CHAPTER III

ISOXABEN DISSIPATION IN FIELD SOIL AS AFFECTED BY APPLICATION TIMING

Abstract: Timing of PRE herbicide application is critical for effective weed management. Field studies were established at Blacksburg, Virginia to determine the effect of spring, fall, and a double (spring followed by fall) application of isoxaben at 0.56, 0.84, and 1.12 kg ai/ha on its dissipation. Isoxaben levels in the top 3.8 cm of the soil profile at 0, 1, 3, 6, and 9 MAT were determined. Isoxaben was extracted using methanol/water, followed by partitioning into dichloromethane, and sample clean-up by deactivated alumina column, with quantitation by HPLC using a UV detector at 254 nm. The half-life of isoxaben was estimated as 2.7 months following spring application, 5.7 months following fall application, and 6.1 months following spring plus fall application. Isoxaben levels in the soil at 3 and 6 MAT following fall application was generally higher than that following spring application. Degradation following fall application may be slower than that following spring application due to lower microbial activity during winter months. Negligible levels of the herbicide remained in the top 3.8 cm of soil nine months following spring or fall application. Double application of isoxaben resulted in higher levels of isoxaben, especially at 9 MAT.

Nomenclature: Isoxaben, N-[3-(1-ethyl-1-methlypropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide.

Additional index words: Preemergence herbicides, herbicide degradation, herbicide breakdown in soil, herbicide residues.

Abbreviations: DT_{50} half-life; HPLC, high performance liquid chromatography; MAT, months after treatment; PRE, preemergence; UV ultra violet

Introduction

Isoxaben is a selective preemergence (PRE) herbicide that controls broadleaf weeds in established turf and ornamentals in the U.S. (Anonymous 1990; Colbert and Ford 1987). In Europe, isoxaben controls several annual broadleaf weeds selectively in cereal crops (Huggenberger et al. 1982). Although its exact mechanism of action in plants is unclear, isoxaben can inhibit cellulose biosynthesis and cell wall formation (Heim et al. 1991). Primary breakdown mechanism of this molecule in soils is through microbial degradation. (Rouchaud et al. 1993 b).

Rouchaud et al. (1993 a, b) have determined the half-life of isoxaben in unamended and amended soils using first-order kinetics. Chemical analyses indicated that isoxaben dissipated with half lives of 2.9 months in unamended plots to 6.6 months in plots treated with various organic fertilizers. During the entire period of their study in Belgium, isoxaben was not detected in the 10 to 20 cm depth of soil layer, which indicated that it remained in the top 10 cm of the soil profile. Huggenberger and Ryan (1985) studied the half-life and mobility of isoxaben in field trials, carried out in France. Isoxaben applied in early winter had a half-life of 2.5 to 4 months. However, the same authors have determined half-life estimates of 5 to 6 months in earlier studies carried out in Indiana (Huggenberger et al, 1982). About 50% of isoxaben remained in the top 2 cm of field soil and 85% of the compound remained within the top 4 cm of the soil column at 4 to 5 months after application. Walker (1987) determined that 30 to 40% of the applied isoxaben remained in the top 5 cm of soil four months after spring treatment.

In the U.S., limited research has been conducted to determine isoxaben breakdown from fall compared to spring application at different application rates. Potential for increased soil residues following repeat application is also not well understood. The objective of this research was to quantify isoxaben residues through chemical analyses over a 9-month period as affected by application timing and rate.

Materials and Methods

General Conditions. A field study was established at Blacksburg, VA in tilled soil using a split-plot design. Main plots were herbicide treatments and subplots consisted of evaluation timings. Each treatment was replicated four times. The study was established in 1994 and was repeated in another field in 1995.

Each study was conducted for 9 months following treatment. Plastic flats (45 cm by 32.5 cm by 7.5 cm) were filled with a Ross silt-loam soil (fine-loamy, mixed, mesic Cumilic Hapludoll) having a pH of 5.4 and an organic matter content of 3%. Flats were imbedded in the field sites at the soil level. Three rates of isoxaben (0.56, 0.84, and 1.12 kg ai/ha) were applied. These rates represent the low, intermediate, and maximum use rates in turf (Anonymous, 1990). The three application timings evaluated were spring, fall, and spring followed by fall (double) application. Isoxaben, formulated as a 75% dry flowable, was applied using a CO_2-pressurized boom sprayer with 8003 flat fan nozzles[4] delivering 230 L/ha with water as the carrier. Flats were taken from the field at 0, 1, 3, 6, and 9 MAT. The flats from spring plus fall treatments were taken at the above time intervals after the second application. Soil samples were collected from the top 3.8 cm of each flat using a 2 cm core sampler. Samples were stored in a freezer until extraction and quantification.

