INFLUENCE OF ISOXABEN APPLICATION TIMING ON DISSIPATION AND BROADLEAF WEED CONTROL IN TURF

by

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Plant Pathology, Physiology, and Weed Science

(ABSTRACT)

Isoxaben is a preemergence (PRE) broadleaf herbicide used in turf and ornamentals. Field, greenhouse, and laboratory research evaluated this herbicide for PRE control of selected broadleaves in turf, suspected postemergence (POST) herbicidal effects, and the influence of application timings and rates on soil residual. During seed germination in moist filter paper, isoxaben concentrations required for 50% inhibition of radicle growth (GR_{50}) were 0.013, 0.010, 0.008, 0.008, and 0.007 μg/ml for dandelion, buckhorn plantain, white clover, black medic, and common lespedeza, respectively. In greenhouse experiments, isoxaben applied POST at 2.24 kg ai/ha suppressed the growth of Florida betony, black medic and white clover by 45, 65, and 66%, respectively, and reduced regrowth of Florida betony by 71%. In soil bioassays, yellow rocket control from isoxaben applied in fall was approximately 20 and 30% greater than spring-applied isoxaben at 3 and 6 MAT, respectively. Buckhorn plantain control from fall treatments at 3 MAT was approximately 15% higher than spring-applied isoxaben at 3 MAT. Application timings did not influence control of spotted spurge, a less sensitive weed. Isoxaben applied to turf in spring at 1.12 kg/ha provided > 90% control of buckhorn plantain, dandelion, and corn speedwell at 4 MAT. Fall applied isoxaben at the same rate provided total control of common chickweed, corn speedwell and henbit at 3 MAT and 80 to 90% control of white sweet clover and buckhorn plantain that germinated the following spring. Double (spring followed by fall) application of isoxaben to turf appeared to enhance broadleaf weed control in some instances. Dissipation of isoxaben in the top 3.8 cm of a Ross silt-loam soil as affected by spring, fall, and spring followed by fall applications was determined using high performance liquid chromatography (HPLC) analysis. Isoxaben residues in soil decreased by 55 and 92% by 3 and 6 MAT, respectively, for spring treatments, and decreased 29 and 52% by 3 and 6 MAT for fall treatments, respectively. A soil-bioassay study correlated well with chemical analysis of isoxaben residues, as the correlation coefficients were 0.85 and 0.89 for yellow rocket and buckhorn plantain, respectively.

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INTRODUCTION

Weed Control in Turf

Weed control in lawns and other turf areas perhaps directly affects more people in the United States than any other crop (Ashton and Monaco 1991). About two million hectares are estimated to be in home lawns and an additional four million hectares are covered by other types of turf. Weeds reduce the aesthetic value of turfgrass, compete for water, nutrients, and sunlight, and may serve as alternate hosts for insect pests or disease agents (Bingham et al. 1995).

Weed control in turfgrasses is achieved through physical and cultural practices that enhance the growth of a dense, vigorous, and competitive turf (Beard 1979). Common weeds in turf are classified based on life cycles as annuals, biennials, and perennials, or on the basis of the subclasses to which they belong, as monocots (grasses and grass like species) and dicots (broadleaves). Weed control can be improved through use of herbicides.

Herbicides used in turfgrass are classified based on weed control spectrum and application timing. Annual grasses are controlled preemergence (PRE) through the use of dinitroaniline family of herbicides or other PRE chemicals, or, postemergence (POST) using several graminicides or arsenicals. Broadleaf weeds attract more attention in turfgrasses because of their contrasting appearance. Control of these weeds before emergence may, therefore, prove to be beneficial.

Chemical Control of Broadleaf Weeds in Turf

Current chemical control strategies for broadleaf weeds in turf primarily utilize POST herbicides. Postemergence control of a wide range of broadleaf weeds is achieved mostly through two or three way combinations of growth-regulator type herbicides (Bingham 1990). However, certain PRE herbicides can be used to control these weeds.

Most of the PRE herbicides used in turfgrasses are targeted to control annual grasses. Some of the PRE crabgrass (*Digitaria* sp.) herbicides, including oxadiazon, benefin, and prodiamine, control certain small-seeded broadleaf weeds (Bingham and Hipkins 1997). However, PRE herbicides that selectively control a wide range of broadleaf weeds in turf are limited. Use of a selective

PRE broadleaf herbicide with extended residual activity could offer several advantages. These benefits may include better turf appearance from control of weeds before emergence, possible reduction in number of herbicide applications, season-long weed control, and consequent decrease in weed seed production.

Isoxaben, (N-[3-(1-ethyl-1-methlypropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide), is a PRE, broad spectrum, residual herbicide discovered in 1979, and used initially and currently in Europe for broadleaf weed control in winter cereals (Huggenberger et al. 1982). Due to PRE control of winter annuals in cereal crops using isoxaben, yield increases of 0.22 t/ha of winter barley (*Hordeum vulgare* L.), and 0.14 t/ha of winter wheat (*Triticum aestivum* L.) when compared to plots that received selective POST herbicide treatments in spring, and increases of 0.30 t/ha of wheat and 0.26 t/ha of barley when compared to untreated plots, were obtained (Huggenburger et al. 1982). In the U.S., this herbicide is registered for control of broadleaf weeds in turf, ornamentals, nursery stock, non-bearing fruit and nut trees, non-bearing vines, Christmas plantations and non-cropland areas (Anonymous 1990). Most established turfgrass species can tolerate isoxaben at normal use rates of 0.56 to 1.12 kg/ha. Isoxaben did not cause any turf injury to ‘Common’ and ‘Tifton 419’ bermudagrass (*Cynodon dactylon* (L.) Pers.), and bahiagrass (*Paspalum notatum* Fluegge.) (Grant et al. 1990). Several cool season grasses including Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), bentgrass (*Agrostis* sp.), and fescue (*Festuca* sp.) tolerated isoxaben up to 2.24 kg/ha (Colbert and Ford 1987).

