

**BASIS FOR SELECTIVITY OF ISOXABEN IN AJUGA
Ajuga reptans), WINTERCREEPER (*Euonymus fortunei*), AND
DWARF BURNING BUSH (*Euonymus alatus* 'Compacta')**

by

Sydha Salihu

Dissertation submitted to the Faculty of the Virginia Polytechnic
Institute and State University in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

in

Weed Science

APPROVED:

Jeffrey F. Derr, Co-chairman

Kriton K. Hatzios, Co-chairman

S. Wayne Bingham

John L. Hess

Alexander X. Niemiera
, , ,

May, 1997

Blacksburg, Virginia

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Sydha Salihu

Jeffrey F. Derr , Co-chairman

Kriton K. Hatzios, Co-chairman

Department of Plant Pathology, Physiology and Weed Science
(ABSTRACT)

Isoxaben is a preemergence herbicide used for broadleaf weed control in turf and ornamentals. Although isoxaben can be used on a number of ornamentals, certain species are injured by isoxaben applications. The objectives of this research were: a) to evaluate the tolerance of ajuga, wintercreeper and dwarf burning bush to isoxaben applications, b) to compare the absorption, translocation and metabolism of isoxaben following root and shoot application in these ornamentals, and c) to examine the effect of isoxaben on glucose incorporation in the roots of these species.

Greenhouse and lathhouse studies demonstrated that ajuga was the most sensitive species compared to wintercreeper and dwarf burning bush following root and shoot exposure to isoxaben at 0.84, 1.69 and 3.39 kg ai/ha. Following root and shoot application, isoxaben at 3.39 kg/ha caused approximately 50% shoot injury in ajuga at 2 months after treatment compared to approximately 30% in dwarf burning bush in sand culture. Wintercreeper was not visually injured by any isoxaben rate. Isoxaben at 3.39 kg/ha reduced wintercreeper root weight by 15% following root application and shoot weight by 10% following shoot application.

Field studies showed that isoxaben applications made one month after bud-break caused 30 to 45% injury to dwarf burning bush. However, the plants outgrew the injury in the following year. Dwarf burning bush was not injured from applications of isoxaben made at the dormant stage or two months after the bud-break stage.

Studies with root-applied radiolabeled isoxaben showed that ajuga and dwarf burning bush had absorbed 34 and 41% of the applied radioactivity, respectively, while wintercreeper had absorbed only 21% at 14 days after treatment (DAT). The percent of absorbed radioactivity which translocated was greater in ajuga (58%) and wintercreeper (50%) than in dwarf burning bush (28%). In the root extracts, metabolism of isoxaben was greater in ajuga than wintercreeper or dwarf burning bush at 3, 7 and 14 DAT. Most of the radioactivity recovered from the shoots of the three species appeared to be polar metabolites of isoxaben, possibly conjugates.

In studies with shoot-applied radiolabeled isoxaben, radioactivity recovered from the treated leaf of ajuga increased

from 46% of applied at 3 days to 64% at 14 days after treatment. In wintercreeper, the most tolerant species, approximately 40% of the applied radioactivity was recovered in the treated leaf at each harvest date. Radioactivity recovered from the treated leaflet increased from 45 at 3 DAT to 70% at 14 DAT in both growth stages of dwarf burning bush. Ajuga and wintercreeper metabolized isoxaben faster than dwarf burning bush. There was no difference in the metabolism of isoxaben between the two growth stages of dwarf burning bush. Incorporation of glucose in the roots of wintercreeper and dwarf burning bush was not inhibited by isoxaben (1 mM). Approximately 10% inhibition of glucose incorporation by isoxaben was observed in the roots of the sensitive species ajuga.

Ajuga absorbed more isoxaben than wintercreeper following root or shoot application, which may explain its greater sensitivity. Wintercreeper absorbed less isoxaben and metabolized more of this herbicide than dwarf burning bush, which may explain its greater tolerance. Absorption and metabolism of isoxaben following shoot application were similar in both growth stages of dwarf burning bush. More information is needed on target site sensitivity in these three species.