Study 1. Application timings evaluated in this study were spring, fall, and spring followed by fall (double application). Soil samples were taken at 0, 1, 3, 6, and 9 months after the second treatment for the double application. Spring treatments were applied on June 17, 1994. Cloud cover was 25%, air temperature was 27 C, and soil temperature at a 5-cm depth was 25 C. Wind velocity at the time of application was 0 to 5 km/h. The first precipitation after treatment occurred 6 days later and totaled 2.8 cm. The fall treatment and the fall component of the double application were applied on October 7, 1994. Cloud cover was 100%, air temperature was 15 C, soil temperature at a 5-cm depth was 22 C, and there was no wind. The first precipitation occurred 6 hours after treatment and totaled 0.5 cm.

Study 2. Spring treatments were applied on June 5, 1995. Cloud cover was 10% with no wind, air temperature was 28 C, and soil temperature at a 5-cm depth was 19 C. A total precipitation of 25.1 cm occurred within two weeks after treatment (Table 1). Fall treatments were applied on October 30, 1995. Cloud cover was 100% with no wind, air temperature was 13 C, and soil temperature at a 5-cm depth was 16 C. The first precipitation after treatment occurred 3 days later and totaled 2.2 cm

Quantification of isoxaben. Soil samples were extracted and quantified for isoxaben using a modified procedure published by Rutherford (1993). The stored sample was allowed to thaw to room temperature and air dried overnight. Any organic debris was

[4] TeeJet 8003 flat fan spray nozzles, Spraying Systems Co., North Ave., Wheaton, IL 60788

removed and the sample was homogenized thoroughly. A 50 g subsample was transferred and boiled with 200 ml of 80:20 methanol/water in a 1000 ml flask. Isoxaben was extracted by refluxing the mixture for a period of one hour. A 100 ml aliquot was collected after the mixture cooled to room temperature. Isoxaben was partitioned in three successive portions of dichloromethane in a separatory funnel after adding 50 ml of a 5% NaCl aqueous solution. The partitioned fraction was then prepared for high performance liquid chromatography (HPLC) analysis by alumina column chromatography. Deactivated alumina (with 4 ml of water added per 100 g alumina) was used for this purpose. Sample clean-up was carried out by elution with successive fractions of 30 ml methylene chloride, 50 ml 80:20 ethyl acetate/methanol, 25 ml 99:1 dichloromethane/methanol, and 50 ml 99:1 dichloromethane/methanol. Elutions were carried out at a flow rate of 0.3 ml/min. The first three eluate fractions, containing only extraneous materials as determined by method standardization, were discarded. The final 50 ml eluate (99:1 dichloromethane/methanol), which contained isoxaben, was then collected. The eluate was brought to dryness using a rotary vacuum evaporator at 40 C and the isoxaben residue was dissolved in 3 ml of 70:30 methanol/water.

Untreated soil samples were fortified with isoxaben to determine percent recovery for the procedure. Fortified samples were maintained under conditions similar to those of unknown samples to determine stability of the compound. Isoxaben was quantified using an HPLC[5] fitted with a 250 mm by 4.5 mm column[6] with an injection port[7] loop size of 50 ul. A flow rate of 1 ml/min for an isocratic mobile phase of 70:30 methanol/water was maintained. Retention time for isoxaben was 8.2 min, with UV detection [8] at 254 nm at room temperature. Running standards of known concentrations ranging from 0.05 µg/ml to 1 µg/ml of isoxaben were injected to develop standard curves. The following equation was used to determine isoxaben concentrations in an unknown soil sample.

$$[U] = \frac{aU \times [S] \times af \times FV}{aS \times g}$$

where, [U] = concentration of unknown sample (µg/g)

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[5] Shimadzu SCL-6B
[6] Sigma Aldrich Spherisorb ODS 2 4.6 mm X 250 mm
[7] Rheodyne 7161
[8] Shimadzu SPD-6A

aU = peak area of unknown sample
[S] = concentration of standard (μg/ml)
af = aliquot factor
FV = Final volume of sample (ml)
aS = peak area of standard
g = weight of soil sample (g)