Preemergence Weed Control Spectrum of Isoxaben

Preemergence activity of isoxaben was documented in several field trials. In a turf study to determine weed control spectrum, isoxaben applied at 0.56 to 0.84 kg ai/ha provided 80 to 100% control of common chickweed (*Stellaria media* L.), lawn burweed (*Soliva pterosperma* Juss. Less.), smallflower buttercup (*Ranunculus arbortivus* L.), large hop clover (*Trifolium campestre* Schreb.), and henbit (*Lamium amplexicaule* L.) (Grant et al. 1990). Complete control of prostrate knotweed (*Polygonium aviculare* L.) that germinated in spring was achieved from isoxaben applied at 0.84 kg/ha during the previous fall (Neal 1997). Greater than 90% control of dandelion (*Taraxacum officinale* Weber. in Wiggers.), mouseear chickweed (*Cerastium vulgatum* L.), speedwells (*Veronica* sp.) and ground ivy (*Glechoma hederacea* L.) was observed up to 8 months after a combination treatment of isoxaben at 1.12 kg/ha with a commercial herbicide mixture containing 2,4-D, mecoprop, and dicamba (Keese and Forth 1997).

Jagschitz and Sawyer (1988) reported the activity of certain preemergence herbicides for extended broadleaf weed control in turf, in field experiments conducted in Rhode Island. Isoxaben applied in fall at 0.14 kg/ha provided almost complete control of henbit, common chickweed, and purslane speedwell (*Veronica peregrina* L.) when evaluated 10 months after treatment (MAT). Oxadiazon applied at 2.8 kg/ha gave almost 90% control of these weeds at 10 MAT. Extended activity of these herbicides may be attributed to the low mean soil temperature at the test site in Rhode Island.

In field nursery crops, Derr (1993) reported that isoxaben controlled most annual broadleaf weeds except morningglory (*Ipomoea hederacea* (L.) Jacq.) and common lambsquarters (*Chenopodium album* L.). Isoxaben provided nearly total control of common groundsel (*Senecio vulgaris*) and Virginia pepperweed (*Lepidium virginicum* L.) in container-grown ornamentals (Gallitano and Skroch 1993).

Isoxaben applied at 0.56 kg/ha controlled creeping woodsorrel approximately 70%, however, greater than 90% control was attained when the rate was increased to 1.12 kg/ha. Isoxaben did not control common mallow (*Malva neglecta* Wallr.) and velvetleaf (*Abutilon theophrasti* Medicus.) (Neal and Senesac 1988).

Isoxaben at 0.84 kg/ha provided excellent control of red sorrel (*Rumex acetosella* L.), smooth pigweed (*Amaranthus hybridus* L.), Pennsylvania smartweed (*Polygonium pensylvanicum* L.) and common ragweed (Setyowati et al. 1995). In this field-ornamental study, isoxaben was not effective in controlling velvetleaf, hairy galinsoga and prickly sida (*Sida spinosa* L.).

Isoxaben is compatible with a wide range of PRE and POST herbicides. Such combinations allow for broader spectrum of PRE control or PRE plus POST weed control. In ornamental grasses, isoxaben applied in combination with prodiamine controlled smooth crabgrass (*Digitaria ischaemum* Schreb. ex Muhl.), prostrate spurge (*Euphorbia humistrata* Englem. ex Gray), common groundsel, and hairy galinsoga (*Galinsoga ciliata* (Raf.) Blake) (Neal and Senesac 1990 a). Isoxaben applied in fall at 0.08 kg/ha in combination with cyanazine at 0.75 kg/ha gave season long control of annual bluegrass (*Poa annua* L.), chamomile (*Matricaria* spp.), and speedwells. This combination treatment applied PRE in spring also controlled common hempnettle (*Galeopsis tetrahit* L.) and prostrate knotweed (Huggenberger et al. 1982). Isoxaben suppressed certain annual grasses when applied PRE. It provided 58-65% control of crabgrass, goosegrass (*Eleusine indica* (L.) Gaertn.), and fall panicum (*Panicum dichotomiflorum* Michx.) (Neal and Senesac 1990 b). Isoxaben, when applied in combination with oryzalin at 4.48 kg/ha provided a broader spectrum of grass control in this study.

Isoxaben possesses a similar spectrum of activity as the triazine herbicides simazine and atrazine, and could therefore be used to control triazine-resistant weeds. Triazine-resistant common groundsel found in apple orchards and conifer nurseries was effectively controlled by isoxaben at 0.5 kg/ha (Himme and Bulcke 1988).

Sensitivity of Weeds to Isoxaben Applied Postemergence

Isoxaben injured certain weeds when applied POST (Neal and Senesac 1994; Elmore and Breuninger 1991). Isoxaben applied POST was more effective on healall (*Prunella vulgaris* L.) and ground ivy under field conditions than under greenhouse conditions. In the field, isoxaben at 2.24 kg/ha controlled ground ivy and healall at 99 and 85%, respectively, which was about 65 and 35% greater control, respectively, than the control observed in the greenhouse. In this study, POST activity was not enhanced by adding clopyralid or triclopyr to isoxaben. Triclopyr and clopyralid alone gave only 10 to 20% control of healall (Neal and Senesac 1994).

Schneegurt et al. (1993) determined POST effects of isoxaben on several broadleaf and grass species. Among the grasses studied, barnyardgrass (*Echinochloa crusgalli* L.) was found to be very sensitive. Isoxaben applied POST at rates greater than 1 kg/ha produced more marked effects when applied both to soil and foliar when compared to any one form of application. Only 0.08% of the absorbed isoxaben translocated to shoot tissues from leaves, which implied that symplastic translocation of isoxaben was minimal. Literature on the POST activity of isoxaben and its effectiveness when tank mixed with growth-regulator type herbicides is limited.