ACKNOWLEDGMENTS

I am grateful to Dr. Jeffrey F. Derr, for the countless hours spent in advising me. I would also like to acknowledge his patience, and encouragement during my entire graduate program. I would like to thank Dr. Kriton K. Hatzios for his inspiration and valuable suggestions during my research. I would like to thank my committee members: Drs. S. Wayne Bingham, John L. Hess and Alex Niemiera, for their willingness to help me whenever I approached them.

The faculty, staff and graduate students of PPWS deserve special thanks for being supportive of me, with special mention of Mr. Lloyd Hipkins, Ms. Judy Fielder and Mr. Phil Keating. I would like to express my gratitude towards Mr. David Quillen of Waynesboro Nursery for his generous donation of plants and his willingness to cooperate with my research. Special thanks goes to my husband, my parents, sister and brother for their love and prayers. Lastly but not least, I would like to mention our precious little Akshay for keeping me sane during this trying period of my life.

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Chapter I Introduction and Literature Review

1. Weed management in nursery crops

The nursery industry, an important part of Virginia's agriculture, includes container production, field production and landscape maintenance. Nursery and greenhouse crops ranked third in cash receipts after tobacco and soybeans, and ahead of peanuts and corn in Virginia in 1989¹. Nationally, farmer cash receipts of nursery and greenhouse crops in 1992 were estimated at \$9.0 billion.

Weed control in nursery crop production is important because weeds compete with plants for moisture, nutrients and space. In a study where weeds were not controlled, growth was reduced by 25 to 53% depending upon crop, weed species and weed densities (Gibson 1985). Herbicides are commonly used in ornamental nurseries because of their effectiveness and lower costs than hand weeding (Weatherspoon 1975). However, herbicide tolerance varies with species and cultivars (Neal and Senesac 1988; Jacobsen and Walls 1987). Therefore, herbicide use in nurseries requires caution because improper application can cause economic loss due to plant damage.

2. Preemergence broadleaf herbicides in nursery crops

There are a number of preemergence herbicides available for controlling annual grasses in container and field-grown nursery crops. However, very few herbicides are available for broadleaf weed control. Simazine (Princep), oxyfluorfen (Goal), oxadiazon (Ronstar) and dichlobenil (Casoron) have been used for preemergence broadleaf control in ornamental nurseries (Derr 1993a). Oxyfluorfen applied postemergence also controls certain annual broadleaf weeds (Neal 1993).

However, there are limitations to the use of these four herbicides in nursery crops. Isoxaben, is an alternative for preemergence broadleaf weed control in container- and field-grown nursery stock.

A. Simazine, Oxyfluorfen, Oxadiazon, and Dichlobenil

Although simazine provides excellent control of most major broadleaf weeds, it is known to injure privet (*Ligustrum ciliatum* L.), golden bells (*Forsythia intermedia* L.), willow (*Salix acutifolia* L.), mock-orange (*Philadelphus* spp.), *Prunus* spp., euonymus [*Euonymus fortunei* (Turcz.) Hand.-Mazz], deciduous azalea (*Rhododendron gandavense* Rehd.) and Japanese andromeda [*Pieris japonica* (Thunb.) D. Don] (Ahrens 1988; Bing 1981). Oxyfluorfen can be applied preemergence and postemergence in conifer species (Creager 1982). Oxyfluorfen provided excellent control of common groundsel (*Senecio vulgaris* L.), common chickweed [*Stellaria media* (L.) Cyrillo.], hairy bittercress (*Cardamine hirsuta* L.) and horseweed (*Conyza canadensis*

¹ Information provided by the Virginia Nurserymen's Association.

