SOME PHYSIOLOGICAL ASPECTS OF SELECTIVE ORCHARDGRASS CONTROL IN KENTUCKY BLUEGRASS WITH BROMACIL

by

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Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Plant Pathology and Physiology

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May, 1972

Blacksburg, Virginia

ACKNOWLEDGMENTS

The author expresses sincere appreciation to his major professor, Dr. S. W. Bingham, Department of Plant Pathology and Physiology, for assistance in the research and writing of this investigation. Thanks go to Drs. C. L. Foy, L. D. Moore, R. E. Schmidt, and D. D. Wolf for serving on the author's graduate committee.

The author thanks the Department of Plant Pathology and Physiology for financial support and the opportunity to conduct this investigation and the Department of Agronomy for the cooperation in conducting various phases of this investigation.

Thanks go to the Forage Testing Laboratory personnel for conducting the Kjeldahl nitrogen determinations.

Thanks go to Mr. John Petty and technicians at the Plant Pathology and Physiology Research Station at Chatham, Virginia, for their assistance with the field research program.

The author wishes to thank E. I. duPont deNemours and Company, Wilmington, Delaware, for radiolabeled bromacil and unlabeled metabolites used in this study.

Thanks go to Mr. Harold Witt for drawing the figures. Appreciation is expressed to Mrs. Sherry Williams for the typing of this manuscript.

The author extends deepest appreciation to his wife, Ginny, for her support, financially and morally, and to her and our daughter, Debby Jane, for understanding, most of the time, when he had to work late and concentrate hard on his studies, research and dissertation.

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I. INTRODUCTION

Programs of herbicide use have led to control of many broadleaf and annual grass weeds in turf. Methods to remove undesirable perennial grasses such as orchardgrass (*Dactylis glomerata* L.) without damage to the desirable grasses have not been found.

In preliminary studies bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) has shown marginal selectivity for the control of annual bluegrass (*Poa annua* L.) and orchardgrass in Kentucky bluegrass (*Poa pratensis* L.) turf.

Bromacil was first introduced in 1962 for nonselective weed control of a wide range of grass and broadleaf weeds. Inhibition of photosynthesis has been found to be the major mode of action of this herbicide.

The present studies were initiated to evaluate:

1. the use of bromacil for the selective control of orchardgrass in Kentucky bluegrass turf;
2. the effect of bromacil on physiological processes in the two grasses; and
3. the basis of herbicidal selectivity in the two grasses.

II. SELECTIVITY OF BROMACIL ON KENTUCKY BLUEGRASS AND ORCHARDGRASS

Abstract

In field studies, photosynthesis was reduced by 5-bromo-3-sec butyl-6-methyluracil (bromacil) to a greater extent in orchardgrass (Dactylis glomerata L.) than in Kentucky bluegrass (Poa pratensis L.). Foliage carbohydrates were depressed initially in bluegrass followed by recovery in 2 weeks to 1 1/2 months after bromacil application. Orchardgrass, however, did not exhibit the same degree of recovery. Application of 0.56 kg/ha bromacil each summer and fall for 2 years gave the best control of orchardgrass. During the second year, increased foliage crude protein was observed in treated bluegrass. Bluegrass foliage yields were equal to or greater than the controls. Orchardgrass yields were drastically reduced by bromacil. Effective seedling orchardgrass control was obtained with minimal bluegrass injury at 0.14 to 0.28 kg/ha of bromacil. Bromacil treated areas were effectively fall-seeded with bluegrass approximately 8 weeks after a 0.56 kg/ha application. Rooting of bluegrass sod was not inhibited at the rates used for selective control of orchardgrass in bluegrass turf.

Additional index words: Photosynthesis, carbohydrates, protein, herbicide, turfgrass.

Introduction

The selective control of undesirable perennial grass in Kentucky bluegrass (*Poa pratensis* L.) turf is an unsolved problem. Currently, orchardgrass (*Dactylis glomerata* L.) control requires either hand pulling or nonselective herbicide spot treatments which are followed by reseeding or resodding. Fults (7) reported that cacodylic acid (hydroxydimethylarsin oxide) and potassium cyanate together at 3.4 kg/ha and 34 kg/ha, respectively, gave selective control of orchardgrass in Kentucky bluegrass turf.

In 1962 the substituted uracils were introduced for nonselective weed control (5). Their effectiveness on a wide range of both annual and perennial weeds made the compounds particularly attractive as industrial herbicides, where complete vegetation control is desirable. Bingeman, et al (4) introduced the compound 5-bromo-3-*sec*-butyl-6-methyluracil, bromacil, for use as an industrial herbicide.

Gibeault (8) reported that when bromacil was applied to a prepared seedbed at 0.9 kg/ha and a mixture of Chewings' fescue (*Festuca rubra* L.) and browntop bentgrass (*Agrostis tenuis* Sibth) was sown about 3 weeks later, a turf relatively free of annual bluegrass (*Poa annua* L.) resulted. Neidlinger, Furtick, and Goetze (14) found that terbacil (3-*tert*-butyl-5-chloro-6-methyluracil) and bromacil at 0.2 and 0.4 kg/ha, respectively, gave selective control of annual bluegrass in both Merion and Newport Kentucky bluegrass when applied preemergence to the three grasses. Peters and O'Leary (15) found that terbacil and bromacil were unsuitable for weed control in mixed stands of alfalfa (*Medicago sativa* L.) and orchardgrass pastures because of orchardgrass injury at 0.6 to 1.1 kg/ha.

The major mode of action of the uracils is the selective inhibition of photosynthesis with no effect on CO_2-fixation in the dark (6, 10). Van Oorschot (20) found that 5-bromo-6-methyl-3-phenyluracil and 3-cyclohexyl-5,6-trimethyleneuracil gave a more pronounced inhibition of CO_2 uptake than transpiration in intact plants.

The studies reported here were conducted to determine the effectiveness of bromacil for the selective removal of orchardgrass from Kentucky bluegrass turf and to evaluate the physiological responses of bromacil on the grasses under field conditions.

Materials and Methods

Effect on Established Turf

Plot establishment. In the fall of 1969, studies were initiated on a Fredrick silt loam soil which had previously been fumigated with methyl bromide. Six plugs (10-cm diameter and 10-cm depth) of Merion Kentucky bluegrass and Virginia Common orchardgrass were transplanted from the Virginia Polytechnic Institute and State University golf course and agronomy farm, respectively, on 51-cm spacings in a 1 m x 1.5 m subplot within treatments. The plugs became established by the summer of 1970 when treatments were first applied. Supplemental irrigation was used to maintain optimum moisture conditions. A split plot design was employed with four replications of each bromacil treatment as main plots. Bromacil was applied with a two-wheeled, compressed-air plot sprayer. Two fan-type, Teejet nozzles were used with an angle and orifice size of 8003 at 51-cm spacings. Bromacil was applied at 428 l/ha spray volume by making two passes over the plots in opposite directions. The bromacil application dates and rates for the 2-year study are presented in Table II-1. Treatment rates were evaluated and adjusted throughout the course of the experiment to obtain optimum selective control of orchardgrass.

Visual evaluation and growth responses. Throughout the course of the experiment the two grass species were evaluated visually, and rated according to herbicide injury symptoms, color, and grass vigor. Yield was obtained during 1970 by weighing leaf clippings from the plugs cut at a 6-cm height. During 1971 yield samples were obtained using a reel mower at the same cutting height. The samples were dried at 80 C for 2 days before being weighed. The number of living plugs per plot and the vigor of the plugs were recorded during April of 1971. The data were used to evaluate the effectiveness of bromacil applications the previous year on the regrowth at the start of the second season.

Physiological responses. The influence of bromacil on photosynthesis of the two species was measured in the field at intervals for 1 month following the initial summer treatments. Using an "open" system, differential gas analysis was used to measure the change in the CO_2 content of air samples obtained from plexiglas chambers (30 cm height x 11.5 cm diameter) placed over the 10 cm diameter plugs of sod.

Table II-1. Bromacil rates and application dates for the 2-year study on Kentucky bluegrass and orchardgrass.

Bromacil treatment number	1970 Summer (July 29)	1970 Fall (Sept 17)	1971 Summer (June 10)	1971 Fall (Sept 29)
	(kg/ha)	(kg/ha)	(kg/ha)	(kg/ha)
1	0.00	0.00	0.00	0.00
2	0.14	0.28	0.56	0.56
3	0.28	0.42	0.56	0.56
4	0.56	0.56	0.56	0.56
5	----	----	1.12	1.12

Light intensities were maintained at 86 to 108 klux with a 1,000 watt "Sylvania Buhor" metal arc lamp. Temperatures in the chambers ranged from 26 to 29 C. The foliage was harvested and oven-dried at 80 C for 2 days and weighed.

The foliar tissue of 1970 photosynthesis samples and 1971 yield samples was ground to pass through a 40-mesh screen and 100 Or 200 mg samples were extracted for water soluble carbohydrates using 30 ml of deionized water in a 100 C water bath for 1 hr. After filtration and dilution, the anthrone sulfuric acid colorimetric method of Morris (12) was used for detecting carbohydrates expressed as percent of oven-dry weight using glucose standards. The Virginia Polytechnic Institute and State University Forage Testing Laboratory analyzed the ground plant samples for nitrogen (protein was calculated as 6.25 x N) by Kjeldahl digestion using the modified Gunning method (3).

Effect on Seedling Grasses

Preemergence and postemergence studies. A split plot experimental design was employed in the summer of 1971 with four replications of each bromacil treatment as main plots. Merion Kentucky bluegrass and Virginia Common orchardgrass were seeded separately (1.2 m by 1.2 m plots) at 5 and 10 g per m^2, respectively. Bromacil was applied preemergence (7 days after seeding) and postemergence (17 days after seeding) when the bluegrass was approximately 1 cm tall and the orchardgrass was approximately 3 cm tall. Application rates ranged from 0.07 to 0.56 kg/ha. The grasses were irrigated frequently and clipped at 4 cm every 2 weeks beginning 1 month after seeding. Leaf clipping yield was measured 3 1/2 months after the treatments were applied and the grasses were well established. A swath (0.5 m by 1.2 m) was cut with a reel mower adjusted to a 4 cm cutting height. Yield samples were dried and weighed as before. Injury evaluation was taken based on turf color, vigor and ground cover.

