over time and to be quite important in the ecology of a mixed stand (10).

This study represents the first step in the overall investigation of allelopathy in cool-season turfgrasses. A second, future step should entail the search for possible toxins. The logical starting place for such a study might be the isolation of the toxin produced by germinating \underline{P} . annua seeds.

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