L.)(Gallitano and Skroch 1993). Oxyfluorfen can injure many broadleaf evergreens and deciduous nursery crops (Kuhns and Haramaki 1980). Oxyfluorfen damaged root systems of azalea (*Rhododendron obtusum* Planch.), cotoneaster (*Cotoneaster acuminata* L.), and euonymus (*Euonymus fortunei* Hand.-Mazz. 'Colorata') (Weller et al. 1984). An emulsifiable concentrate formulation of oxyfluorfen was more injurious to woody plants but provides superior weed control when compared to the granular formulation (Weller et al. 1984). Oxadiazon at 4.5 kg ha⁻¹ provided excellent control of common groundsel, prostrate spurge (*Euphorbia humistrata* Engelm. ex Gray), hairy bittercress, Virginia pepperweed (*Lepidium virginicum* L.) and horseweed (Gallitano and Skroch 1993). The wettable powder (WP) and emulsifiable concentrate (EC) formulation of oxadiazon at 4.5 and 9 kg ha⁻¹ caused greater injury than the granular formulation to 'Compacta' Japanese holly (*Ilex crenata* Thunb.) and 'Hershey Red' azalea 30 days after treatment (Kalmowitz and Whitwell 1988). However, the granular formulations of oxadiazon at 8.96 kg ha⁻¹ has been reported to reduce growth and root development in 'Coral Bells' azalea and 'Formosa' azalea (*Rhododendron indicum* 'Sweet') (Singh et al. 1981).

Dichlobenil provides good control of many annual and biennial broadleaf and grass weeds such as annual bluegrass (*Poa annua* L.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], pineappleweed [*Matricaria matricarioides* (Less.) C.L.Porter], common purslane (*Portulaca oleracea* L.), and wild carrot (*Daucus carota* L.), as well as some perennial weeds including dandelion (*Taraxacum officinale* Weber.), Canada thistle [*Cirsium arvense* (L.) Scop.] and quackgrass [*Elytrigia repens* (L.) Nevski] (Ahrens 1994). Substantial losses of dichlobenil occur due to volatilization when surface applied to wet soil. To reduce volatilization in dry soils, dichlobenil has to be incorporated.

B. Isoxaben

Isoxaben, evaluated under the code name EL-107, is a selective preemergence herbicide developed for the control of broadleaf weeds in ornamentals, turf, landscape plantings and in orchard crops (Colbert and Ford 1987). In the United States, isoxaben is commercially marketed as a 75 percent dry flowable formulation (Gallery) and in combination with trifluralin (Snapshot 2.5 TG). It was also formerly marketed in combination with oryzalin (Snapshot 80 DF). Isoxaben is available commercially in Europe as Flexidor for season-long broadleaf weed control in small grains (Huggenberger et al. 1982). Isoxaben can be applied preemergence at 0.56 to 1.12 kg ai ha⁻¹ in established turf, landscape ornamentals, nursery crops, nonbearing vines, Christmas tree plantations, nonbearing fruit and nut trees, and noncropland areas (Ahrens 1994). Desirable characteristics of isoxaben use are the high degree of safety in a variety of ornamental crops and excellent annual broadleaf weed control (Gilliam 1989; Neal and Senesac 1988). Most groundcovers,

perennial flowers and woody nursery crops have excellent tolerance to this herbicide. However, certain nursery species can be injured by isoxaben applications.

Isoxaben is an alternative to simazine for controlling broadleaf weeds in ornamentals because of their similar weed control spectrum (Neal 1993). Effective alternative herbicides to simazine would be desirable because of its potential to contaminate ground and surface waters (Koterba et al. 1993; Bruggman et al. 1995) and the occurrence of triazine-resistant weeds in nurseries (Bandeem 1982).

3. Weed control and nursery crop tolerance with isoxaben

A. Preemergence effects of isoxaben

a. Broadleaf weed control

Setyowati et al. (1995) found that isoxaben at 0.84 kg ha⁻¹ provided excellent control of common chickweed, henbit (*Lamium amplexicaule* L.), red sorrel (*Rumex acetosella* L.), smooth pigweed (*Amaranthus hybridus* L.), Pennsylvania smartweed (*Polygonum pennsylvanicum* L.), and common ragweed (*Ambrosia artemisiifolia* L.). Colbert and Ford (1987) obtained greater than 90% control of many broadleaf weeds including pigweeds (*Amaranthus* spp.), common chickweed, common groundsel and common lambsquarters (*Chenopodium album* L.), with 0.84 to 1.1 kg ha⁻¹ isoxaben in field grown ornamentals, turf and orchard crops. However, Derr (1993b) reported that isoxaben gave poor control of common lambsquarters and morningglory (*Ipomoea* spp.). Isoxaben controlled most broadleaf weeds at less than 0.56 kg ha⁻¹ in field soil and a soilless potting medium (Neal and Senesac 1990b). In field tests, isoxaben was not effective in controlling velvetleaf (*Abutilon theophrasti* Medic.), galinsoga and prickly sida (*Sida spinosa* L.) (Neal and Senesac 1990a; Setyowati et al. 1995).