Reseeding studies. The effect of bromacil residue remaining in the soil on Nerion Kentucky bluegrass establishment from seed was evaluated. The experimental design was a randomized complete block with plot size of 0.6 m by 2.4 m replicated three times. Bromacil was applied at 0.14, 0.28, 0.56, and 1.12 kg/ha at 0, 2, 4, and 8 weeks prior to seeding in August, 1971. Estimates of percent ground cover were made 7 weeks after seeding.

Effect on Rooting

In the fall of 1971 sections of Merion Kentucky bluegrass sod, 9.3 dm^2 and 2.5 cm thick, were placed into wooden frames with 0.6 cm hardware cloth bottoms. The frame was placed in direct contact with the soil surface that had just been treated with bromacil rates ranging from 0.14 to 0.56 kg/ha. The treatments were replicated three times. Before the rooting force was measured using a modified vertical force technique of King and Beard (11), the sod was allowed to root into the soil through the hardware cloth for 1 month. Clipping yields, weight of soil held by roots, and dry weight or roots in soil mass were also obtained.

The residual effect of repeated bromacil treatments on rooting of bluegrass sod was evaluated in November, 1971. Two 10-cm diameter and 2.5 cm thick bluegrass sod plugs were removed from selected treatments (Table II-1, treatments 1, 4, and 5). The total amount applied was 2.24 kg/ha of bromacil. The plugs were placed in wooden frames with 10-cm diameter holes covered on the lower side by 0.6 cm mesh hardware cloth. The plugs were then placed on a greenhouse bench filled with 20 cm of soil and covered with 0.6-cm mesh hardware cloth. The sod was allowed 2 months to develop roots through the hardware cloth. The extent of root growth of the various plugs was measured by the kg weight required to pull the plugs from the soil in the bench.

Data were analyzed by analysis of variance and Duncan's multiple range comparisons.

Results and Discussion

Effects on Established Turf

Visual evaluation and growth response. In the summer, 1970, after the initial application of bromacil, there was appreciable injury to orchardgrass at the 0.56 kg/ha rate (Table II-2). The 0.14 kg/ha of bromacil was ineffective in controlling orchardgrass. Bluegrass exhibited initial leaf tip dieback but recovered within 1 month. Yield of bluegrass was not affected while yield of orchardgrass was reduced 62% by 0.56 kg/ha of bromacil. Due to the low levels of injury observed on bluegrass, the rates were increased for the fall application. Following the 1970 fall repeat treatment with 0.56 kg/ha

Table II-2. Clipping yield and injury of Kentucky bluegrass and orchardgrass after the 1970 summer and fall applications of bromacil.

Bromacil treatment number	Rate	Yield[a] Blue-grass	Yield[a] Orchard-grass	Injury ratings[b] Blue-grass	Injury ratings[b] Orchard-grass
	(kg/ha)	(g/plot)	(g/plot)		
		Summer			
1	0.00	29 a[c]	73 a	0.0 a	0.0 c
2	0.14	26 a	62 a	1.0 a	0.8 c
3	0.28	26 a	61 a	0.8 a	3.5 b
4	0.56	24 a	28 b	1.4 a	8.5 a
		Fall[d]			
1	0.00	24 a	42 a	0.0 b	0.0 c
2	0.28	23 a	21 b	1.0 a	5.5 b
3	0.42	19 a	9 c	1.2 a	8.2 a
4	0.56	20 a	4 c	1.2 a	9.2 a

[a] g foliage dry wt/plot with plot area equals 1.6 m^2, and clipping height = 6 cm. Accumulative yield for 4 harvests during the first month following summer application and 1 harvest at 1 month following fall application.

[b] 0 = no injury; 10 = death. Based on grass vigor, color and injury symptoms 1 month following application.

[c] Data followed by a common letter in each column within seasons are not significantly different at the 5% level.

[d] Fall retreatments were applied to the same plots which had received summer applications.

bromacil, selective control of orchardgrass was obtained with minor injury to bluegrass (Fig. II-1). Bluegrass foliage yields were unaffected whereas orchardgrass foliage yields were reduced 90%. The tolerance of bluegrass to bromacil in these initial observations is similar to that reported by Neidlinger et al. (14) in Merion and Newport Kentucky bluegrasses where Merion was the most tolerant cultivar.

A few plugs of orchardgrass survived the first year of high bromacil treatment and were less vigorous than bluegrass (Table II-3). This was due in part to the depletion of the carbohydrate reserves in orchardgrass which resulted in less vigorous regrowth in the spring. Similar results were reported by Swann and Buchholtz (17) where depletion of carbohydrate reserves by atrazine contributed to the elimination of quackgrass. Hasting and Kust (9) found that though herbicide applications in early autumn reduced root carbohydrates, weeds could survive the winter but did not compete effectively the following spring.

Since bromacil at 0.56 kg/ha gave effective orchardgrass control with minimal bluegrass injury during 1970, the retreatment of plots during 1971 was made with the 0.56 kg/ha rate which was used as a maintenance treatment. No significant injury was observed on bluegrass with 0.56 or the 1.12 kg/ha rate when evaluated 2 1/2 weeks to 1 1/2 months following application of bromacil (Table II-4). Orchardgrass was severely injured by bromacil treatments. The 0.56 kg/ha treatment of bromacil repeated for the third time (Treatment 4) gave complete control of orchardgrass with minimal injury to bluegrass.

Figure II-1. Effect of bromacil on established bluegrass (foreground) and established orchardgrass (background). 1 = control. 4 = 0.56 kg/ha summer, 1970, plus 0.56 kg/ha fall, 1970, applications. The reduced growth of 6 orchardgrass plugs was observed in late fall, 1970, with treatment 4.

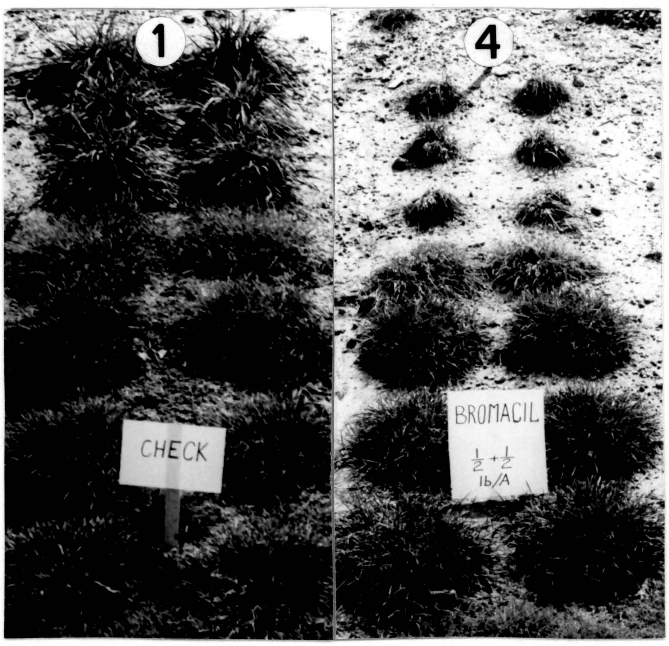
1
4
CHECK
BROMACIL
1/2 + 1/2
lb/A

Table II-3. Effect of bromacil applied during 1970 on number and vigor of Kentucky bluegrass and orchardgrass plugs on April 1, 1971.

Bromacil treatment number	Rates[a]	Blacksburg Live plugs[b]	Blacksburg Injury ratings[c]
	(kg/ha)		
	Bluegrass		
1	0.00	6.0 a[d]	0.5 a
2	0.42	6.0 a	0.3 a
3	0.70	6.0 a	0.5 a
4	1.12	6.0 a	0.5 a
	Orchardgrass		
1	0.0	5.8 a	0.8 c
2	0.42	5.0 b	6.5 b
3	0.70	2.8 c	9.1 a
4	1.12	0.5 d	9.8 a

[a] Accumulative rate of bromacil for summer and fall 1970.

[b] Number of live plugs of the 6 transplanted of each species.

[c] 0 = no injury; 10 = death. Based on grass vigor, color and injury symptoms.

[d] Means in the same column within grasses followed by a common letter are not significantly different at the 5% level.

By the summer of 1971 bluegrass plugs had spread by rhizome growth to cover uniformly the entire plot area whereas orchardgrass plugs retained a bunch growth habit and produced many tillers. Yield of bluegrass was initially reduced by 1.12 kg/ha of bromacil (Table II-4). By the mid-July harvest (1 1/2 months following treatment) all bromacil treatments were significantly out-yielding the control plots. Control plots were not as vigorous in July as indicated by visual injury ratings. This was due partially to natural infestation of crabgrass [Digitaria sanguinalis (L.) Scop.] which invaded control plots and competed with bluegrass for water and nutrients. Crabgrass was not observed in treated plots. Crabgrass was removed from control plots by hand prior to yield sampling. Yield of orchardgrass, however, was reduced 67% or more by 0.56 kg/ha bromacil treatments. Yield and injury trends following the fall 1971 treatment of bromacil were similar to those for the summer application.

Physiological responses. For 1 month following the summer treatment, 1970, bromacil affected photosynthesis in both grasses, but the effect was more pronounced in orchardgrass than in bluegrass. Photosynthesis was reduced 38% in bluegrass at 0.56 kg/ha compared to a 67% reduction in orchardgrass (Table II-5). Photosynthetic CO_2-fixation was reduced by a lower herbicide rate in orchardgrass than in Kentucky bluegrass. Analysis of foliar carbohydrates showed little effect on bluegrass at 0.56 kg/ha rate compared to a 57% reduction in orchardgrass. The low overall reduction of photosynthesis in bluegrass tends to suggest a tolerance mechanism. Photosynthesis as measured by

Table II-4. Effect of bromacil on Kentucky bluegrass and orchardgrass following summer application, June 10, 1971.

Bromacil treatment number	Rate[c]	Injury ratings[a] June 28	Injury ratings[a] July 7	Injury ratings[a] July 22	Yield[b] June 28	Yield[b] July 7	Yield[b] July 22
	(kg/ha)				(g/ plot)	(g/ plot)	(g/ plot)
		Bluegrass					
1	0.00	0.0 a[d]	1.2 a	1.8 b	30 a	17 a	17 b
2	0.56	0.2 a	0.5 a	0.2 b	29 ab	19 a	23 a
3	0.56	0.2 a	0.8 a	0.2 b	30 a	21 a	21 a
4	0.56	0.8 a	0.5 a	0.0 b	33 a	22 a	24 a
5	1.12	0.8 a	1.2 a	0.0 b	25 b	19 a	23 a
		Orchardgrass					
1	0.00	0.0 d	0.0 c	0.0 c	14 a	7 a	15 a
2	0.56	7.8 c	7.8 b	6.5 b	3 b	1 b	5 b
3	0.56	9.5 a	9.8 a	9.2 a	1 b	1 b	1 c
4	0.56	10.0 a	10.0 a	10.0 a	0 b	0 b	0 c
5	1.12	8.8 b	9.6 a	9.7 a	1 b	1 b	1 b

[a]0 = no injury; 10 = death. Based on grass color, vigor and injury symptoms.