Since isoxaben possesses a different mode of action than growth-regulator type herbicides, combining the two may be effective for POST broadleaf control. Isoxaben applied alone POST at 1.12 kg/ha did not reduce yellow woodsorrel or spotted spurge cover (Elmore and Breuninger 1991). However, tank mixing isoxaben with triclopyr gave better control of yellow woodsorrel but did not improve control of spotted spurge. The phytotoxic action of POST growth-regulator type herbicides is based on their ability to mimic the activity of endogenous auxins in plants. Sensitive plants exhibit uncontrolled growth leading to plant death. Induction of abscisic acid (ABA) was a common effect in plants treated with these herbicides (Grossmann et al. 1996). The authors suggested that this may play a role in growth inhibition of susceptible plants.

Tank mixing two or more herbicides could result in equal to, greater than, or less than the expected, additive effects (Devine et al. 1993). Synergism was reported when auxin-type herbicides were combined with herbicides having a different mode of action. Field bindweed (*Convolvulus arvensis* L.) control was enhanced when glyphosate was tank mixed with 2,4-D or dicamba (Flint and Barrett 1989). The combination treatment caused greater inhibition of root and shoot growth compared to injury from individual components. The authors attributed this effect to increased uptake and transport of glyphosate due to the activity of auxin-type herbicide. Simazine and oxadiazon improved the POST efficacy of 2,4-D, dicamba, and mecoprop when applied as a tank-mix for the control of Virginia buttonweed although these PRE herbicides applied alone did not control this particular weed (Merrit et al. 1986). The combination treatment improved the control of common chickweed than that obtained when mecoprop was applied alone.

Mode of Action and Basis for Selectivity of Isoxaben

The exact mechanism of action of isoxaben in plants is still unclear but is thought to affect cellulose biosynthesis and cell wall formation. Isoxaben severely inhibited the incorporation of radiolabelled glucose into the cell wall (Heim et al. 1991). Cabanne et al. (1987) compared its behavior in wheat, a tolerant monocot, and rape, a sensitive dicot, under controlled conditions. Rape absorbed more isoxaben compared to wheat and translocated less to the shoots. They concluded that selectivity was partly due to differential absorption into the roots where the herbicide exerts its phytotoxic effect and partly due to differential translocation to the shoot where the herbicide is metabolized. Catchweed bedstraw (*Galium aparine* L.), redroot pigweed (*Amaranthus retroflexus* L.), and velvetleaf tolerated isoxaben following POST application through decreased sensitivity at the target site (Schneegurt et al. 1994).

Growth Inhibition by Isoxaben

Petri-dish bioassays were used to determine the concentration of a herbicide that inhibited growth of a weed species by half

(GR_{50}). Petri-dish studies indicated that radicle length of scentless chamomile (*Matricaria perforata* Merat), lady's thumb, common chickweed, Persian speedwell (*Veronica persica* Poir.), and field violet (*Viola arvensis* Murr) was inhibited by 50% when exposed to isoxaben concentrations ranging from 0.08 to 0.10 ppm (Huggenberger and Ryan 1985). Lefebvre et al. determined that 0.0057 ppm of isoxaben inhibited growth of rape (*Brassica napus* L.) by 50%. Isoxaben did not prevent the germination of cucumber (*Cucumis sativus* L.) , lettuce (*Lactuca sativa capitata* L.) or radish (*Raphanus sativus* L.) seeds at concentrations up to 3.32 ppm. Wheat (*Triticum aestivum* L.) tolerated up to 1.2 ppm of isoxaben before its growth was reduced by 40%.

Attributes of Isoxaben Injury

Injury from isoxaben to cucumber seedlings (*Cucumis sativus* L.) in a bioassay study were swelling of the apical region and a progressive disappearance of the meristematic region of the root (Lefevbre et al. 1987). Isoxaben caused swelling of roots and hypocotyls, asymmetrical growth of tissues, browning of roots and vascular tissue in the hypocotyl and subsequent plant death in common chickweed (*Stellaria media* L), and lady's thumb (*Polygonum persicaria* L.) seedlings (Huggenberger and Ryan 1985). Following POST application, isoxaben caused leaf malformation, general inhibition of growth and a reduction in height and vigor of susceptible weed species (Schneegurt et al. 1994).

Dissipation of Isoxaben in Soils

Degradation of a herbicide is a process by which the molecule is structurally transformed through biotic and abiotic processes to inorganic end products (Devine et al. 1993). Dissipation includes degradation along with other physical processes that may remove the herbicide from the site of application. Processes that govern herbicide dissipation include leaching, microbial and thermal degradation, chemical decomposition, runoff, volatilization, and plant uptake.

Isoxaben has a low water solubility of 1.0 mg/L and a vapor pressure less than 3.9×10^{-7} mm Hg at 26 C (Rutherford 1983). Therefore, the primary mechanism of isoxaben dissipation in soils is microbial activity (Rouchaud et al. 1993b). Microbes derive carbon through degradation of herbicide molecules (Landis and Yu 1995).

Isoxaben is a nonionic molecule, classified as a benzamide (Ahrens 1994). Isoxaben requires activation after application either through a light cultivation or a minimum precipitation of 1.3 cm within three weeks after treatment. Isoxaben adsorbs to the soil and possesses moderate to long residual activity (5 to 6 months).