The aliquot factor was 2 for all calculations because only half the extract was used for quantification. The final sample volume was adjusted so that the peak fell within the standard curve. Fortified samples were used to determine average percent recovery. Herbicide concentrations determined for the unknown samples were subjected to analysis of variance (ANOVA) and means were separated by the Least Significant Difference (LSD) test at the 0.05 probability level. Data from the two studies were analyzed separately due to the impact of a high rainfall following spring 1995 application. Differences in isoxaben residues at various months after treatment as affected by the three application timings were determined in study 1. A factorial analysis of variance was performed to determine effects of application timings and rates at each evaluation interval. Differences in degradation pattern of isoxaben at different time intervals following a fall application were determined in study 2. Regression models for herbicide breakdown were fitted to depict isoxaben dissipation following each application timing. The natural logarithms of average isoxaben levels for each application timing were regressed against time (MAT) to determine the rate constants as a first order kinetic dissipation. Quadratic regressions using untransformed isoxaben levels were also performed against time to obtain non-linear models. Based on these models, the DT_{50} (half-life) values for each application timing was calculated.

Results and Discussion

The procedure used to extract isoxaben from soil samples had a recovery of 115% (Table 2). Storage fortification samples indicated that there was no breakdown of isoxaben in frozen soil samples during the one-year storage period (data not shown). The detection limit of isoxaben from soil sample was 0.005 μg/g.

Study 1 Mean isoxaben residues in the soil following spring and fall application did not differ at 0 MAT, indicating consistent applications (Table 3). Double application resulted in higher mean residues compared to single applications at 0 MAT.

This implied that residues from spring applications were still present at the time of fall application. At 1 MAT, no differences were seen between spring and fall application; however, double application did result in higher levels of the compound in the soil. Isoxaben residues differed among application timings at 3, 6, and 9 MAT. At 3 MAT, mean residue levels in flats that received double application of isoxaben were higher compared to those that received single spring application. Isoxaben applied at 0.56 and 1.12 kg/ha during fall or spring followed by fall resulted in higher residue levels than after spring application when evaluated 3 MAT. A similar trend was observed at 6 MAT, where fall treatments at 0.84 and 1.12 kg/ha resulted in higher residues of isoxaben in the soil compared to spring treatments. At this evaluation timing, isoxaben applied twice resulted in higher mean residues compared to a single spring or fall application. At nine months following spring or fall application, isoxaben dissipated to low levels (<0.1 µg/g) at all rates, but were numerically higher in fall treatments. The double application, however, resulted in higher levels of the compound than single spring or fall applications.

Isoxaben applied in spring underwent a different rate of dissipation than following fall application. During the first month after application, dissipation rates were not substantially different between spring and fall application. However, between 1 and 6 months after treatment, isoxaben breakdown rates were faster after spring application compared to fall application.

<u>Study 2</u>

A total precipitation of 25 cm occurred within 2 weeks after spring treatment (Table 1); no isoxaben was detected at 1 MAT following spring application (data not shown). It was suspected that excessive rainfall following spring treatments caused runoff of isoxaben. Isoxaben residues in runoff at a container nursery were recorded as 9.2% of the applied isoxaben following the first irrigation (Wilson et at. 1997). Therefore, only results from the fall treatments from 1995 are reported. Isoxaben residues in the soil decreased with time following a single fall application (Table 4). Breakdown of isoxaben following fall application appeared to be slower during 1 to 6 months after herbicide application compared to 6 to 9 MAT. This is perhaps due to slower decomposition during the winter months when soil temperatures are not favorable for microbial activity. Walker (1987) determined that the half-life of isoxaben was 2.5 months at a mean soil temperature of 20 C and 5.2 months at a mean soil temperature of 10 C.

Based on observed values, the percentage of isoxaben remaining in the top 3.8 cm of soil following spring treatment was 45% at 3 MAT and 8% at 6 MAT of the residue seen at 0 MAT. Following fall application, residue levels were 71% and 48% of isoxaben at 0 MAT, respectively, at 3 and 6 MAT. Spring followed by fall application resulted in isoxaben residues of 73% and 57% at 3 and 6 MAT, respectively, of the residue seen at 0 MAT. Several experiments have been conducted in Europe to determine the soil half-life of isoxaben. Rouchaud et al. (1993 a, b) determined that the half-life of isoxaben in unamended soils was 2.9 months when applied in fall and that the dissipation rate of isoxaben was greater in spring and summer months than during winter. Garcia et al. (1992) determined that the half-life of isoxaben in the soil was more than 6 months following fall application. Colbert and Ford determined the half-life of fall-applied isoxaben to be approximately 5 months, with 20% of the applied herbicide persisting after 12 months.