[b]Plot area = 1.6 m^2; clipping height = 6 cm; g dry weight yield.

[c]Rate of bromacil application during summer 1971. These were retreatments to the same plots receiving 1970 applications except treatment 5 which received the first application.

[d]Means in the same column within grasses followed by a common letter are not significantly different at the 5% level.

Table II-4. Effect of bromacil on Kentucky bluegrass and orchardgrass following summer application, June 10, 1971. (Cont'd.)

Bromacil treatment number	Rate[c]	Content in leaves[e] Carbohydrates June 28	Carbohydrates July 7	Carbohydrates July 22	Crude protein June 28	Crude protein July 7	Crude protein July 22
	(kg/ha)	(%)	(%)	(%)	(%)	(%)	(%)
		Bluegrass					
1	0.00	11.7 ab[f]	11.1 a	12.2 abc	17.7 c	17.7 c	17.2 c
2	0.56	12.4 a	11.6 a	12.1 bc	19.9 b	19.9 b	18.5 b
3	0.56	11.1 ab	11.4 a	11.7 c	20.3 b	19.6 b	18.9 b
4	0.56	12.2 a	11.5 a	13.2 ab	21.7 b	20.0 b	19.6 b
5	1.12	10.4 b	11.5 a	13.5 a	22.4 a	21.5 a	20.2 a
		Orchardgrass					
1	0.00	10.6	8.4	8.6	18.7	20.7	18.4
2	0.56	5.3	7.3	8.1	24.8	26.5	24.8
3	0.56	4.7	7.1	10.3	29.9	25.1	26.9
4	0.56	---	---	----	----	----	----
5	1.12	5.9	5.7	7.5	30.2	23.2	29.4

[c]Rate of bromacil application during summer 1971. These were retreatments to the same plots receiving 1970 applications except treatment 5 which received the first application.

[e]Based on dry weight of foliage.

[f]Means in the same column followed by a common letter are not significantly different at the 5% level. Due to missing plots, orchardgrass carbohydrate and protein data were not analyzed statistically.

Table II-5. Effect of bromacil on photosynthesis, carbohydrates, and protein content of Kentucky bluegrass and orchardgrass foliage during 1 month after application in the summer 1970.[a]

Bromacil treatment number	Rate	Photosynthesis	Content in leaves[b] Carbohydrates	Protein
	(kg/ha)	(mg CO_2/hr/g leaf dry wt)	(%)	(%)
		Bluegrass		
1	0	2.6 a[c]	9.1 a	20.2 a
2	0.14	2.7 a	7.8 a	21.2 a
3	0.28	2.3 a	8.7 a	20.8 a
4	0.56	1.6 b	7.8 a	21.4 a
		Orchardgrass		
1	0	1.2 a	6.1 a	13.6 a
2	0.14	1.0 ab	4.0 b	13.8 a
3	0.28	0.8 bc	3.4 b	14.6 a
4	0.56	0.4 c	2.6 b	14.7 a

[a]Data average of 4 sampling dates: 3, 7, 14, and 28 days after application.

[b]Based on dry weight of foliage.

[c]Means in the same column within grasses followed by a common letter are not significantly different at the 5% level.

CO_2 uptake was greater in Kentucky bluegrass than orchardgrass and this was also reflected in the higher water soluble carbohydrates in bluegrass foliage.

Bluegrass foliar carbohydrate levels from bromacil treatments were equivalent to the control at all harvest dates 1 month after the summer application in 1971 (Table II-4). If foliar carbohydrate level was initially depressed, it recovered by the first June harvest 18 days after treatment. Orchardgrass foliar carbohydrate levels were still depressed 18 days after treatment, but by 1 1/2 months the carbohydrate levels had also returned to control levels.

Eleven days following the fall, 1971 bromacil application, the bluegrass foliar carbohydrate levels were reduced 27 and 36% for the 0.56 and 1.1 kg/ha application, whereas orchardgrass foliar carbohydrate levels were reduced 75 and 82% for these treatments, respectively. The recovery of the carbohydrate level at a faster rate in bluegrass than in orchardgrass demonstrates that bluegrass probably has a greater physiological tolerance to the herbicide. Van Oorschot (20) suggested that if species recovered photosynthetic capability following initial depression, that the resistance was due to a greater capacity to inactivate the herbicide.

Throughout the experiment it was observed that foliar carbohydrate level of bluegrass was always 2 to 3% higher than that of orchardgrass. If a plant has a high carbohydrate reserve, the maintenance of a toxic concentration of bromacil will reduce the carbohydrate reserve, but with physiological inactivation of the herbicide, the plant will utilize the remaining reserve to recover. If the carbohydrate level is low, as in orchardgrass, the plants would have difficulty recovering. These results are supported by the work of Thompson, Slife and Butler (18) where the environment influenced the tolerance of corn to atrazine by affecting plant carbohydrate level and the detoxication mechanism.

The foliar protein content was similar to the control for both grasses following the initial bromacil application in 1970 (Table II-5). The crude protein content of bluegrass increased 2 to 6% following the bromacil treatments during the summer of 1971, indicating increased nitrogen uptake and fixation (Table II-4). The results were similar to those reported by Allison and Peters (1) where simazine at sub-herbicidal rates, 0.07 to 0.56 kg/ha, resulted in increased crude protein of reed canarygrass (<u>Phalaris arundinacea</u> L.). Estimated yield of crude protein per hectare was found to be increased with simazine application in some instances. The increased crude protein content in bluegrass with equal or increased foliar yields per plot indicate that bromacil can also be used at low rates to increase crude protein in forage. The bluegrass after recovery from initial chlorosis responded by having a slightly darker green color with bromacil treatments when compared to the control. This would indicate increased foliar nitrogen. The protein content of orchardgrass was also increased with 1971 bromacil application, but since forage yields were reduced, the yield of crude protein per hectare is negated. The crude protein content of bluegrass following the fall, 1971, application was about 3% more than the control when measured 12 days following treatment. Orchardgrass protein tended to decrease due to catabolism of protein, since carbohydrate levels were so low.

In higher plants, 40% of the leaf nitrogen is in the chloroplast (13). Nitrogen is fixed in the chloroplast stroma, structural protein, and chlorophyll and thus exerts a positive effect on photosynthesis. However, if leaves lose photosynthetic activity but retain their respiratory activity, the lack of respiratory substrate induces protein breakdown producing amino acids, amides, and ammonium ions (13). Ammonium ions are toxic to the cell and eventually kill the leaves. The low carbohydrate content in orchardgrass and subsequent formation of toxic substances, possibly ammonium ions, may account in part for orchardgrass injury symptoms.

Effect on Seedling Grasses

Preemergence and postemergence studies. Application of 0.14 to 0.28 kg/ha of bromacil effectively controlled orchardgrass seedlings with little injury to bluegrass when applied either pre- or early postemergence to the grasses (Table II-6). These results of bluegrass tolerance are similar to those of Neidlinger et al. (14) where annual bluegrass was selectively controlled in Merion and Newport Kentucky bluegrass. During the early weeks of seedling establishment, it was observed that the bromacil treatment did not allow bluegrass seedlings to establish as rapidly as control treatments. Many of the seedlings emerged, turned chlorotic, and died. The result was a thinner stand in the treated plots, but as time progressed, the thinner stand was

Table II-6. Injury and yield of seedling bluegrass and orchardgrass approximately 3 1/2 months after summer application of bromacil.

Bromacil treatment rate	Preemergence		Postemergence	
	Injury rating[a]	Yield[b]	Injury rating	Yield
(kg/ha)		(g/plot)		(g/plot)
		Bluegrass		
0.00	1.1 bc[c]	7.0 bc	0.5 b	18.9 b
0.07	0.2 c	23.4 a	---	----
0.14	0.5 c	23.4 a	0.8 b	30.4 a
0.28	1.9 b	19.6 a	1.1 b	21.1 b
0.56	8.4 a	3.0 c	5.8 a	9.7 c
		Orchardgrass		
0.00	0.0 d	26.4 a	0.0 b	41.8 a
0.07	4.0 c	18.6 a	---	----
0.14	7.7 b	5.9 c	9.6 a	3.8 b
0.28	9.9 a	0.1 c	9.9 a	0.8 b
0.56	10.0 a	0.0 c	10.0 a	0.0 b

[a] 0 = no injury; 10 = total kill. Based on turf color, vigor and ground cover.

[b] Plot yield = clipping cutting height 4 cm (0.5 m x 1.2 m plot area), g dry wt yield.

[c] Means in the same column within grasses followed by a common letter are not significantly different at the 5% level.

advantageous because the remaining plants were more vigorous and proceeded to tiller more.

In control plots, however, the bluegrass plants remained short, tillered little and were much less vigorous, due in part to plant competition. This reduced vigor was reflected in the yields obtained 3 1/2 months after application. Bluegrass foliage clippings from the control plots yielded significantly less than the 0.14 kg/ha treatment plots.

Bromacil at low rates was beneficial to bluegrass growth. Orchardgrass reproduces from seed which is the source of each orchardgrass clump in an established lawn. Bromacil reduces the possibility that new seedlings may become established.

Reseeding studies. Bromacil at 0.56 kg/ha was the most effective rate in controlling established orchardgrass but was very injurious to seedling bluegrass. A study was initiated in late summer, 1971, to determine the waiting interval required after treating an area with bromacil until the area may be successfully seeded to bluegrass. As shown in Table II-7, it took approximately 8 weeks after the 0.56 kg/ha application before successful bluegrass seeding was accomplished. Eight weeks was not long enough for the 1.12 kg/ha of bromacil to dissipate to obtain successful bluegrass establishment. All other rates did not hinder bluegrass establishment from seed. The dissipation of bromacil may be attributed to microbial degradation and leaching in low organic soils (16, 19).