Isoxaben provided nearly total control of common groundsel and Virginia pepperweed in container trials and the control was consistent in both years the research was conducted (Gallitano and Skroch 1993). Isoxaben at 0.56 kg ha⁻¹ provided equal or superior control of common chickweed, wild mustard [*Brassica kaber* (DC.) L.C.Wheeler], horseweed, common groundsel and dandelion as compared to oxyfluorfen plus oryzalin, oxadiazon or simazine in containers (Neal and Senesac 1990b). Gilliam et al. (1989) reported that isoxaben provided inferior control (76% control of broadleaf weeds) when compared to oryzalin plus oxyfluorfen treatments, but controlled spotted spurge (*Euphorbia maculata* L.) equal to the combination herbicide. Ahrens (1988) reported that isoxaben plus oryzalin at 0.57 plus 1.71 kg ha⁻¹ gave excellent preemergence control of most broadleaf weeds. The combination of isoxaben and oryzalin provided good control of horseweed and musk thistle (*Carduus nutans* L.) (Gallitano and Skroch 1993; Monks et al. 1991). Isoxaben in combination with prodiamine controlled prostrate spurge, common groundsel and

hairy galinsoga [*Galinsoga ciliata* (Raf.) Blake] in container trials (Neal 1997b).

Herbicides are often used in combination with organic mulches in landscape plantings. Isoxaben provided better control of weeds when placed under a mulch than on top and it provided 40% more control than mulch alone (Derr 1993c; Kuhns and Micheal 1992). Isoxaben applied in combination with postemergence herbicides in the fall adds residual control of broadleaf weeds through spring in winter cereals (Drinkall and Farrant 1987). Populations of flexuous bittercress (*Cardamine flexuosa* With.) and a mixture of hairy bittercress and flexuous bittercress which came from a nursery with regular applications of isoxaben, were less sensitive to this herbicide as compared to a population of hairy bittercress which came from a site with no isoxaben applications (Eelen and Bulcke 1997).

b. Grass and sedge control

Isoxaben is not a very effective herbicide for controlling grasses. Neal and Senesac (1990a) reported poor annual grass control (<70%) with isoxaben at 1.1 kg ha⁻¹. Higher rates of isoxaben were required for annual grass control than for broadleaf weed control (Neal and Senesac 1988). The rate of isoxaben could be reduced when used in combination with oryzalin. Isoxaben combined with trifluralin or oryzalin improved control of annual grasses (Hood and Klett 1992; Neal and Senesac 1990a). Isoxaben combined with preemergence herbicides such as oryzalin or prodiamine gave greater control of smooth crabgrass [*Digitaria ischaemum* (Shreb.) Muhl.] than isoxaben applied alone (Neal and Senesac 1990b). Kuhns and Michael (1992) observed that isoxaben plus oryzalin applied to bare soil controlled annual grasses 80%. Isoxaben did not control yellow nutsedge (*Cyperus esculentus* L.) when placed either above or below the mulch.

B. Postemergence effects of isoxaben

Isoxaben is phytotoxic when applied postemergence to certain weed species (Roberts et al. 1993; Schneegurt et al. 1994a). Schneegurt et al. (1994a) in their study on the postemergence activity of isoxaben on redroot pigweed found that the response to isoxaben was more dependent on the growth stage of the plant at treatment time than on the rate of isoxaben. Furthermore, herbicidal activity was enhanced by soil and foliar application, but phytotoxicity was observed with foliar interception alone. Neal and Senesac (1991b) reported that isoxaben at 2.2 kg ha⁻¹ applied postemergence provided poor control of ground ivy (*Glechoma hederacea* L.) and healall (*Prunella vulgaris* L.) (only 35% and 50%, respectively) under greenhouse conditions. But when applied postemergence in the field, isoxaben provided up to 99% and 85% control of ground ivy and healall, respectively. However, the control obtained from postemergence applications of isoxaben on other weeds was poor. Chandran and Derr (1997) reported that isoxaben applied postemergence provided poor to no control of dandelion, white clover (*Trifolium repens* L.),

buckhorn plantain (*Plantago lanceolata* L.), common yellow woodsorrel (*Oxalis stricta* L.), common lespedeza (*Lespedeza striata* Thunb.), black medic (*Medicago lupulina* L.) and spotted spurge. Elmore and Breuninger (1993) reported poor postemergence control of creeping woodsorrel (*Oxalis corniculata* L.) by isoxaben.