Data from the two studies were averaged across the three rates of application to estimate the half-life (DT_{50}) of isoxaben following the three application timings. Quadratic regressions using untransformed data resulted in a better fit with the observed values and were, therefore, used to calculate the DT_{50} values. Based on this, calculated DT_{50} values were 2.7 months following spring application, 5.7 months following fall application, and 6.1 months following spring followed by fall application.

Seasonal variations in soil temperature may influence degradation processes. Microbial degradation is proposed to be the primary mode of degradation mechanism of the isoxaben molecule (Rouchaud et al. 1983). Microbial activity is affected strongly by soil temperature. Soil temperatures during the 6 months following fall application would be considerably lower than the 6-month period following spring application. Isoxaben dissipated faster during the first six months following spring application, as opposed to fall application, indicating that soil temperature played an important role in its degradation. Levels of herbicide in the top 3.8 cm of soil nine months following single applications were negligible. Timing of herbicide application is critical for effective weed mangagement. Weed control may improve due to buildup of isoxaben residues in the soil following multiple applications.

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Table 1. Precipitation and mean monthly air temperature data at Blacksburg for the studies conducted in 1994 to 1996[a].

Month	1994		1995		1996	
	Precptn. (cm)	Temp. (C)	Precptn. (cm)	Temp. (C)	Precptn. (cm)	Temp. (C)
January	-	-	19.6	1.2	15.4	-1.4
February	-	-	5.2	0.8	6.4	0.6
March	-	-	4.6	7.1	8.1	1.8
April	-	-	2.5	10.9	5.8	9.5
May	3.5	13.6	9.7	15.2	13.1	15.9
June	4.9	21.8	24.7	19.3	9.9	-
July	14.9	23.2	4.5	21.8	6.2	20.6
August	10.5	17.3	2.4	22.0	15.3	20.6
September	2.5	10.9	5.4	16.6	11.0	16.9
October	0	17.3	11.1	12.1	-	-
November	1.3	8.3	8.6	4.1	-	-
December	5.1	2.8	3.9	-0.1	-	-

[a] Hadean, K.D. 1995. Climatological data annual summary. National Oceanic and Atmospheric Admn. Ashville, NC 28801. Vol 105(13)1-9. Blank cells represent months outside the study period or unavailable data.

Table 2. Percent recovery of isoxaben for 1 ml standard solutions applied to 50 g soil.

Fortification standard (µg/ml)	Isoxaben in soil (µg/g)		Recovery (%)
	Expected	Observed	
0.00	0.00	0.00	-
0.00	0.00	0.00	-
0.25	0.005	0.0054	110.9
0.25	0.005	0.0049	98.8
0.50	0.01	0.0140	131.5
0.50	0.01	0.0130	125.5
1.25	0.025	0.0279	111.9
1.25	0.025	0.0278	111.1
Mean recovery from fortified samples			114.9

Table 3. Effect of isoxaben application timing on its dissipation from the top 3.8 cm of the soil.

Months after Treatment	Application timing	Isoxaben in soil[a] Rate of application (kg/ha) 0.56	0.84	1.12	Mean
			μg/g		
0	Spring	1.03	1.78	2.15	1.65
0	Fall	0.59	1.75	2.76	1.70
0	Spring + Fall	1.61	2.63	3.88	2.70
	L.S.D (0.05)	1.09	1.48	1.62	0.52
1	Spring	0.85	1.49	1.73	1.36
1	Fall	0.82	1.20	2.03	1.35
1	Spring + Fall	1.50	2.22	3.20	2.31
	L.S.D (0.05)	0.44	0.73	0.64	0.32
3	Spring	0.56	1.12	1.44	1.04
3	Fall	0.76	1.16	2.02	1.31
3	Spring + Fall	1.02	1.66	2.07	1.58
	L.S.D (0.05)	0.18	0.75	0.29	0.29
6	Spring	0.04	0.05	0.42	0.17
6	Fall	0.29	0.87	1.38	0.85
6	Spring + Fall	0.80	1.24	2.01	1.35
	L.S.D (0.05)	0.54	0.19	0.80	0.18
9	Spring	0.01	0.02	0.02	0.02
9	Fall	0.06	0.05	0.07	0.06
9	Spring + Fall	0.33	0.55	0.74	0.54
	L.S.D (0.05)	0.18	0.18	0.27	0.09

[a] When untreated plots were assayed, levels of isoxaben found were less than 0.03 μg/g at all evaluation intervals.

Table 4. Dissipation of fall-applied isoxaben from the top 3.8 cm of the soil in the second study.