Effect on Rooting

Ashton, Cutter and Huffstutter (2) reported that bromacil was inhibitory to root elongation at concentrations ranging from 10^{-6} to 10^{-3} M. Bromacil at 0.14 to 0.56 kg/ha had minor effects on the re-rooting of bluegrass sod into treated soil under field conditions. The weight required to pull the sod vertically and the weight of roots in the soil mass were not affected by increasing bromacil concentration. Approximately 50 kg weight was required to pull rooted sod after 4 weeks. The weight of the soil mass held by the roots of sod from treated areas was less than the control, indicating slight rooting inhibition. The rooting ability of the bluegrass sod which had received an equivalent of 2.2 kg/ha bromacil in the two-year study was not affected. These results indicate that bluegrass rooting is probably not seriously affected by bromacil applications at the rates used to control orchardgrass and that treated areas may be effectively resodded.

The repeated application of bromacil was effective in reducing the growth of orchardgrass in Kentucky bluegrass turf. Tolerance of bluegrass was related to higher carbohydrate levels and the minor effect on the photosynthetic mechanism. Orchardgrass susceptibility was related to low carbohydrate levels and greater photosynthesis inhibition. Treated areas were effectively resodded immediately following treatment or seeded 8 weeks following application of bromacil at rates which gave selective control in field studies.

Table II-7. Residual effect of bromacil on establishment of bluegrass from seed in the fall of 1971. Percent ground cover was evaluated 7 weeks after seeding.

Bromacil rate	Bluegrass ground cover — Weeks bromacil applied before seeding: 0	2	4	8
(kg/ha)	(%)	(%)	(%)	(%)
0.00	100 a[a]	100 a	100 a	100 a
0.14	100 a	100 a	100 a	100 a
0.28	98 a	100 a	100 a	100 a
0.56	62 bcd	73 abc	87 ab	98 a
1.12	38 d	35 d	38 d	50 cd

[a]Data followed by a common letter are not significantly different at the 5% level.

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III. PHYSIOLOGICAL EFFECTS OF BROMACIL ON KENTUCKY BLUEGRASS AND ORCHARDGRASS

Abstract

Bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) did not affect germination of Kentucky bluegrass (*Poa pratense* L. 'Merion') or orchardgrass (*Dactylis glomerata* L. 'Virginia Common') but reduced the growth of emerging shoots of orchardgrass more than bluegrass. Fresh weight gain, root growth, and transpiration were reduced in orchardgrass plants at 0.125 ppmw bromacil whereas 1.0 ppmw gave reductions in bluegrass. Photosynthesis was inhibited initially in both grasses; however, bluegrass recovered in 6 days. Water soluble carbohydrate content in bluegrass was greater and was affected less than in orchardgrass by bromacil treatment. Root absorption and translocation of 2-^{14}C-bromacil to the shoot was directly related to transpiration in both grasses. Bromacil was absorbed and translocated acropetally in sheath and foliar treatments in both grasses. Higher metabolic conversion of 2-^{14}C-bromacil occurred in bluegrass as compared to orchardgrass with 1.0 ppmw treatment. The major metabolites were 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil and an unknown. Trace amounts of 3-*sec*-butyl-6-methyluracil and 5-bromo-3-*sec*-butyl-6-hydroxymethyluracil were also detected. Bluegrass tolerance involves high carbohydrate levels in tissues, hydroxylation of bromacil, and rapid recovery of photosynthesis.

Introduction

Hoffmann et al. (12) working with Chlorella pyrenoidosa Chick, Euglena gracilis Klebs, and isolated spinach (Spinacia oleracea L.) chloroplasts concluded that the herbicidal activity of bromacil was due to inhibition of photosynthesis. Hilton et al. (10) found that the effect of uracil herbicides on the growth of nonphotosynthetic systems required 1000 times more herbicide than the inhibition of the Hill reaction in isolated chloroplasts. Bromacil at 1.4×10^{-6} M concentration gave 50% inhibition of the Hill reaction. Exogenously applied carbohydrates partially counteracted the toxicity of bromacil to intact plants. Couch and Davis (6) found that 1 ppmw bromacil reduced photosynthetic $^{14}CO_2$ fixation in corn (Zea mays L.), cotton (Gossypium hirsutum L.), and soybeans [Glycine max (L.) Merr.], but had no effect on $^{14}CO_2$ fixation in the dark. Jordan et al. (13) found that bromacil did not affect the growth of tobacco (Nicotiana tabacum L.) callus tissue in the dark at concentrations below 10^{-3} M, indicating that bromacil was not toxic in the dark at concentrations comparable to those normally used for selective weed control.

Flax (Linum usitatissimum L.) had moderate recovery of photosynthesis after a temporary exposure to 5-bromo-6-methyl-3-phenyluracil at 2×10^{-5} M concentration, but recovery of photosynthesis was absent in French bean (Phaseolus vulgaris L.) (19). Over the 25-hr test period, transpiration was reduced 25% in French bean compared to approximately 10% in flax. Van Oorschot (20) also found that inhibitors of photosynthesis reduced CO_2 uptake more rapidly than transpiration indicating that reduction in transpiration was probably a secondary effect of photosynthesis inhibition.

Gardiner et al. (8) isolated and identified six metabolites of bromacil from animal urine. The major metabolite was found to be 5-bromo-3-*sec*-butyl-6-hydroxymethyluracil. The uptake, translocation, and metabolism of substituted uracils in plants has been studied but not extensively (2, 3, 9). Gardiner et al. (9) reported that one mode of degradation of bromacil in soils and plants proceeded through hydroxylation of the side-chain alkyl groups presumably followed by ring opening and then complete metabolism to carbon dioxide, ammonia, and hydrobromic acid. The major metabolite in 'Hamlin' orange (*Citrus sinensis* L.) trees maintained for 4 weeks in sand on a nutrient solution containing 10 ppmw of 2-^{14}C-bromacil was the same as found in animal urine. The tolerance of peppermint (*Mentha peperita* L.) to 3-*tert*-butyl-5-chloro-6-methyluracil (terbacil), a closely related uracil, was found related to rate of metabolism (3).

In field studies by the authors, effective selective control of orchardgrass in Kentucky bluegrass turf was obtained with repeated applications of bromacil.[1] The objectives of this research were: (a) to explore the basis of bromacil selectivity between Kentucky bluegrass and orchardgrass observed in field studies, (b) to determine

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[1]Shriver, J. W., and S. W. Bingham. 1972. Selective control of orchardgrass in bluegrass with bromacil. p. 4. Weed Sci. Soc. Amer. Abstr.

the effect of bromacil on germination, photosynthesis, and transpiration of the grasses, and (c) to evaluate uptake, translocation, and degradation of bromacil in relation to selective effects on these grasses.

Materials and Methods

Kentucky bluegrass and orchardgrass were used as test plants, and were grown from seed in Weblite (expanded shale from Weblite Corporation, Roanoke, Va.) unless otherwise indicated. The experiments were conducted in controlled growth chambers. Environmental conditions were 8 hr, 25 C day and 16 hr, 15 C night; and a light intensity of 16 klux at plant leaf surface with cool white flourescent lamps and incandescent bulbs.

Effect on germination. The effect of 0, 0.125, 0.25, 0.5, 1, 5, 10, and 50 ppmw bromacil on germination of bluegrass and orchardgrass was observed for 41 and 23 days for bluegrass and orchardgrass, respectively. Fifty seeds of each grass, replicated three times, were placed in 20 mm petri dishes on germination blotter pads and watered with 5 ml of bromacil solutions. Water was added periodically to petri dishes if drying was observed. At the termination of the experiment, percent germination was determined and emerging leaf shoots were harvested, dried at 80 C for 48 hr, and weighed.

Effect on seedling plants. Bluegrass and orchardgrass plants were allowed to become established for 3 months in Weblite at which time they were separated into individual plants having 4 to 5 leaves.

Roots were washed, then placed for 3 days in one-tenth strength Hoagland and Arnon's nutrient solution (11). Two plants of bluegrass or orchardgrass were placed in aluminum foil-wrapped 22 ml plastic vials with slotted cork stoppers containing 20 ml of 0 to 50 ppmw bromacil. Treatment solutions were replicated three times. Plant fresh weight gain and transpirational water loss by gravitational measurement were monitored for 15 days following treatment, and the water level was adjusted periodically. Transpirational water loss was checked against a control vial without plants. Plants were visually examined for injury after 15 days.

Effect on photosynthesis. Ten-cm diameter plugs of bluegrass and orchardgrass obtained from field plots were cultured in plastic pots (15-cm diameter) containing a loam soil in a controlled environmental growth chamber (16 hr day, 22 C; 8 hr night, 20 C). The light intensity in the chamber, supplemented with a 1,000-watt "Sylvania BU-HOR" metal arc lamp, was approximately 33.4 klux at plant surface. Four pots of each species were selected for soil treatments of 0, 0.14, 0.28, and 1.12 kg/ha bromacil based on surface area of pots using 50 ml of water injected uniformly into the soil. Differential gas analysis using a Beckman IR-215 CO_2 gas analyzer was used to measure the CO_2 content of air samples obtained periodically from an "open" system glass chamber placed periodically over the pots 0 to 6 days following treatment (7). Corrections were made for ground respiration using a pot containing soil without plants. At termination of the experiment, the leaf area was determined and net photosynthesis was expressed as μg $CO_2/hr/cm^2$ leaf area. The specific leaf weight (SLW) mg leaf dry wt/cm^2 leaf area, was also determined. The water soluble carbohydrates were extracted from 100 mg of oven-dried leaf tissue using 30 ml water in a 100 C water bath for 1 hr. After filtration and dilution, carbohydrate content was determined using the anthrone sulfuric acid colorimetric test (14).

Absorption and translocation. Two seedling plants of bluegrass and orchardgrass having 4 to 5 leaves, were treated via the roots in 22 ml vials with 15 ml of nutrient solution containing 0, 0.25, 0.5, and 1.0 ppmw bromacil. The treatments were replicated three times and the plants were grown for 6 days. All treatments except controls contained 0.25 ppmw 2-^{14}C-bromacil (0.015 μc). Technical bromacil was used to obtain higher treatment concentrations. Transpirational water loss was determined. Bromacil uptake was determined by counting aliquots of the treatment solutions at 0 and 6 days in "Aqua-sol™" (New England Nuclear xylene-based liquid scintillation counting solution) with a liquid scintillation spectrometer. Count data of ^{14}C-radioassays were corrected for background, counter efficiency, and quenching. The plants were harvested, separated into roots and shoots, mounted on paper, dried at 80 C for 48 hr, rehumidified, pressed, and exposed to GAF HR3000 X-ray film for 4 weeks. The X-ray film was developed in the conventional manner and translocation patterns were evaluated.