C. Phytotoxicity of isoxaben

The efficacy of a herbicide depends partly on its ability to cause minimal crop injury. Most woody ornamentals are tolerant to isoxaben applications (Gilliam et al. 1989; Mervosh and Ahrens 1997; Neal and Senesac 1990a; Neal and Senesac 1991a; Reeder et al. 1994; Wilson et al. 1994) but broadcast spray applications can injure certain nursery crops (Table 1) (Colbert and Ford 1987; Derr 1993b; Fuller 1990; Setyowati et al. 1995; Staats and Klett 1993). Derr and Salihu (1996) reported that isoxaben at 1.12 kg ha⁻¹ reduced new root growth of Japanese holly after one application and the shoot growth of azalea after three applications. Isoxaben does not injure most ornamental grasses (Neal and Senesac 1991a). However, isoxaben reduced the shoot fresh weight of fountain grass (*Pennisetum* spp.) in one study (Wilson et al. 1994). Some of the herbaceous perennials sensitive to isoxaben applications belong to the family Compositae (Table 1) (Porter 1996; Senesac and Neal 1988; Jacobsen and Walls 1987). Other sensitive species are in the plant families Labiatae, Begoniaceae, Balsaminaceae, and Aizoaceae.

Injury caused by isoxaben include both root and shoot effects. Roberts (1991) reported that the primary response of susceptible plants to isoxaben was the swelling of the seedling root tip. Other root symptoms are nubbing, stunting and discoloration. Roberts et al. (1993) reported the foliar symptoms of isoxaben as cell swelling, resulting in swollen and split stems and petioles, and formative effects on leaves. The foliar injury symptoms like leaf browning, curled leaves and meristematic shoot death were seen in plants with little cuticle development. Growth stage might be important in the tolerance of nursery crops to isoxaben.

4. Other uses of isoxaben

A. Weed control in turfgrass

Isoxaben has also been evaluated in turf for broadleaf weed control. 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), and is registered for most turfgrasses species cultivated in the U.S. (Anonymous, 1994). Grant et al. (1990) observed that isoxaben fall applied at 0.56 to 1.12 kg ha⁻¹ provided control of a number of broadleaf weed species found commonly in turf which included henbit, common chickweed, lawn burweed (*Soliva pterosperma* (Juss.) Less.), short buttercup (*Ranunculus parviflorus* L.), hop clover (*Trifolium aureum* Pollich), curly dock (*Rumex crispus* L.),

Carolina geranium (*Geranium carolinianum* L.), and buckhorn plantain. Isoxaben controls prostrate spurge very effectively in turf (Jagshitz and Sawyer 1989). Excellent control of prostrate knotweed (*Polygonum aviculare* L.) was achieved in spring from isoxaben applied at 0.84 kg ha⁻¹ during the previous fall (Neal and Senesac 1997a). Keese and Forth (1997) reported greater than 90% control of dandelion, speedwell (*Veronica* spp.) and ground ivy up to 8 months after a combination treatment of 1.12 kg ha⁻¹ of isoxaben with a commercial herbicide mixture containing 2,4-D, mecoprop and dicamba. Up to 98% control of lawn burweed was achieved at 6 months after isoxaben application (Keese and Forth, 1997).