Evaluation interval (MAT)	Isoxaben in soil — Rate of application (kg/ha) 0.0	0.56	0.84	1.12
	µg/g			
0	0.01	0.92	1.53	2.98
1	0.01	0.78	1.48	2.36
3	0.01	0.67	1.01	1.88
6	0.0	0.31	0.78	1.17
9	0.01	0.01	0.03	0.06
L.S.D (0.05)	0.02	0.34	0.36	1.02

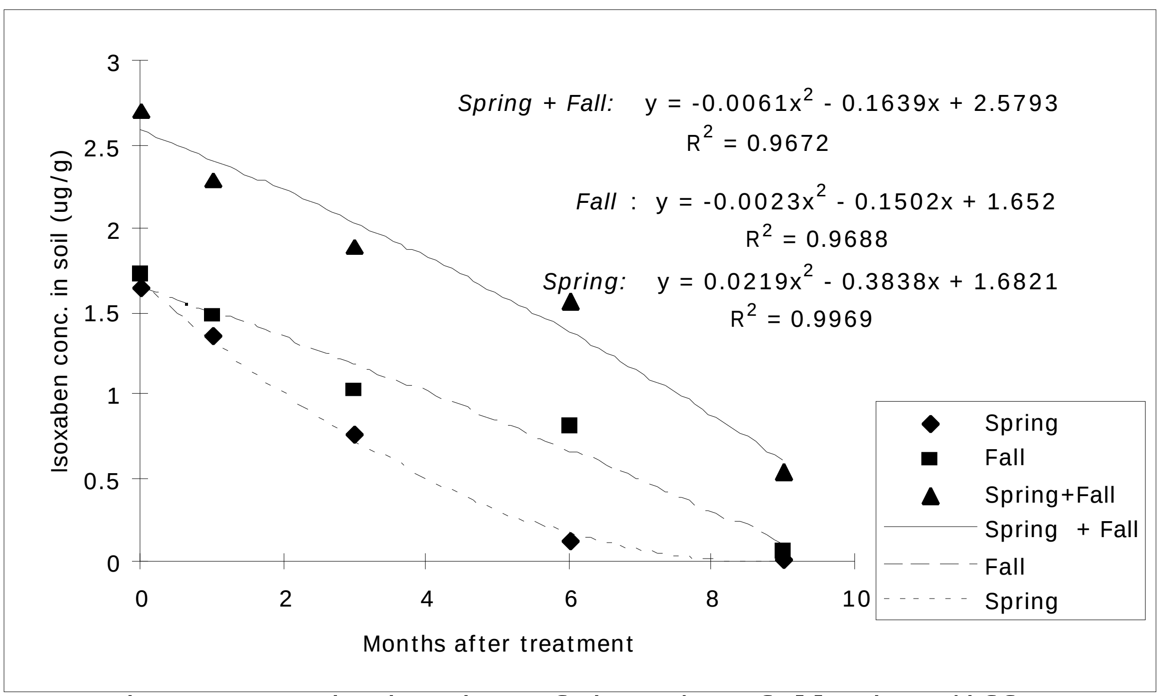

Figure 1. Dissipation of isoxaben following different application timings as predicted by regression.

CHAPTER IV

DISSIPATION OF ISOXABEN IN SOILS USING BIOASSAYS

Abstract: A soil bioassay experiment was conducted in Blacksburg, Virginia to determine the effect of isoxaben application timings and rates on duration of weed control. Flats containing soil were imbedded into the field. Isoxaben was applied in spring, fall, and spring followed by fall (double application) at 0.56, 0.84, and 1.12 kg ai/ha. Flats were moved to a greenhouse at 0, 1, 3, 6, and 9 MAT and seeded with yellow rocket, buckhorn plantain, and spotted spurge for the bioassays. Weed counts from treated flats were compared to those in untreated flats to determine percent control. Fall and spring followed by fall applications gave approximately 20% greater control of yellow rocket at 3 MAT and 30% greater control at 6 MAT, compared to a single spring application. Compared to spring application, fall and spring plus fall-applied isoxaben at 3 MAT gave about 15 and 20% greater buckhorn plantain control, respectively, and no improvement in spotted spurge control for any evaluation timing. Greater than 70% yellow rocket control was achieved with all isoxaben rates six months following fall treatment. The medium and high rate of isoxaben gave >70% control of buckhorn plantain for the first three months following fall application. Similar control of yellow rocket and buckhorn plantain was seen at the three isoxaben rates. Isoxaben gave longer term weed control compared to oxadiazon for the broadleaves tested. **Nomenclature:** Isoxaben, N-[3-(1-ethyl-1-methlypropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; oxadiazon, 3-[2,4-dichloro-5-(1-methylethoxy)phenyl]-5-(1,1-dimethylethyl)-1,3,4-oxadiazol-2-(3*H*)-one; buckhorn plantain, *Plantago lanceolata* L. # [9] PLALA; spotted spurge, *Euphorbia maculata* L. # EPHMA; yellow rocket, *Barbarea vulgaris* R. Br. # BARVU.
Additional index words: preemergence herbicides, residual activity, broadleaf weed control, soil bioassays.
Abbreviations: MAT, months after treatment; S, spring application; F, fall application; SF, spring plus fall application.