Soil persistence of isoxaben has been reported by several authors. Rouchaud et al. (1993 a, b) determined the half-life of isoxaben in unamended soils as 2.9 months and in plots treated with pig-slurry as 6.6 months. The study indicated that organic fertilizers increased the persistence of isoxaben by adsorbing the herbicide molecules. After isoxaben was fall-applied at 125 g/ha of isoxaben, they quantified 33% and 25% of the herbicide in the top 10 cm of soil at 6 and 9 MAT, respectively, in unamended soils. They also found that the dissipation rate of isoxaben was greater in spring and summer months than during winter. Isoxaben was not detected in the 10 to 20 cm depth of soil during the study. They identified six metabolites of isoxaben that were formed after application of isoxaben in winter.

The herbicidal activity of isoxaben was not lost from shallow incorporation of this compound into the soil, and greater than 75% of the compound remained in the top 0 to 7.5 cm (weed germination zone) profile of the soil (Colbert and Ford 1987). Their research indicated that the soil half-life of isoxaben was approximately 5 months, with 20% of the applied herbicide persisting after 12 months.

Isoxaben runoff was monitored in container nursery production after isoxaben plus trifluralin was applied as a granular formulation (Wilson et al. 1996). Greatest quantities of isoxaben in runoff were noted during the first irrigation following application. The runoff water contained about 0.75 μg/ml of isoxaben during the first 15 min following herbicide application, which amounted to 9.2%. The isoxaben in runoff water collected in a pond dissipated via photodegradation to negligible quantities within 60 days after treatment. The authors noted that isoxaben dissipation was significantly higher under light conditions as opposed to darkness.

Walker (1987) assessed the persistence and movement of isoxaben in a sandy loam soil under field conditions. 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. The half-life increased by almost 70% at 20 C when the soil moisture was decreased by 60%. His studies showed that 30 to 40% of the initial dose remained in the top 5 cm of soil, either four months after spring treatment or six months after fall treatment.

Soil mobility and half-life studies using isoxaben have produced variable results. Huggenberger and Ryan (1985) found that about 50% of isoxaben remained in the top 2 cm of field soil and that 85% of the compound remained within the top 4 cm of the soil column at 4 to 5 MAT. In this study, isoxaben applied in early winter lasted in the soil with 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 with similar application timings (Huggenberger et al. 1982). In a different study, Winkler and Huggenberger (1986) reported that only 25% of isoxaben remained in the top 2 cm of soil 4 to 5 months after application. The remaining fraction was believed to be degraded or leached further down the soil profile. It is speculated that such variations may be due to differences in microbial activity of these locations. Dissipation of most herbicides is governed largely by the geographical location and prevailing environmental conditions (Cheng and Lehmann 1985). They have emphasized the risks of generalizing dissipation results from a particular location under given environmental conditions.

Quantification and Bioavailability of Herbicides

Isoxaben in the soil can be quantified by extracting and purifying the parent compound, and using high pressure liquid chromatography (HPLC) to determine the actual concentrations. Rutherford (1993) has described analysis of isoxaben extracted from commercial formulations, soil, water, grain, and straw.

An alternate method but less accurate method is through the use of soil bioassays. Soil bioassays have advantages in that they do not require sample cleanup and may provide information about levels of herbicides in the soil that are bioavailable (O’Bryan et al. 1994). Hsio and Smith (1983) used oat seedlings to assay residues of diclofop and sethoxydim in treated soils, and maize seedlings to determine residues of the herbicide chlorsulfuron. Their detection limits were 0.001, 0.2, and 0.05 ppm for chlorsulfuron, diclofop, and sethoxydim, respectively. Dev et al. (1992) used a root growth bioassay with sorghum (*Sorghum halepense* L.) and wheat (*Triticum aestivum* L.) to detect concentration of pendimethalin and fluchloralin in soils. Root growth of pre-germinated seedlings was dependent on herbicide concentration. The phytotoxic residue levels of a sulfonyl urea herbicide (DPX-A7881) in the soil were determined by a lentil radicle bioassay (Beckle and McKerder 1988). Residues in the soil caused a reduction in taproot length and the number of primary lateral roots. The persistence of imidazolinone herbicides and clomazone in soils was monitored using corn root growth as an indicator of soil residue levels (Loux et al. 1988). Garcia et al. (1992) used a turnip (*Brassica rapa*) bioassay to determine isoxaben levels in field soil samples.

Photodegradation of Isoxaben

Although degradation of isoxaben in soils is primarily through microbial activity, solar radiation can transform this molecule in an aqueous system (Mamouni et al. 1992). Aqueous isoxaben underwent transformation to form eight product with three of them being isoxaben isomers of unknown phytotoxicity and five being breakdown products (Mamouni et al. 1992). In comparison to biotic degradation, 2,6-dimethoxybenzamide was the only common breakdown product (Rouchaud et al. 1993b). Both studies acknowledged that the presence of organic matter or humic acid increased the stability of the parent molecule, either due to adsorption of the herbicide molecule to, or absorption of light energy by, organic matter. Solar breakdown of isoxaben applied in the field was considered negligible. Isoxaben runoff collected in pond water dissipated faster under light conditions as opposed to darkness (Whitwell and Riley 1996).

Rationale and Objectives

Objective 1. Radicle elongation following germination is critical for root development and subsequent plant growth. The concentration of isoxaben that effectively inhibits radicle elongation of a given broadleaf species can be used to determine weed species sensitivity. The concentration of isoxaben required to inhibit radicle elongation by 50% for common broadleaf weeds was unknown. The first objective was to determine the response of broadleaf weed germination and radicle elongation to varying concentrations of isoxaben. Dose response curves were used to calculate GR_{50} values (concentration of isoxaben required to reduce radicle length by 50%). Common weeds in turf were chosen for this experiment.

Objective 2. Although isoxaben is a preemergence herbicide, it injures certain weeds when applied postemergence. The postemergence effect of isoxaben in certain broadleaf weeds in turf was unknown. The possible benefits of combining isoxaben with growth-regulator type herbicides has not been elucidated.