B. Weed control in cereals

Used for preemergent control of annual broadleaf weeds in cereals, isoxaben at 0.125 kg ai ha⁻¹ controls more than 30 species of weeds common to grain crops (Huggenberger et al. 1982). Anderson et al. (1984) reported greater than 90% control of redroot pigweed (*Amaranthus retroflexus* L.), common lambsquarters, common ragweed, field pennycress (*Thlaspi arvense* L.), common chickweed and shepherd's purse [*Capsella bursa-pastoris* (L.) Medic.] by isoxaben at 0.2 kg ha⁻¹. Excellent control of cruciferae weeds was achieved with isoxaben at rates as low as 0.05 kg ha⁻¹. Isoxaben did not injure spring and winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and sunflower (*Helianthus annuus* L.) and controlled several cool-season annual weeds such as wild mustard and field pennycress (Banks et al. 1986). Isoxaben combined with other herbicides and applied in fall gave residual control of broadleaves through spring without injury to winter cereals (Drinkall and Farrant 1987). Isoxaben applications increased yields of winter barley and wheat) due to broadleaf weed control as compared with the untreated controls (Huggenberger and Gueguem 1987).

5. Physical and chemical properties of isoxaben

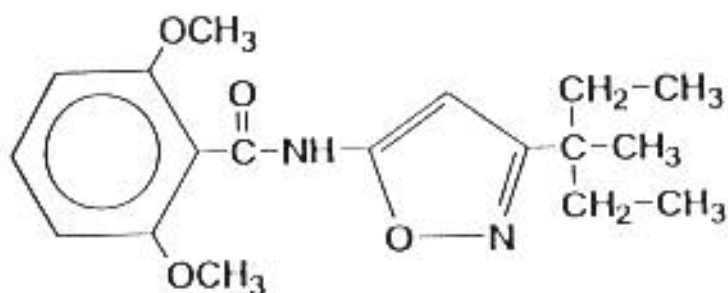


Figure 1. Chemical structure of isoxaben.

The chemical name of isoxaben is N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl] 2,6-dimethoxybenzamide and it belongs to the family benzamide (Figure 1). It is a white crystalline

solid with a melting point of 176 to 179° C. It has very low vapor pressure (less than 3.9×10^{-7} mm Hg at 25° C), undergoes microbial degradation in soil, and is degraded by UV light in aqueous solution (Ahrens 1994). Isoxaben is soluble in organic solvents like acetone, acetonitrile and dichloromethane. It is classified as generally nontoxic, having an oral LD₅₀ in rat of more than 10,000 mg kg⁻¹. It has a water solubility of 1 part per million (ppm) at 25°C and leaches very little in soil. Detection limit for residues of isoxaben and its major soil metabolite was 0.05 ppm by High Pressure Liquid Chromatography (HPLC) (Rutherford 1990).

6. Behavior of isoxaben in plants

A. Mode of action of isoxaben

Although the exact target site of action for isoxaben is yet to be discovered, the general effect is thought to be inhibition of cell wall biosynthesis in susceptible plants (Heim et al. 1990b). The only other herbicide that is an effective and specific inhibitor of cellulose synthesis in higher plants is 2,6 dichlorobenzonitrile (dichlobenil, also referred to as DCB) (Hogetsu et al. 1974). A dichlobenil receptor which may function as a regulatory protein for β -glucan synthesis in plants has been identified (Delmer et al. 1987).

Lefebvre et al. (1987) studied the effects of isoxaben on plant and cell growth, cell division and some cellular biosynthetic processes of maple (*Acer pseudoplatanus* L.) and soybean [*Glycine max* (L.) Merr.] in tissue culture. Isoxaben inhibited the incorporation of glucose into molecules insoluble in aqueous solutions or organic solvents, probably components of the cell wall. Isoxaben did not interfere with respiration nor with the synthesis of proteins, fatty acids or nucleic acids.

Further experiments were carried out to determine whether these effects were due to a primary or a secondary site of action. Heim et al. (1990b) used a novel experimental system in which hydroponically-grown seedlings of mouse-ear cress (*Arabidopsis thaliana* L.) were used in radiolabeling experiments. Isoxaben did not cause a generalized herbicidal effect, but it uniquely or specifically inhibited cell wall biosynthesis and did so at physiologically relevant concentrations. Heim et al. (1989, 1990a) also isolated mutants of mouse-ear cress with increased resistance to isoxaben. Furthermore, Heim et al. (1991) used wild type and resistant mutants of mouse-ear cress to demonstrate that the herbicidal action of isoxaben is due to its effect on cellulose biosynthesis.