Foliar treatments of 2-^{14}C-bromacil were made 2 cm from the collar either on the upper or lower leaf surface. Treated leaves were taped onto metal supports to avoid movement. Small lanolin rings on the leaves were used to contain 2.5 μg 2-^{14}C-bromacil (0.10 μc) treatments in 10 μl water with 0.1% Tween 80 (nonionic surfactant containing polyoxyethylene sorbitan monooleate). Because of bromacil's relatively high water solubility, 815 ppm, an aqueous surfactant solution was used. Sheath treatments were made by applying 10 μl of treatment solution directly above a lanolin ring at the base of the shoot. The treated plants with their root systems in nutrient solution were placed in the growth chamber for 6 days. At termination of the experiment the treated areas of the leaf or sheath were removed and assayed for radioactivity. The remaining plant parts were exposed to X-ray film for 4 weeks. The autoradiographs were evaluated for translocation patterns, and the count data were used to determine absorption.

<u>Degradation of bromacil</u>. Bluegrass and orchardgrass plants (3 to 10 g fresh weight) were grown in foil-wrapped glass jars for 7 days with root systems in 50 ml of one-tenth strength nutrient solution containing 1 ppmw 2-^{14}C-bromacil (0.2 μc). Treatments were duplicated. The plants were harvested and separated into roots and shoots. The plant material was extracted for 3 min in 50 ml of ethanol in an Omni-mixer submersed in a 3 C ice water bath similar to the method of Barrentine and Warren (3). The liquid was decanted, and the residue was extracted for 3 min with an additional 50 ml of ethanol. The ethanol extracts were filtered through Whatman No. 1 filter paper, then evaporated to dryness. The dry residue was suspended in 5 ml

of ethanol, transferred to a graduated centrifuge tube, and evaporated to a final volume of 2 ml. Aliquots of the plant extract were spotted on Eastman Chromagram 6060 sheets coated with 100 μ silica gel with flourescent indicator for thin-layer chromatography (TLC). The plant extracts were over-spotted with 20 μl containing 20 μg of technical unlabeled reference compounds: (a) bromacil, (b) 3-sec-butyl-6-methyluracil, (c) 5-bromo-3-sec-butyl-6-hydroxymethyluracil, (d) 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil, and (e) 6-methyluracil (9). The plates were developed one and/or two-dimensionally (15 cm) with two solvent systems, i.e., petroleum ether:chloroform: 95% ethanol (7:2:1, v:v:v) or benzene:acetone (2:1, v:v). The plates were then exposed to X-ray film for 4 weeks. The unlabeled references were visualized under UV light and compared with radioactive areas on the plates. The plates were sectioned and the sections were assayed for radioactivity in 15 ml of Aquasol™. The plant residue remaining after ethanol extraction was ground to pass through a 60-mesh screen, and assayed for radioactivity using liquid scintillation spectrometry.

An additional metabolism experiment was conducted in which the plants were treated for 10 days with their root systems in 32.5 ml of one-tenth strength nutrient solution containing 10 ppmw of 2-^{14}C-bromacil (1.5 μc). The plants were harvested, extracted, and chromatographed as above.

Results and Discussion

Effect on germination. Bromacil did not affect germination of bluegrass or orchardgrass seeds, even at 50 ppmw (Table III-1). Orchardgrass seeds germinated rapidly with some seeds germinating in 3 days and maximum germination occurring in 8 days. Leaf tip dieback in orchardgrass was first observed about 14 days after the start of the experiment. Shoot growth of orchardgrass at 23 days showed that all bromacil treatments significantly reduced shoot growth as measured by reduced dry weight with 0.5 ppmw giving maximum reduction. Bluegrass seeds germinated from 11 days until harvest 41 days later. Treatments of 5 ppmw caused chlorosis and inhibition of shoot growth, and few secondary leaves emerged within 25 days. At termination of the experiment bluegrass shoot yields were reduced at 1 ppmw while 5 ppmw gave maximum growth inhibition (Table III-1). The delayed action of bromacil was similar to that reported for uracil herbicides by Hilton et al. (10) working with barley (*Hordeum vulgare* L.) where the early growth of germinating seedlings was relatively insensitive to uracil herbicides. As long as the seed supplied adequate carbohydrates, new shoot growth occurred, but with the inhibition of photosynthesis, growth stopped and old leaf tissue died.

Effect on seedling plants. Pancholy and Lynd (18) found that plant fresh weight was a better indicator of toxicity than dry weight because of the delayed action of bromacil on growth of oats (*Avena sativa* L.). In this study, the fresh weight gain was a good indicator for determining growth of the plant during the bromacil bioassay

Table III-1. Effect of bromacil on shoot growth of Kentucky bluegrass and orchardgrass after 41 and 23 days, respectively.

Bromacil	Germination		Shoot yield of germinated seedlings	
	Bluegrass	Orchardgrass	Bluegrass	Orchardgrass
(ppmw)	(%)	(%)	(mg dry wt)	(mg dry wt)
0.0	53 a[a]	81 a	3.1 ab	28.7 a
0.125	52 a	85 a	3.0 ab	22.5 b
0.25	56 a	78 a	3.5 a	16.2 c
0.5	59 a	81 a	2.8 b	10.1 d
1.0	57 a	81 a	1.6 c	8.6 d
5.0	43 a	84 a	0.3 d	6.7 d
10.0	51 a	81 a	0.5 d	7.3 d
50.0	57 a	79 a	0.6 d	6.9 d

[a] Data in the same column followed by a common letter are not significantly different at the 5% level.

(Table III-2). The lowest concentration tested, 0.125 ppmw, gave 90% reduction in growth of orchardgrass after 15 days. A more gradual reduction in bluegrass growth was observed with increasing concentrations of bromacil. One ppmw was required to obtain a significant reduction in growth. Root growth of orchardgrass was inhibited by all concentrations of bromacil. Inhibition of bluegrass roots was observed only with concentrations greater than 1 ppmw bromacil. Differential tolerance of the two grasses to bromacil is also dramatized by the greater reduction in transpiration in orchardgrass compared to bluegrass.

Effect on photosynthesis. Suppression of net photosynthesis occurred in both grasses following soil treatments with bromacil (Figures III-1 and III-2). Bluegrass photosynthesis recovered to control levels at the lower rates by 6 days. However, orchardgrass did not show this recovery in photosynthesis. Water soluble carbohydrate content in leaf tissue of untreated plants at the termination of the experiment showed that bluegrass had a higher carbohydrate level than orchardgrass (Figure III-3). Carbohydrates in orchardgrass were reduced equally by all rates of bromacil whereas 1.12 kg/ha of bromacil reduced carbohydrates in bluegrass to the same level. The specific leaf weight was inhibited by bromacil more so in orchardgrass than bluegrass. The recovery of photosynthesis by bluegrass suggests a greater physiological inactivation of bromacil in bluegrass compared to orchardgrass. These results are similar to those observed by Van Oorschot (19) working with uracils and a moderately resistant

Table III-2. Effect of bromacil on transpiration and fresh weight of Kentucky bluegrass and orchardgrass 15 days after treatment.

Bromacil	Water transpired		Fresh wt gain[a]	
	Bluegrass	Orchardgrass	Bluegrass	Orchardgrass
(ppmw)	(ml/two plants)	(ml/two plants)	(g/two plants)	(g/two plants)
0.0	15.4 a[b]	15.0 a	0.84 a	0.98 a
0.125	12.2 b	6.8 b	0.61 a	0.09 b
0.25	10.4 b	5.1 bc	0.63 a	0.11 b
0.5	11.8 b	4.6 bc	0.71 a	0.06 b
1.0	11.7 b	4.2 c	0.29 b	0.10 b
5.0	6.3 cd	4.8 bc	0.17 b	0.03 b
10.0	4.7 d	3.5 c	0.13 b	0.13 b
50.0	7.0 c	3.2 c	0.20 b	0.21 b

[a]Starting fresh weight was 0.60 g/two plants for bluegrass and 0.37 g/two plants for orchardgrass.

[b]Data in the same column followed by a common letter are not significantly different at the 5% level.

Figure III-1. Effect of soil-applied bromacil on net photosynthesis of orchardgrass for 6 days following application.

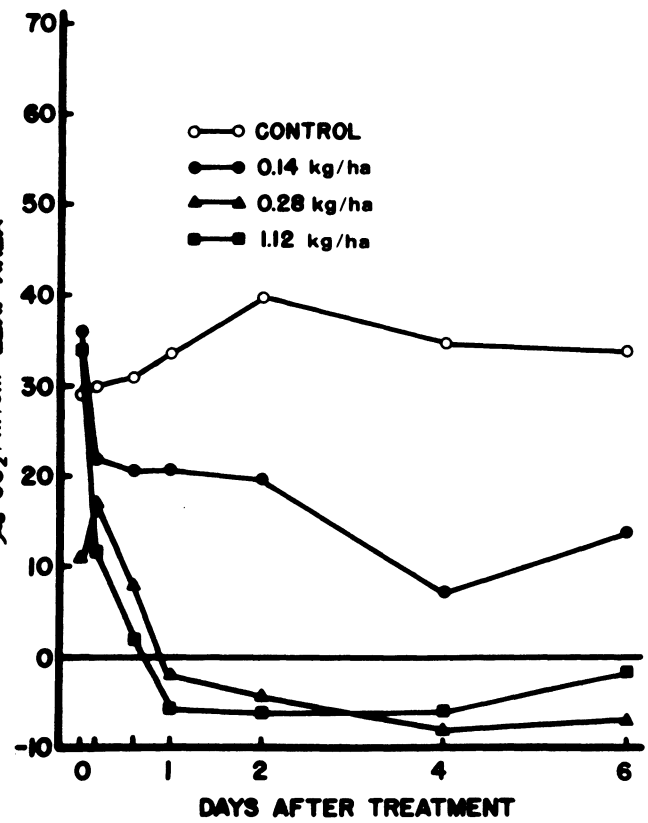

70
60
50
40
30
20
10
0
-10
CONTROL
0.14 kg/ha
0.28 kg/ha
1.12 kg/ha
µg CO_2 / hr / cm^2 LEAF AREA
0
1
2
4
6
DAYS AFTER TREATMENT

Figure III-2. Effect of soil-applied bromacil on net photosynthesis of bluegrass for 6 days following application.