The second objective was to determine postemergence effects of isoxaben on certain broadleaf weeds in turf when applied alone or as a tank mix with growth-regulator type herbicides.

Objective 3. Isoxaben is known to possess residual activity for broadleaf weed control. In the U.S., research on the effectiveness of isoxaben for long term weed control as affected by timing and rates of application was limited. The effect of double application on its soil residual was also not elucidated. The third objective was to determine the effect of application timing and rates on residual weed control which was approached with three separate experiments.

Firstly, to determine the longevity of isoxaben in soil as affected by spring, fall, and spring plus fall treatments through chemical analyses.

Secondly, to determine differences in weed control as affected by isoxaben application timings using soil-bioassays in a greenhouse, which made it possible to measure plant growth responses in winter.

Thirdly to determine the effect of isoxaben application timings and rates on duration of broadleaf weed control in turfgrass.

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CHAPTER I

EFFECT OF ISOXABEN ON RADICLE ELONGATION IN BROADLEAF WEEDS

Abstract: Suppression of radicle elongation is a good indicator of species sensitivity to isoxaben. A bioassay was conducted to predict isoxaben concentrations that reduced radicle elongation of certain broadleaf weeds by half (GR_{50}). The radicle length of black medic, buckhorn plantain, common lespedeza, dandelion, and white clover was measured after a 48 or 120 hour period exposure to isoxaben concentrations of 0.005 to 10.24 µg/ml. GR_{50} values were calculated for each species by performing linear regression on the natural logarithm of radicle length against that of herbicide concentration. Based on reduction of radicle length, GR_{50} values (in µg/ml) were determined as: dandelion, 0.013; buckhorn plantain, 0.010; white clover, 0.008; black medic, 0.008; and common lespedeza, 0.007. In general, the leguminous weeds were more sensitive to isoxaben than dandelion and buckhorn plantain at low concentrations of the herbicide. Isoxaben caused swelling of meristematic tissues and discoloration of the elongating primary roots. All five species studied demonstrated initial signs of germination even at the highest rate of isoxaben but further radicle growth was arrested. **Nomenclature:** Isoxaben, N-[3-(1-ethyl-1-methlypropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; black medic, *Medicago lupilina* L. #[1] MEDLU; buckhorn plantain, *Plantago lanceolata* L. # PLALA; dandelion, *Taraxacum officinale* Weber. # TAROF; common lespedeza, *Lespedeza striata* Thunb. # LESST; white clover, *Trifolium repens* L. # TRFRE.
Additional index words: Preemergence herbicide, GR_{50}, broadleaf weed, bioassay, EL-107.
Abbreviations: GR_{50} , Growth reduction by 50%; HAT, hours after treatment; PRE preemergence; POST postemergence.

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[1] 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

Bioassays are inexpensive, yet effective tools to evaluate the phytotoxicity of a given herbicide, determine its breakdown in soil, and to detect bioavailable fractions of the herbicide before planting a sensitive crop (Lavy and Santelmann 1986). Bioassay techniques have been developed for a number of preemergence (PRE) and postemergence (POST) herbicides which included soil bioassays, foliar bioassays, *in-situ* bioassays, and the use of lower orders of autotrophic organisms as well as microorganisms (Leonard et al. 1962; Muzik and Whitworth 1963; Blackman and Robertson-Cuninghame 1955; Murray et al. 1969; Addison and Bardsley 1968; Holly and Roberts 1963).

One type of bioassay to determine herbicide concentrations in an aqueous solution utilized petri-dishes (Swanson 1946). Petri-dish bioassays utilized growth parameters like primary root and shoot lengths to determine phytotoxicity of herbicides. This technique was used to study 2,4-D (Ready and Grant 1947), diphenamid (Horowitz and Hulin 1974) and certain herbicides in the dinitroaniline family (Jacques and Harvey 1974).

Biosassays were used to determine sensitivity of certain winter annuals in cereal crops to isoxaben (Lefebvre et al. 1987; Huggenberger and Ryan 1985). Bioassays for isoxaben were developed using seeds of rape (*Brassica napus* L.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa capitata* L.), and radish (*Raphanus sativus* L.) (Lefebvre et al. 1987). Wheat, (*Triticum aestivum* L.), a tolerant species, was used for comparison in this study. Huggenberger and Ryan (1985) measured radicle growth of lady’s thumb (*Polygonum persicaria* L.), common chickweed (*Stellaria media* L.), Persian speedwell (V*eronica persica* L.), and field violet (*Viola arvensis* L.) as affected by various concentrations of isoxaben using petri-dish and sand culture tests.

Sensitivity of common broadleaf weeds in turfgrasses to isoxaben is not well documented. The objective of this study was to compare the sensitivity of black medic, buckhorn plantain, dandelion, common lespedeza, and white clover, which are common turf weeds, to isoxaben. The concentration of isoxaben that reduced radicle growth by 50% (GR_{50}) was determined for each species using a petri-dish bioassay.

Materials and Methods

A 10.24 µg ai/ml stock suspension of isoxaben was prepared using the commercial 75% dry flowable formulation of the herbicide in distilled water. Through serial dilutions, 11 concentrations ranging from 0.005 to 5.12 μg/ml, were prepared. Petri-dishes with diameters of 10 cm were used for the study. One piece of circular filter paper was placed in each petri-dish and 10 ml of each suspension was added. Twenty seeds each of dandelion, buckhorn plantain, black medic, common lespedeza, and white clover were placed on the filter paper in separate petri-dishes. Lids were fastened with parafilm to facilitate air exchange and to prevent vapor loss. The study was repeated twice in a one-week period. The repeated studies were analyzed as replications. The petri-dishes were placed in an incubator maintained at 30 C throughout the study period. Radicle length was measured for each species after allowing sufficient time for radicle growth (see results). Data were subjected to an analysis of variance and means were separated using Least Significant Differences (LSD) at $p = 0.05$. Radicle growth inhibitions to varying concentrations of isoxaben were subjected to regression analysis to determine dose-responses. Natural logarithms of radicle lengths were also regressed against that of isoxaben concentrations to generate linear models. Based on these linear models, the concentration of isoxaben that inhibited radicle length of each weed species by 50% (GR_{50}) was calculated.