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[9] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds. Revised 1989. WSSA, 1508 West University Ave., Champaign, IL 61821-3133

Introduction

Isoxaben is a selective preemergence herbicide that controls broadleaf weeds in turf and ornamentals in the U.S. (Colbert and Ford 1987; Anonymous 1990). Although its exact mechanism of action in plants is still unclear, it is linked to processes involving cellulose biosynthesis and cell wall formation (Heim et al. 1991).

Annual broadleaf weeds were more sensitive than annual grasses to isoxaben, based on differences in absorption, translocation and target-site sensitivity (Cabanne et al. 1987). Isoxaben was effective in controlling broadleaves like speedwells (*Veronica* sp.), purple deadnettle (*Lamium purpureum* L. # LAMPU), violets (*Viola* sp.) and burning nettle (*Utrica urens* L. # URTUL), but ineffective in controlling several annual grasses in an apple orchard (Himme and Bulcke 1988). Isoxaben applied in fall at 0.52 to 0.84 kg/ha provided 80 to 100% control of common chickweed (*Stellaria media* L. Vill. # STEME), lawn burweed (*Soliva pterosperma* (Juss.) Less. # SOVPT), smallflower buttercup (*Ranunculus arbortivus* L. # RANAB), hop clover (*Trifolium campestre* Schreb. # TRFAU), and henbit (*Lamium amplexicaule* # LAMAM) (Grant et al. 1990). Control of parsley-piert (*Aphanes arvensis* L. # APHAR) and Carolina geranium (*Geranium carolinianum* L. # GERCA) ranged from 47 to 80% at these rates. In container and field-grown ornamentals, isoxaben at 0.56 kg/ha provided variable control of common groundsel (*Senecio vulgaris* L. # SENVU), dandelion (*Taraxacum officinale* Weber. # TAROF), yellow woodsorrel, and witchgrass (*Panicum capillare* L. # PANCA) (Neal and Senesac 1988). Only fair control of creeping woodsorrel was reported with isoxaben at 0.56 kg/ha; however, greater than 90% control of this weed was attained when the isoxaben rate was increased to 1.12 kg/ha.

The herbicidal activity of isoxaben was not lost following shallow incorporation into the soil, and greater than 75% of the compound remained in the top 0 to 7.5 cm of the soil (Colbert and Ford 1987). Soil degradation of isoxaben is primarily through microbial activity (Rouchaud et al. 1993). Solar breakdown of isoxaben has also been reported in aquatic systems (Mamouni et al. 1992). Wilson et al. (1996) documented that isoxaben collected from ponds after a runoff event dissipated faster under light conditions as opposed to darkness. In field degradation studies, the top 2 and 4 cm of soil contained 50 and 80%, respectively, of isoxaben at 4 to 5 MAT. Isoxaben applied in early winter had a half-life of 2.5 to 4 months (Huggenberger and Ryan 1985). In a different study, the half-life of fall applied isoxaben in unamended soils was 2.9 months (Rouchaud et al. 1993). Application timing of isoxaben affected duration of weed control (Huggenberger et al. 1982; Walker 1987). Walker documented the half-life of isoxaben as 2.5 months at a mean soil temperature of 20 C and 5.2 months at a mean soil temperature of 10 C.

A turnip (*Brassica rapa* L.) bioassay was used to determine isoxaben levels in field soil samples (Garcia et al. 1992). Lefebvre et al. (1987) determined that 0.0057 µg/ml of isoxaben inhibited radicle growth of the rape (*Brassica napus* L.) by 50%. Wheat (*Triticum aestivum* L.), a tolerant species, exhibited only 40% reduction in radicle length at 1.2 µg/ml of isoxaben.