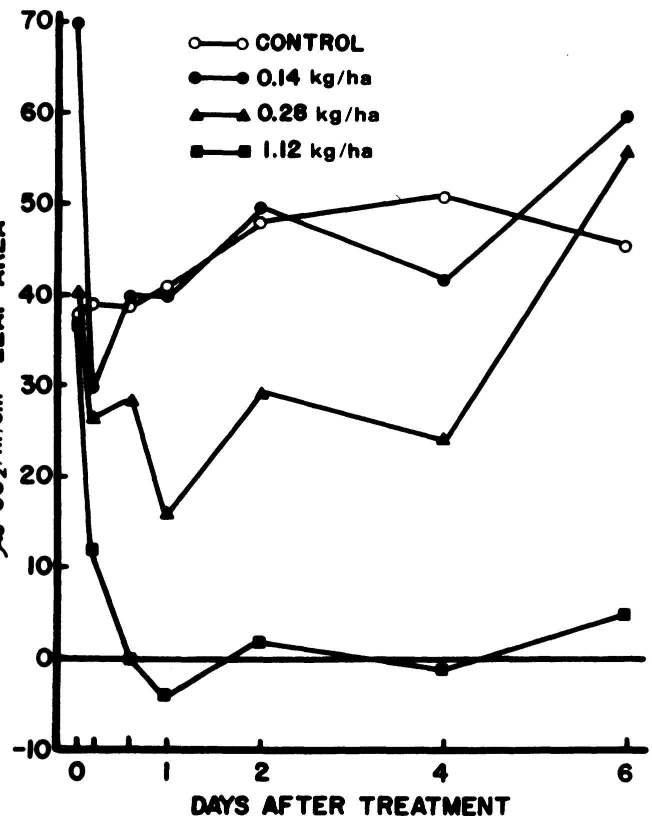
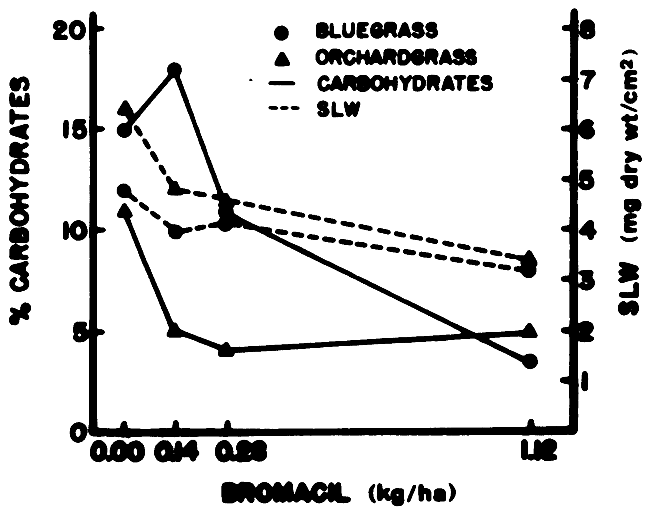

CONTROL
0.14 kg/ha
0.28 kg/ha
1.12 kg/ha
μg CO_2/hr/cm² LEAF AREA
70
60
50
40
30
20
10
0
-10
0
1
2
4
6
DAYS AFTER TREATMENT

Figure III-3. Effect of a 6-day bromacil soil treatment on bluegrass and orchardgrass percent foliage carbohydrates and specific leaf weight (SLW).


BLUEGRASS
ORCHARDGRASS
CARBOHYDRATES
SLW
% CARBOHYDRATES
SLW (mg dry wt/cm²)
BROMACIL (kg/ha)
0.00
0.14
0.28
1.12
0
5
10
15
20
1
2
3
4
5
6
7
8

plant, flax, which showed recovery of photosynthesis following treatment. High water soluble carbohydrate content in bluegrass leaves would tend to protect and allow the plant to recover after inactivation of the herbicide.

Absorption and translocation. Increasing herbicide concentration reduces transpiration of the intact plants and uptake of 2-^{14}C-bromacil from nutrient solution (Table III-3). The reduction of transpiration over the experimental time period results in reduced bromacil uptake in orchardgrass compared to bluegrass. Bromacil uptake is related to transpiration rate, similar to results of Van Oorschot (20).

Reduction in transpiration in the intact plant may relate to reduced root permeability. Schreiber et al.[2] rated reduced root permeability as a major influence on bromacil uptake by wheat (*Triticum aesativum* L.) plants under soil moisture stress conditions. Ashton et al. (1) found that 10^{-3} M bromacil caused oat root tips to become smaller and necrotic, and to collapse. Epidermal cell walls also became thickened, supporting the concept of reduced root permeability (1). Murata (15) explained that root systems of plants required adequate supplies of carbohydrates to maintain root activity. Exogenously supplied carbohydrates partially nullified the toxicity symptoms in intact barley plants (10). Initially bluegrass had a

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[2]Schreiber, J. D., V. V. Volk, and L. Boersma. 1972. Bromacil uptake by wheat under soil moisture stress conditions. p. 71. Weed Sci. Soc. Amer. Abstr.

Table III-3. Effect of bromacil on transpiration and 2-^{14}C-bromacil uptake from nutrient solution after 6 days by Kentucky bluegrass and orchardgrass.

Bromacil	Water transpired		^{14}C-bromacil uptake/two plants[a]	
	Bluegrass	Orchardgrass	Bluegrass	Orchardgrass
(ppmw)	(ml/two plants)		(dpm x 10^3)	
0.0	13.3 a[b]	13.1 a	--	--
0.25	11.9 a	6.8 b	18 a	11 a
0.50	9.5 b	5.7 b	16 a	9 b
1.00	6.4 c	4.9 b	12 b	9 b

[a]15 ml nutrient solution contained 0.25 ppmw 2-^{14}C-bromacil (0.015 c or 33.8 dpm x 10^3)

[b]Data in the same column followed by a common letter are not significantly different at the 5% level.

higher leaf carbohydrate level than did orchardgrass. Inhibition of photosynthesis by bromacil reduced the supply of carbohydrates available to the roots. The permeability concept is supported by these data where transpiration and bromacil uptake in bluegrass were not influenced to the extent as in orchardgrass.

The general distribution patterns of ^{14}C from 2-^{14}C-bromacil root treatments showed concentration of ^{14}C in the foliage more so than the roots in both grasses (Figure III-4). These results are similar to those observed by Barrentine and Warren (3) with terbacil in peppermint and ivyleaf morningglory [_Ipomoea hederacea_ (L.) Jacq.].

Bromacil has shown effective foliar activity against weeds 5 to 20 cm high when applied with 0.5% wetting agent (17). When foliarly applied in isoparaffinic oil or nonionic surfactant, terbacil reduced the fresh weight of giant foxtail (_Setaria faberii_ Herrm.) 50 and 80%, respectively (2). In these studies, 2-^{14}C-bromacil was absorbed and translocated acropetally in both sheath and upper foliar applications in both grasses (Figures III-5 and III-6). Results are similar to those of Wax and Behrens (21) with 2-chloro-4-ethylamino-6-isopropylamino-_s_-triazine (atrazine) on quackgrass (_Agropyron repens_ L.) and Bayer and Yamaguchi (4) with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (diuron) on barley. With lower leaf surface treatment, translocation patterns were similar. These results of acropetal movement indicate that bromacil diffuses predominantly along the cell walls and enters the xylem and does not readily enter the phloem in these two grasses.

The upper leaf surface of bluegrass allowed more penetration of bromacil than did the lower leaf surface (Table III-4). Orchardgrass,

Figure III-4. Autoradiograms (above) of bluegrass (B) and orchardgrass (O) plants (below) showing distribution of ^{14}C 6 days after root treatment with 0.25 ppmw 2-^{14}C-bromacil (0.015 μc) in nutrient solution.

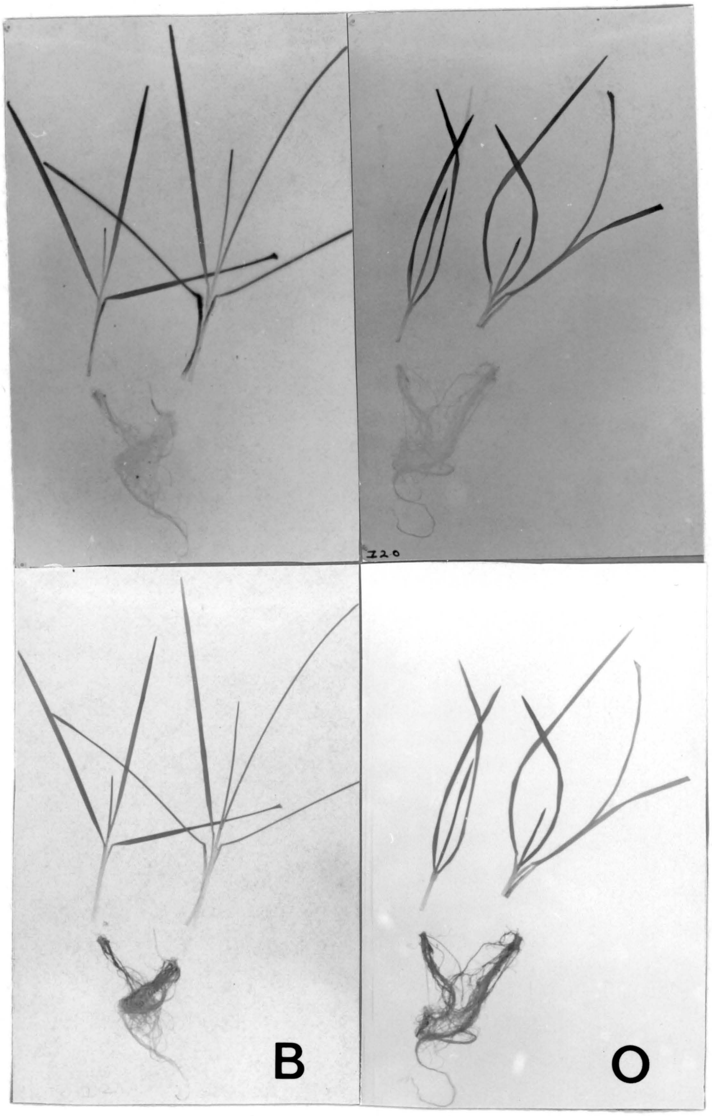
B
O

Figure III-5. Autoradiograms (above) of bluegrass (B) and orchardgrass (O) plants (below) showing ^{14}C distribution 6 days after foliar treatment of the upper leaf surface with 10 μl droplet containing 2.5 μg (0.01 μc) of 2-^{14}C-bromacil in a 0.1% Tween 80 aqueous solution.