Results and Discussion

All five weed species germinated greater than 80% (data not shown). Time required for adequate radicle growth varied among the species tested. Radicle length was measured after 48 hours for dandelion, white clover, and black medic, and after 120 hours for common lespedeza and buckhorn plantain. Mean radicle length of untreated seeds was measured as 18.6, 29.5, 15.8, 27.4, and 24.8 mm for dandelion, buckhorn plantain, white clover, black medic, and lespedeza, respectively. Differential sensitivity was exhibited by the weed species at lower isoxaben concentrations (Table 1). At isoxaben concentrations of 0.005, 0.01 and 0.02 μg/ml, reductions in radicle elongation of buckhorn plantain and dandelion were lower than that of lespedeza, white clover, and black medic. At isoxaben concentrations of 0.04, and 0.08 μg/ml, dandelion was the least sensitive species. Above concentrations of 0.08 μg/ml, similar reductions in radicle length were observed among the 5 species.

Regression analysis of radicle response to isoxaben concentrations indicated that of all five species were affected by isoxaben concentrations in a quadratic manner. All weed species responded with a drastic decrease in radicle elongation as isoxaben concentration increased from 0.01 to 0.04 µg/ml. At isoxaben concentrations above 0.16 to 0.32 µg/ml, decreases in radicle lengths were not noticeable for all species. Isoxaben levels of 0.32 µg/ml in an aqueous medium caused greater than 80% reduction in radicle elongation of the five species studied.

When the natural logarithm of radicle length response was regressed against that of isoxaben concentration, a linear model was a good fit for all five weed species. GR_{50} values (in µg/ml) were determined as: dandelion, 0.013; buckhorn plantain, 0.010; white clover, 0.008; black medic, 0.008; and common lespedeza, 0.007. These predicted values agree with the observed radicle response of the respective species at isoxaben concentrations of 0.005 to 0.02 µg/ml.

Seeds of all five weed species tested showed signs of imbibition and rupture of testa, even at the highest concentration of isoxaben. Injury was manifested as progressive swelling and slight to moderate discoloration of the emerging radicle with increasing levels of isoxaben, until radicle emergence was minimal.

The results of this study are in agreement with other research findings. Lefebvre et al. (1987) determined that isoxaben at 0.0057 µg/ml inhibited growth of rape, a sensitive broadleaf species, by 50%. They also observed that the most characteristic symptom was swelling of the apical regions and a progressive disappearance of the meristematic region. Isoxaben did not prevent germination of cucumber, lettuce, or radish seeds at concentrations up to 3.32µg/ml. Wheat, a tolerant species, exhibited only 40% reduction in radicle elongation at 1.2 µg/ml of isoxaben. Huggenberger and Ryan (1985) described injury symptoms as swelling and browning of vascular tissues in roots and hypocotyls, asymmetrical growth of tissues, and subsequent plant death. They noted GR_{50} values ranging from 0.05 to 0.11µg/ml in various winter annuals in sand culture studies.

Based on differences in sensitivity to low concentrations of isoxaben and the predicted GR_{50} values, dandelion and buckhorn plantain may be categorized as less sensitive species than lespedeza, white clover, and black medic. In this study, black medic and buckhorn plantain were considered to be the best species for petri-dish bioassays of isoxaben.

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Table 1. Effect of isoxaben on radicle elongation of five broadleaf weeds in a petri-dish bioassay.

Isoxaben Concentration[b] (µg/ml)	Reduction in Radicle Elongation[a] Dandelion	Buckhorn plantain	White clover	Black medic	Common lespedeza	L.S.D (0.05)
	%					
0	0	0	0	0	0	-
0.005	18	13	34	35	31	11
0.01	29	34	51	54	49	16
0.02	54	56	73	73	72	15
0.04	61	75	79	79	81	13
0.08	66	77	75	73	96	7
0.16	89	80	79	85	95	8
0.32	91	90	83	89	92	4
0.64	92	91	83	90	97	4
1.28	92	94	83	95	97	3
2.56	93	96	91	94	97	3
5.12	91	97	91	93	96	3
10.24	92	94	91	93	98	7
Quadratic	**	**	**	**	**	
GR_{50}[c]	0.013	0.010	0.008	0.008	0.007	
k	-0.3437	-0.4306	-0.321	-0.2731	- 0.4861	
b	0.7449	0.7285	0.4946	1.3	0.121	
R^2	0.81	0.96	0.91	0.98	0.79	

[a] Compared to radicle length of untreated seeds: 18.6, 29.5, 15.8, 27.4, and 24.8 mm for dandelion, buckhorn plantain, white clover, black medic, and lespedeza, respectively. Radicle length was measured after 48 hours for dandelion, white clover, and black medic, and after 120 hours for common lespedeza and buckhorn plantain.

[b] Results from the isoxaben concentrations were subjected to quadratic regression. The '**' mean significant regression at 0.01 level.

[c] Concentration of isoxaben (µg/ml) that reduced radicle elongation by 50%, as determined by regression analysis of log-transformed data.