Limited research was conducted in the U.S. to determine duration of weed control as an effect of isoxaben application timings and rates. The primary objective of the study was to elucidate differences in residual weed control as an effect of spring, fall, and spring followed by fall application. Isoxaben rates were varied to evaluate duration of control of common turf broadleaf weeds.

Materials and Methods

General Conditions. A field study was established at Blacksburg, VA in tilled soil using a split-plot design. Main plots were herbicide treatments and subplots consisted of evaluation timings. All treatments were replicated four times. The study was established in 1994 and was repeated in another field in 1995. Each study lasted for a period of 9 months following treatment. Plastic flats (45 cm by 32.5 cm by 7.5 cm) were filled with a Ross silt-loam soil (fine-loamy, mixed, mesic Cumilic Hapludoll) having a pH of 5.4 and an organic matter content of 3%. Flats were imbedded in the field sites at the soil level. Three rates of isoxaben (0.56, 0.84, and 1.12 kg ai/ha) were evaluated for PRE weed control and rate response. These rates represent the low, intermediate, and maximum use rates in turf (Anonymous, 1990). The three application timings evaluated were spring, fall, and spring followed by fall (double application). A 2% granular formulation of oxadiazon was applied at an intermediate rate of 3.34 kg/ha for comparison. Isoxaben, formulated as a 75% dry flowable, was applied using a CO_2-pressurized boom sprayer with

8003 flat fan nozzles[10] delivering 230 L/ha with water as the carrier. Oxadiazon was applied using a shaker jar.

Following herbicide treatments, the flats were transferred from the field to a greenhouse at 0, 1, 3, 6, and 9 MAT. For spring plus fall treatments, the flats were transferred to the greenhouse at the above time intervals after the second application. They were then seeded with buckhorn plantain (0.75 g), spotted spurge (0.5 g), and yellow rocket (0.5 g) after disturbing the soil surface to reduce compaction. Flats were misted at frequent intervals until seedlings were established. Subsequently, they were irrigated to field capacity on a daily basis. After seedling emergence, the flats were fertilized with a 20-20-20 liquid fertilizer to provide nitrogen at 5 g/m^2. Average day/night temperatures in the greenhouse were 35/25 C, and ambient light level at noon was approximately 650 $\mu mol/m^2/sec$.

Weed counts were taken 3 weeks after seeding and shoot fresh weights were recorded at 8 weeks after seeding. Weed counts taken at three weeks after seeding were more indicative of the preemergence effects, and therefore, only weed counts are reported for weed control. Percent weed control was calculated by comparing weed counts from untreated plots to treated plots. Data were subjected to a two-way analysis of variance (ANOVA) and the means were separated by the Least Significant Difference (LSD) test at the 0.05 probability level. Data from the first study was analyzed to determine differences in weed control as affected by the herbicide rates and application timings at 0, 1, 3, 6, and 9 MAT. The second study was analyzed to determine weed control responses following a single fall application of three rates of isoxaben. Three species of weeds were chosen based on their sensitivity to isoxaben: yellow rocket (most sensitive), buckhorn plantain (intermediate), and spotted spurge (least sensitive).

Study 1. Application timings evaluated in this study were spring, fall, and spring followed by fall. Spring treatments were applied on June 17, 1994. Cloud cover was 25%, air temperature was 27 C, and soil temperature at a 5 cm depth was 25 C. Wind velocity at the time of application was 0 to 5 km/h. First precipitation after treatment occurred 6 days later and totaled 2.8 cm. Fall, and spring followed by fall treatments were applied on October 7, 1994. Cloud cover was 100%, air temperature was 15 C, soil temperature at a 5 cm depth was 22 C, and there was no

[10]TeeJet 8003 flat fan spray nozzles, Spraying Systems Co., North Ave., Wheaton, IL 60788

wind. First precipitation after treatment occurred 6 hours later and totaled 0.5 cm.

Study 2. Spring treatments were applied on June 5, 1995. Cloud cover was 10% with no wind, air temperature was 28 C, and soil temperature at a 5 cm depth was 19 C. A total precipitation of 25.1 cm fell within two weeks after treatment. Fall treatments were applied on October 30, 1995. Cloud cover was 100% with no wind, air temperature was 13 C, and soil temperature at a 5-cm depth was 16 C. First precipitation after treatment occurred 3 days later and totaled 2.2 cm.