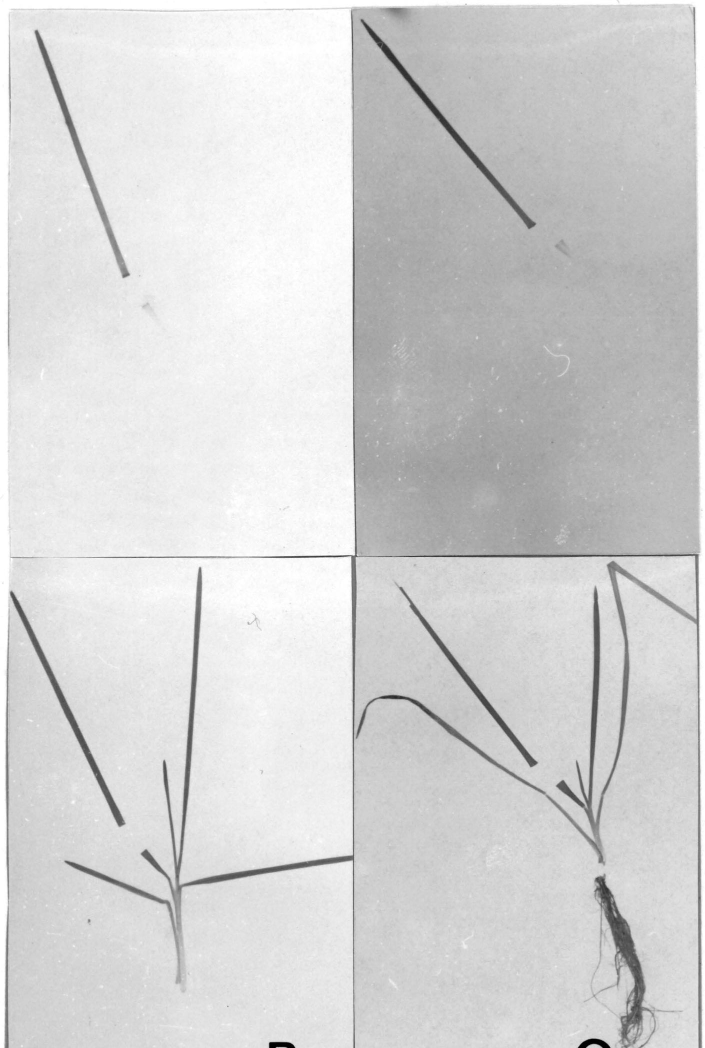
B
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Figure III-6. Autoradiograms (above) of bluegrass (B) and orchardgrass (O) plants (below) showing ^{14}C distribution 6 days after sheath treatment with a 10 μl droplet containing 2.5 μg (0.01 μc) of 2-^{14}C-bromacil in a 0.1% Tween 80 aqueous solution.

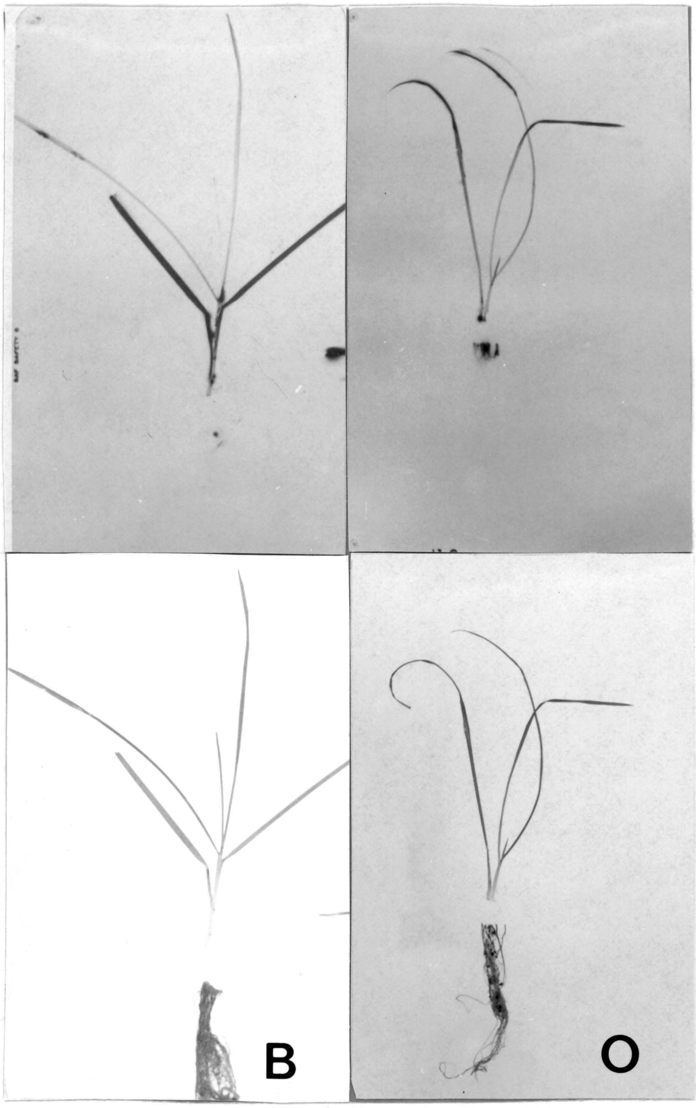
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Table III-4. Absorption of 2-^{14}C-bromacil in bluegrass and orchardgrass from various leaf treatments using 10 μl droplets containing 2.5 μg (0.01 μc or 21.9 x 10^3 dpm) in 0.1% Tween 80 aqueous solution.

Leaf surface treated	^{14}C absorption into plant material	
	Bluegrass	Orchardgrass
	(dpm x 10^3)	(dpm x 10^3)
Upper	3.6 b[a]	7.3 b
Lower	0.9 c	5.0 b
Sheath	20.9 a	15.2 a

[a]Data in the same column followed by a common letter are not significantly different at the 5% level.

on the other hand, absorbed similar amounts of bromacil through both leaf surfaces. Sheath treatments near the crown area of both grasses gave the greatest absorption. Barrentine and Warren (2) postulated that alterations in the cuticle with various carriers modified the penetration of terbacil into leaves. The cuticle of the leaf sheath is thin compared to the leaf blade and allowed penetration of large quantities of bromacil.

Root uptake of bromacil into bluegrass was greater than that into orchardgrass, but translocation patterns were similar in the two grasses. Bluegrass tolerance to bromacil as compared to orchardgrass was not explained on the basis of differential uptake and translocation as previously suggested for terbacil (3).

Degradation of bromacil. Recovery of radioactivity from the root-applied 2-^{14}C-bromacil removed by the plants ranged from 65 to 88% for bluegrass and 61 to 71% for orchardgrass for 1.0 ppmw bromacil treatments for 7 days. These losses might have occurred during extraction and sampling or by the degradation of the ring structure evolving $^{14}CO_2$ during the 7-day experiment. In both grasses the shoots contained more radioactivity than the roots. Both roots and shoots contained metabolites of 2-^{14}C-bromacil (Table III-5). TLC separations of 2-^{14}C-bromacil from ^{14}C-metabolites showed that bluegrass contained only 19% intact bromacil whereas orchardgrass had 77% intact herbicide after 7 days' treatment. Similar trends, although less pronounced, were reported for terbacil in resistant peppermint and susceptible ivyleaf morningglory (3).

Table III-5. Bromacil and metabolites in ethanol extracts of bluegrass and orchardgrass treated for 7 days with 1 ppmw 2-^{14}C-bromacil (0.2 μc)[a] in 50 ml solution were separated using ascending thin layer chromatography with petroleum ether:chloroform:95% ethanol (7:2:1, v/v/v) solvent system.

TLC plate[b]		Radioactivity in ethanol extracts			
Section	Rf	Bluegrass[c]		Orchardgrass[d]	
		Shoot	Root	Shoot	Root
		(10^2 dpm)	(10^2 dpm)	(10^2 dpm)	(10^2 dpm)
1	.61 - .69	199	109	735	363
2	.52 - .60	20	4	46	10
3	.46 - .51	9	0	1	5
4	.40 - .45	26	2	3	1
5	.32 - .39	487	46	85	12
6	.16 - .31	142	17	22	6
7	.08 - .15	9	4	5	3
origin	0 - .07	162	22	55	61
Total		1054	204	952	461

[a]Dosages represented 50 μg of 2-^{14}C-bromacil 453 x 10^3 dpm.

[b]Section of TLC plate was identified as 1 = bromacil, 2 = 3-sec-butyl-6-methyluracil, 3 = 5-bromo-3-sec-butyl-6-hydroxymethyluracil, 5 = 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil, 7 = 6-methyluracil.

[c]Fresh weight of bluegrass plants was 5.4 g.

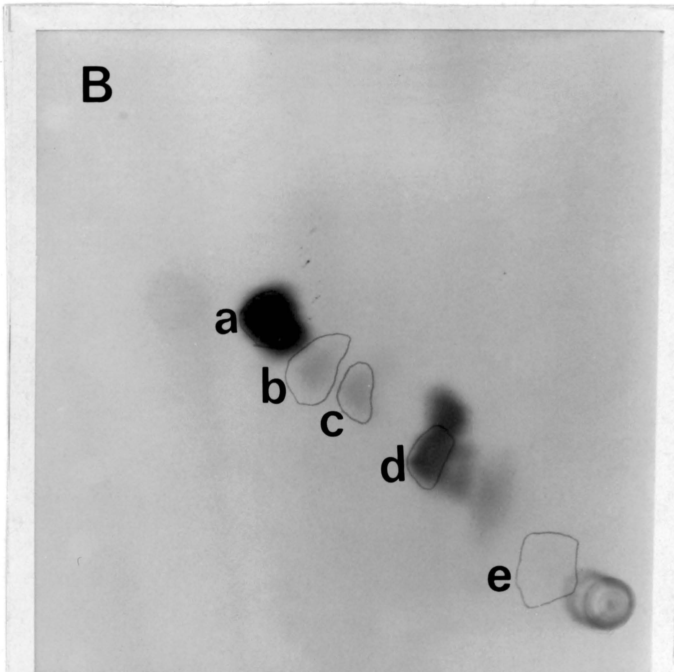
[d]Fresh weight of orchardgrass plants was 10.5 g.