CHAPTER II

SENSITIVITY OF BROADLEAF WEEDS TO ISOXABEN APPLIED POSTEMERGENCE

Abstract. Isoxaben, used primarily as a PRE broadleaf herbicide, was evaluated for POST herbicidal effects on certain broadleaf weeds in turf. Isoxaben was applied POST either alone or as a tank mix with growth-regulator type herbicides to dandelion, white clover, buckhorn plantain, common yellow woodsorrel, common lespedeza, black medic, spotted spurge, and Florida betony in two greenhouse experiments. Isoxaben applied at 0.56, 1.12, and 2.24 kg ai/ha caused <40% injury to all the weed species tested, except for Florida betony. Florida betony injury increased with increasing rates of isoxaben, ranging from 34% injury at 0.56 kg/ha to 62% injury at 2.24 kg/ha. Isoxaben at 2.24 kg/ha reduced Florida betony shoot regrowth by 71%. The 3-way tank mix of isoxaben (1.12 kg/ha) with 2,4-D (1.12 kg/ha) and dicamba (0.37 kg/ha) resulted in a faster decline of buckhorn plantain, yellow woodsorrel, Florida betony, and dandelion at 3 WAT compared to the 2-way tank mix of 2,4-D (1.12 kg/ha) plus dicamba (0.37 kg/ha). Although isoxaben is not effective as a POST herbicide for the weed species evaluated in this greenhouse experiment, tank mixing this herbicide with growth-regulator type herbicides may provide PRE and POST weed control by a single application.
Nomenclature: Isoxaben, N-[3-(1-ethyl-1-methlypropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; 2,4-D, (2,4-dichlorophenoxy) acetic acid; dicamba, 3,6-dichloro-2-methoxybenzoic acid; black medic, *Medicago lupilina* L.#[2] MEDLU; buckhorn plantain, *Plantago lanceolata* L. # PLALA; dandelion, *Taraxacum officinale* Weber. # TAROF; common lespedeza, *Lespedeza striata* Thunb. # LESST; yellow woodsorrel, *Oxalis stricta* L. # OXAST; spotted spurge, *Euphorbia maculata* L. # EPHMA; white clover, *Trifolium repens* L. # TRFRE; Florida betony, *Stachys floridana* Shuttlew. # STAFL.
Additional index words. Tank mixes, EL-107, Gallery.
Abbreviations. PRE, preemergence; POST, postemergence; WAT, weeks after treatment.

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[2] 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

Broadleaf weeds form an important group of pests in turfgrasses. Postemergence (POST) control of a wide range of broadleaf weeds is achieved mostly through two-, or three-way combinations of growth-regulator type herbicides (Bingham 1990). Isoxaben is one of the few herbicides registered for selective control of broadleaf weeds preemergence (PRE) in turfgrasses, although some PRE herbicides used for annual grass control supress certain broadleaf weeds grown from seeds (Anonymous 1990; Bingham and Hipkins 1997).

Isoxaben applied POST supressed the growth of ground ivy (*Glechoma hederacea* L. # GLEHE) and healall (*Prunella vulgaris* L. # PRUVU) (Neal and Senesac 1994). Postemergence activity was not enhanced by combining clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) or triclopyr ([(3,5,6-trichloro-2-pyridinyl)oxyl]acetic acid) with isoxaben. Isoxaben applied POST at 1.12 kg/ha did not reduce yellow woodsorrel and spotted spurge cover significantly (Elmore and Breuninger 1991). However, isoxaben combined with triclopyr gave better control of yellow woodsorrel but not spotted spurge, when compared to application of triclopyr alone.

Postemergence control of certain weeds common in row crops was observed with isoxaben in a greenhouse study (Schneegurt et al. 1993). Isoxaben reduced barnyardgrass (*Echinochloa crus-galli* L. # ECHCG) growth greater than 90%. Isoxaben applied only to the soil or to the foliage gave less control than application to both leaves and soil.

Synergism was reported with auxin-type herbicides when combined with herbicides with different modes of action. Isoxaben is thought to affect processes involving cellulose biosynthesis and cell wall formation in plants (Heim et al. 1991). Isoxaben combined with growth-regulator type herbicides may provide dual benefits by enhancing POST control as well as providing residual control. Growth-regulator type herbicides exert their phytotoxicity by causing uncontrolled growth leading to plant death (Devine et al. 1993), although there are reports that these herbicides can induce abscisic acid synthesis which causes plant death (Grossmann et al. 1996). Field bindweed (*Convolvulus arvensis* L. # CONAR) control was enhanced when glyphosate (*N*-(phosphonomethyl)glycine) was tank mixed with 2,4-D or dicamba, due to increased uptake and transport of glyphosate (Flint and Barrett 1989). Ioxynil (4-hydroxy-3,5-diiodobenzonitrile), when combined with mecoprop [(±)-2-(4-chloro-2-methylphenoxy)propanoic acid], improved control of common chickweed (*Stellaria media* L. Vill. # STEME) compared to mecoprop applied alone (Merritt et al. 1986).

Research on the effects of isoxaben applied POST to turfgrass weeds is limited. The benefits of combining this herbicide with growth-regulator type herbicides require further investigation. The objectives of this research were to determine POST herbicidal effects of isoxaben, applied either alone or in combination with growth-regulator type herbicides, on common broadleaf weeds in turf.

Materials and Methods

Experiments were conducted in a greenhouse with average day/night temperatures of 35/25 C, and the ambient light level at noon was approximately 650 µmol/m^2/sec. Experimental design was a randomized complete block and the study was repeated. Plastic flats (45 cm by 33 cm by 8 cm) were filled with pine bark and sand (4:1 vol/vol). Spotted spurge was seeded three weeks prior to seeding other weed species to compensate for slower germination. White clover, black medic, common lespedeza, buckhorn plantain, spotted spurge, and dandelion were seeded in rows. After germination, the rows (33 cm) were thinned to about 25 seedlings per species. Ten transplants each of yellow woodsorrel and Florida betony were also included in this study. Yellow woodsorrel plants were collected from a nearby field and Florida betony plants were transplanted from containers kept in a lathhouse. A 20-20-20 liquid fertilizer solution was applied weekly after seedling emergence to provide N at 5 g/m^2.