Results and Discussion

Yellow Rocket. Isoxaben applied at all three timings in 1994 gave good to excellent control of yellow rocket at 0 and 1 MAT (Table 1). Isoxaben at all rates applied in spring gave lower weed control compared to fall, and spring followed by fall, at 3 MAT. Similar results were observed at 6 MAT for isoxaben applied at 0.84 and 1.12 kg/ha. At 9 MAT, double application at 1.12 kg/ha gave better control compared to single spring application, although these control levels were unacceptable. Oxadiazon gave good to excellent control at 0 and 1 MAT at all three application timings but control decreased at later evaluation intervals. However, isoxaben gave acceptable yellow rocket control for 6 MAT. In general, similar yellow rocket control was obtained from all three rates of isoxaben.

Following a single fall application of isoxaben in 1995, greater than 75% yellow rocket control was achieved at 6 MAT for all rates of isoxaben (Table 2). However, poor control was obtained at 9 MAT. These results were comparable to that from the previous year. In general, there was little decrease in yellow rocket control from 0 to 6 MAT in both studies. The 6 to 9 month period corresponds to the months of May to July when soil temperature and precipitation levels were higher (data not shown). Herbicide dissipation rates through microbial degradation and soil leaching may be higher during this time period. As noted with the first study, yellow rocket control did not differ among isoxaben rates. Following fall application, oxadiazon gave lower levels of yellow rocket control compared to isoxaben at essentially all evaluation intervals.

Buckhorn plantain. Isoxaben applied at 0.84 and 1.12 kg/ha gave greater than 90% control of buckhorn at 0 MAT (Table 3). Control levels decreased numerically at 1 MAT regardless of application timings. Differences in control among application timings were apparent at 3 MAT, when the double application of isoxaben gave better control compared to single spring application. At this time, fall-applied isoxaben tended to give better control than a spring application, especially at the highest rate tested. These trends were also observed for the 6 MAT evaluation. At 9 MAT, poor control of buckhorn plantain was seen at all rates of isoxaben. Isoxaben applied at 1.12 kg/ha gave good to excellent control of buckhorn plantain at 0 and 1 MAT. Compared to yellow rocket (Table 1), control of buckhorn plantain started to diminish at 3 MAT for the spring application timing, and at 6 MAT for the fall application timing. Application rates of isoxaben did not influence buckhorn plantain control at 0 MAT. These results may indicate that buckhorn plantain was less sensitive to isoxaben than yellow rocket.

Fall application of isoxaben in 1995 revealed no differences in weed control among 0, 1 and 3 MAT for the highest rate tested (Table 4). However, all three rates of isoxaben gave lower weed control at 6 MAT compared to 3 MAT. These results suggest that the maximum use rate is required to maintain buckhorn plantain control for 3 MAT.

Spotted spurge. All isoxaben rates gave less than 90% control of spotted spurge 0 MAT after spring or fall application (Table 5). This demonstrates that this species is more tolerant to isoxaben than yellow rocket and buckhorn plantain. Less than 80% control of spotted spurge was obtained at 1 MAT from all application timings and rates. Poor control of this weed was also seen at 3 MAT from all isoxaben treatments. For 6 and 9 MAT, spotted spurge in the untreated flats failed to germinate for the fall and spring plus fall treatments. It is speculated that the same trend would be exhibited at these evaluation timings as well.

After fall 1995 application of isoxaben, unacceptable control of spotted spurge was determined for all application rates of isoxaben used (Table 6). No differences in control were observed among application rates at all evaluation timings.

Percent control data obtained from the soil bioassays for yellow rocket and buckhorn plantain were correlated with quantified soil residues of isoxaben determined by HPLC analysis. Correlation coefficients of 0.85 for yellow rocket and 0.89 were determined for buckhorn plantain. This suggests that yellow rocket and buckhorn plantain may serve as useful weed species when using soil bioassays to study isoxaben.

Application timing influenced yellow rocket control at 3 and 6 MAT, where fall-applied isoxaben gave longer residual control than spring control. Buckhorn plantain control was higher at 3 MAT from fall-applied isoxaben as opposed to spring control. After a single fall treatment, isoxaben controlled yellow rocket acceptably for 6 MAT, whereas, buckhorn plantain control was acceptable only for 3 MAT. Timing of herbicide application did not influence spotted spurge control due to the low sensitivity of this weed to isoxaben. These results reflect the sensitivity of the respective species to isoxaben and the effect of application timings. The primary mechanism of isoxaben dissipation in soils is microbial activity (Rouchaud et al. 1993). The cooler winter months following fall application may have caused slower microbial degradation of the herbicide. This would explain the longer weed control from fall application compared to spring treatment. Double application of isoxaben demonstrated better weed control compared to spring application, and numerical increases compared to fall treatments.

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