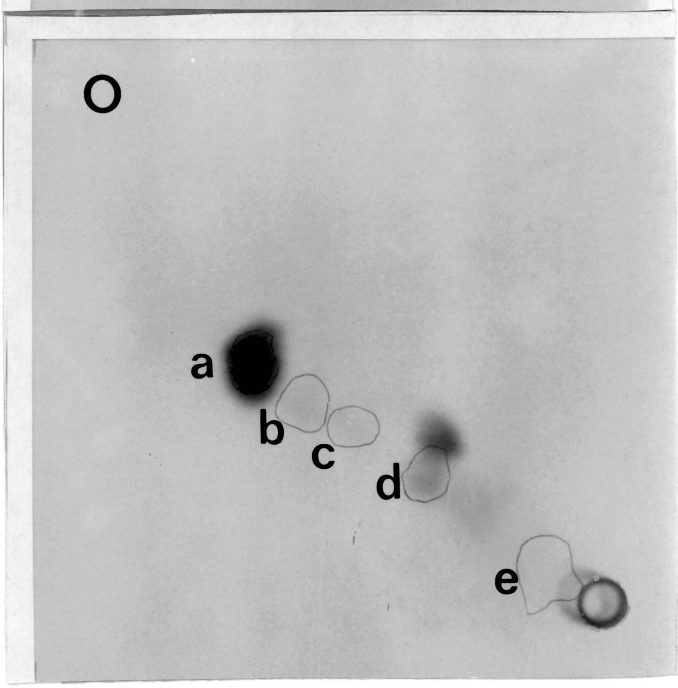
Two-dimensional TLC showed the presence of various metabolites and intact bromacil in both species following 10 ppmw treatment (Figure III-7). A major metabolite of bromacil in both grasses was (d) 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil. This metabolite accounted for 9% and 3% of the radioactivity in the bluegrass and orchardgrass, respectively. An unidentified major metabolite, which separated slightly above 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil, was common in both grasses. Trace amounts of 3-sec-butyl-6-methyluracil and 5-bromo-3-sec-butyl-6-hydroxymethyluracil and 2 or 3 other unidentified metabolites were also found. After treatment with 10 ppmw bromacil for 10 days, approximately 75% of the shoot radioactivity in both grasses was bromacil. The low conversion at high bromacil levels supports the fact that 10 ppmw is not a selective treatment compared to the higher conversion occurring at lower treatment levels.

Gardiner et al. (8) suggested that metabolic conversion occurred through hydroxylation of the side-chain alkyl groups. The route of hydroxylation varies between plants. In orange plants, the major metabolite was 5-bromo-3-sec-butyl-6-hydroxymethyluracil, whereas in grasses, 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil was the major identified metabolite.

Photosynthesis recovery data suggest that metabolites are less phytotoxic than intact bromacil and known phytotoxicity of metabolites

Figure III-7. Autoradiograms of thin layer chromatograms showing $2\text{-}^{14}C$-bromacil and metabolites in ethanol extracts of bluegrass (B) and orchardgrass (O) shoots. Plants were root treated for 10 days with 10 ppmw $2\text{-}^{14}C$-bromacil in nutrient solution. Chromatograms were developed two-dimensionally, 15 cm in the first dimension with benzene:acetone (2:1, v/v) and 15 cm in the second dimension with petroleum ether:chloroform:95% ethanol (7:2:1, v/v/v). Nonradiolabeled reference compounds were cochromatogrammed and their locations circled as follows: a = bromacil; b = 3-sec-butyl-6-methyluracil; c = 5-bromo-3-sec-butyl-6-hydroxymethyluracil; d = 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil; e = 6-methyluracil.


B
a
b
c
d
e


O
a
b
c
d
e

supports this concept.[3] Negi et al. (16) found that resistant plants converted at least twice as much atrazine to hydroxyatrazine as susceptible plants. Hydroxyatrazine was nonphytotoxic. Broadhurst et al. (5) reported the capability of bluegrass to convert 3,6-dichloro-o-anisic acid (dicamba) to 5-hydroxy dicamba in roots and shoots. The reduced influence of bromacil on growth and transpiration of bluegrass, the high carbohydrate content and recovery of photosynthesis of bluegrass leaves, and the conversion of bromacil to hydroxylated compounds are all manifested in bluegrass tolerance observed at low bromacil levels.

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[3] J. A. Gardiner. 1972. Personal communication. Industrial and Biochemical Department, Experimental Station, E. I. duPont de Nemours & Co., Inc., Wilmington, Delaware 19898.

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IV. SUMMARY

The effectiveness of bromacil for the selective removal of orchardgrass from Kentucky bluegrass turf and some physiological responses of the grasses to bromacil at rates ranging from 0.14 to 1.12 kg/ha were evaluated in field studies at Blacksburg, Virginia, during 1970 and 1971. For 1 month following initial bromacil application to established turf, photosynthesis was reduced to a greater extent in orchardgrass than Kentucky bluegrass.

During the second year of retreatment, foliage carbohydrates were depressed initially in bluegrass followed by recovery 2 weeks to 1 1/2 months following treatment. Orchardgrass foliage carbohydrate levels were lower than bluegrass and orchardgrass did not exhibit the same degree of recovery after bromacil application. Application of 0.56 kg/ha bromacil each summer and fall for 2 years gave the best selective control of orchardgrass. During the second year, foliage crude protein increased in treated bluegrass and the foliage yields were equal to or greater than the controls. Orchardgrass yields were drastically reduced by bromacil.

Preemergence or early postemergence application of bromacil at 0.14 and 0.28 kg/ha controlled orchardgrass with minimal bluegrass injury. Higher rates of application were detrimental to bluegrass survival. Treated areas were effectively fall seeded with bluegrass approximately 8 weeks after a 0.56 kg/ha application.

No significant inhibition of bluegrass rooting into treated soil was observed at 0.56 kg/ha of bromacil, the rate used selectively to control orchardgrass. Retreatment of bluegrass sod for two years in the summer and fall with a total of 2.2 kg/ha bromacil had no effect on the sod's ability to root into untreated soil at 2 months after the final fall application.

Studies conducted in controlled environmental growth chambers showed that bromacil had no effect on germination at 50 ppmw, but reduced the growth of emerging shoots at 0.125 ppmw in orchardgrass and 0.5 ppmw in bluegrass. Fresh weight gain, root growth, and transpiration of 3-month-old plants were reduced at 0.125 ppmw with orchardgrass while 1.0 ppmw caused a reduction in bluegrass.

After initial suppression, bluegrass photosynthesis recovered to control levels in 6 days following 0.28 kg/ha application of bromacil. Orchardgrass, however, did not recover. After 6 days, carbohydrate content of controls was greater in bluegrass than in orchardgrass and was not reduced as drastically by bromacil treatment. Root absorption and translocation of 2-^{14}C-bromacil from nutrient solution was directly related to transpiration rate in both grasses with orchardgrass removing less due to a greater reduction in transpiration. Sheath and foliar applications of 2-^{14}C-bromacil in 0.1% Tween 80 water solution showed absorption and translocation acropetally in both grasses. In bluegrass the upper leaf surface absorbed more bromacil than the lower leaf surface. Orchardgrass, however, absorbed the same through either surface. Sheath treatments showed the greatest absorption probably due to thinner cuticle. Differential absorption and translocation patterns did not account for the selectivity between the two grasses. Metabolism of 2-^{14}C-bromacil was greater in bluegrass than orchardgrass with 1.0 ppmw treatment. After 7 days, ^{14}C in bluegrass ethanol shoot extracts was only 19% intact bromacil versus 77% in orchardgrass. Two major metabolites were detected in plant extracts, one of which was identified as 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil. Trace amounts of 3-_sec_-butyl-6-methyluracil and 5-bromo-3-_sec_-butyl-6-hydroxymethyluracil were also detected. The reduced influence of bromacil on growth and transpiration of bluegrass, the high carbohydrate content and recovery of photosynthesis of bluegrass leaves, and the conversion of bromacil to hydroxylated compounds are all manifested in bluegrass tolerance observed at low bromacil levels.

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SOME PHYSIOLOGICAL ASPECTS OF SELECTIVE ORCHARDGRASS CONTROL IN KENTUCKY BLUEGRASS WITH BROMACIL

by

John Wade Shriver

(ABSTRACT)

The effectiveness of 5-bromo-3-*sec*-butyl-6-methyluracil (bromacil) for the selective removal of orchardgrass (*Dactylis glomerata* L.) from Kentucky bluegrass (*Poa pratensis* L.) turf and some physiological responses of the grasses to bromacil were evaluated in field studies. Bromacil reduced photosynthesis to a greater extent in orchardgrass than Kentucky bluegrass turf. Foliage carbohydrates were depressed initially in bluegrass followed by recovery in 2 weeks to 1 1/2 months following bromacil application. Orchardgrass, however, did not recover. Application of 0.56 kg/ha bromacil each summer and fall for 2 years gave the best control of orchardgrass. During the second year, increase in foliage crude protein was observed in treated bluegrass. Bluegrass foliage yields were equal to or greater than the controls. Orchardgrass yields were drastically reduced by bromacil. Effective seedling orchardgrass control was obtained with minimal bluegrass injury at 0.14 to 0.28 kg/ha of bromacil. Bromacil treated areas were effectively fall-seeded with bluegrass approximately 8 weeks after a 0.56 kg/ha application. Rooting of bluegrass sod was not inhibited at the rates used for selective control of orchardgrass in bluegrass turf.

In controlled environmental growth chamber studies, bromacil had no effect on germination of Merion Kentucky bluegrass or Virginia Common orchardgrass, but reduced the growth of emerging shoots of orchardgrass more than bluegrass. Fresh weight gain, root growth, and transpiration were reduced in orchardgrass plants at 0.125 ppmw bromacil whereas 1.0 ppmw gave reductions in bluegrass. Photosynthesis was inhibited initially in both grasses, however, bluegrass recovered in 6 days. Foliage carbohydrate content was greater and was affected less by bromacil treatment in bluegrass than orchardgrass. Root absorption and translocation of 2-^{14}C-bromacil to the shoot was directly related to transpiration in both grasses. Bromacil was absorbed and translocated acropetally in sheath and foliar treatments in both grasses. Higher metabolic conversion of 2-^{14}C-bromacil occurred in bluegrass as compared to orchardgrass with 1.0 ppmw treatment. The major metabolites were 5-bromo-3-(2-hydroxy-1-methylpropyl)-6-methyluracil and an unknown. Trace amounts of 3-*sec*-butyl-6-methyluracil and 5-bromo-3-*sec*-butyl-6-hydroxymethyluracil were also detected. Bluegrass tolerance to bromacil involves high carbohydrate levels in tissues, hydroxylation of bromacil, and rapid recovery of photosynthesis.