At the time of POST herbicide application, all seedlings had reached the four- to six-leaf stage. At this time Florida betony was about 10 to 15 cm tall and yellow woodsorrel was approximately 4 to 5 cm tall. Isoxaben was applied at 0.56, 1.12 and 2.24 kg ai/ha using the commercial 75% dry flowable formulation. A tank mix of 2,4-D plus dicamba at 1.12 plus 0.37 kg/ha, respectively, was applied for comparison. In addition, a three-way tank mix of isoxaben plus 2,4-D plus dicamba at 1.12, 1.12 and 0.37 kg/ha, respectively, was evaluated to determine any complementary effects. All herbicides were applied using a CO_2-pressurized boom sprayer with 8003 flat fan nozzles[3] delivering 230 L/ha with water as the carrier.

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

The first experiment was treated on July 16, 1996 and the second experiment was treated on October 1, 1996. The flats were initially irrigated one day after treatment and daily thereafter. Injury ratings were taken on a 0 to 100 scale (0= no injury or growth suppression; 100 = plant death) at 3 weeks after treatment (WAT). Shoot fresh weights were recorded 6 WAT. Florida betony was allowed to regrow for a period of 4 weeks after recording the initial shoot fresh weights. Regrowth weights were recorded at this time. Percent shoot fresh weight reductions were calculated for each species by comparing the treated plants to untreated plants. All 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 collected from the two studies were combined after testing for homogeneity. Data on percent injury and shoot fresh weight reduction for the three rates of isoxaben were subjected to linear regression.

Results and Discussion

Isoxaben applied at 0.56 and 1.12 kg/ha gave less than 25% injury of all weed species at 3 WAT except Florida betony (Table 1). Percent injury caused by the high rate of isoxaben at 3 WAT to the weed species tested were: Florida betony (62%), lespedeza (38%), white clover (30%), dandelion (21%), black medic (17%), buckhorn plantain (15%), and spotted spurge (9.5%). Florida betony injury increased linearly with increasing rates of isoxaben. The two-way tank mix of 2,4-D plus dicamba injured black medic and white clover >90%, and injured dandelion, lespedeza, and Florida betony from 80 to 90%. This tank mix caused less injury to buckhorn plantain (74%), yellow woodsorrel (54%), and spotted spurge (45%). Addition of isoxaben to the tank-mix generally enhanced percent injury numerically.

Injury symptoms from isoxaben were most pronounced on dandelion, white clover, and Florida betony. Injury symptoms were manifested as swelling of shoot tips, growth suppression, and traces of leaf puckering. Our observations were similar to that of Schneegurt et al. (1993), who have documented the morphological effects of POST application of isoxaben as stunting, leaf malformation, general inhibition of growth, and a reduction in height and vigor of susceptible weed species. In another study, isoxaben applied POST at 2.4 kg/ha gave 99% and 85% control of ground ivy, and healall, respectively, in the field but only 35% and 50% in the greenhouse (Neal and Senesac 1994).

Mean shoot fresh weights for untreated weeds were: dandelion, 9.2 g; lespedeza, 0.9 g; white clover, 8.8 g; black medic, 3.1 g; yellow woodsorrel, 5.3 g;, spotted spurge, 0.7 g; buckhorn plantain, 5.7 g; and Florida betony, 7.2 g, with a regrowth weight of 5.8 g. Isoxaben at all three rates caused less than 30% reduction of dandelion, yellow woodsorrel, and spotted spurge shoot weight (Table 2). Isoxaben applied at 2.24 kg/ha reduced white clover and black medic shoot weight by about 65%. The high rate of isoxaben reduced buckhorn plantain and Florida betony shoot fresh weight by 40 to 45% (Table 2). For most species, isoxaben injury did not increase with increasing rate. However, shoot fresh weight of Florida betony, dandelion, and lespedeza decreased linearly as isoxaben rates increased. The 2-way tank mix of 2,4-D plus dicamba gave > 80% shoot weight reduction of all weeds except yellow woodsorrel (67%) and spotted spurge (46%). Isoxaben, when added to this tank mix did not decrease shoot fresh weights in any of the weed species below that seen in the 2,4-D plus dicamba treatment.

Florida betony, white clover, and black medic were most sensitive to isoxaben applied POST; while buckhorn plantain, lespedeza, and dandelion were moderately sensitive. Yellow woodsorrel and spotted spurge were least sensitive to isoxaben applied POST.

Isoxaben applied POST reduced growth of velvetleaf (*Abutilon theophrasti* Medik. # ABUTH), redroot pigweed (*Amaranthus retroflexus* L. # AMARE), ivyleaf morningglory (*Ipomoea hederacea* L. Jacq. # IPOHE), speedwell (*Veronica* spp.), and common cocklebur (*Xanthium strumarium* L. # XANST) (Schneegurt et al. 1994). These effects were greater than those seen in the current study by approximately 20 to 30%. This greater injury could be due to increased uptake as a result of an adjuvant or their use of more sensitive species. Translocation following shoot uptake was only 0.08% of applied isoxaben which implied that symplastic translocation of isoxaben was minimal. Research on the effect of adding an adjuvant to isoxaben applied POST for common weeds in turf would generate useful information.

Isoxaben, when added to the tank mix, tended to accelerate progression of injury caused by the growth-regulator type herbicides used. Isoxaben applied POST at 2.24 kg ai/ha suppressed the growth of Florida betony, black medic, and white clover, by 45, 65, and 66%, respectively, and reduced regrowth of Florida betony by 71%. Further studies to determine sensitivity of these broadleaf weeds to POST application of isoxaben in the field may generate useful information.

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