Ultrastructural and immunocytochemical studies of cell plates in tobacco (*Nicotiana tabacum* L.) cells revealed that isoxaben and dichlobenil affect two different stages of cell plate formation (Vaughn 1997). Isoxaben-treated cells developed thin cell plates and had no increase in callose or xyloglucan

levels, while dichlobenil-treated cells had a thickened cell plate and were enriched with callose and xyloglucan.

B. Selectivity of isoxaben

Isoxaben is not a very effective herbicide for controlling annual grasses, but does control many broadleaf weeds. Cabanne et al. (1987) suggested that differences in absorption and translocation of isoxaben partly explained the selectivity of isoxaben between dicots and monocots. Heim et al. (1993) reported that marsh bentgrass (*Agrostis palustris* (L.) Huds. var Penncross) did not readily metabolize isoxaben, but glucose incorporation was inhibited less than that seen in mouse-ear cress, a dicot. The mechanism of differential isoxaben tolerance in catchweed bedstraw (*Galium aparine* L.), redroot pigweed and velvetleaf is not due to decreased uptake or metabolic detoxification of isoxaben but due to target site insensitivity (Schneegurt et al. 1994a). Corio Costet et al. (1991a, 1991b) observed the effect of isoxaben on the incorporation of glucose into acid-insoluble cell wall material in wheat and sensitive and tolerant soybean cultures. They concluded that the differential effect was not due to reduced absorption or accelerated detoxification of isoxaben but that the tolerance to isoxaben was correlated to a lesser effect of isoxaben on the synthesis of acid-insoluble cell wall material. In mutants of mouse-ear cress resistant to isoxaben, the herbicide did not inhibit the synthesis of acid-insoluble cell wall material but at the same time, the uptake, accumulation and metabolism by these mutants is indistinguishable from the sensitive wild type plant (Heim et al. 1991).

The absorption and translocation of isoxaben in redroot pigweed was poor when compared to that for mobile herbicides like glyphosate, sethoxydim and mefluidide (Schneegurt et al. 1994b). The potential for postemergence application of isoxaben is limited by poor absorption and translocation.

7. Behavior of isoxaben in soils

According to Huggenberger and Ryan (1985), isoxaben has a high level of biological activity, moderate chemical persistence and relatively low mobility in soil. Mamouni et al. (1992) reported that isoxaben undergoes photodegradation to form eight photoproducts in aquatic systems. Rouchaud et al. (1993a) observed in their study that isoxaben was metabolized in soil into nontoxic products and that organic fertilizer treatments increased the soil persistence of isoxaben. The rates of soil dissipation for isoxaben were greater in the spring and summer season compared to winter (Rouchaud et al. 1993b). This result was in agreement with that of Walker (1987), who observed faster breakdown for spring-applied isoxaben compared to fall application.

Jament and Thoisy-Dur (1988) assessed the movement of isoxaben in soil. They concluded that isoxaben was less mobile than atrazine and therefore it would not be a potential

environmental hazard due to leaching toward groundwater. When sampled at soil depths up to 90 cm neither isoxaben nor its hydroxy metabolite showed significant leaching potential². Walker (1987) reported that isoxaben did not leach deeper than 6 to 8 cm. However, isoxaben can move off-site in irrigation runoff water. Approximately 9% of the applied isoxaben was found in the runoff water from a container nursery (Wilson et al. 1996). Isoxaben was found in the runoff up to five days after treatment in a container study (Briggs et al. 1997).

8. Objectives

There is limited information available on the reasons for differential tolerance of woody nursery crops to isoxaben. In this research, the woody species wintercreeper and dwarf burning bush, and the herbaceous perennial ajuga were used.

Wintercreeper euonymus is tolerant to isoxaben while dwarf burning bush and ajuga are sensitive to isoxaben applications (Anonymous 1994). The basis for this differential response to isoxaben is unclear, and research in this area would be useful in maximizing the tolerance of nursery crops to this herbicide. By adjusting the timing and rate of application of isoxaben, we may be able to alter the sensitivity of certain ornamentals to isoxaben. The specific objectives of this research were to:

1. To compare the response of ajuga, wintercreeper and dwarf burning bush following treatments with foliar and soil applied isoxaben.
2. To determine the response of dwarf burning bush to isoxaben at three different growth stages.
3. To determine the basis of differential tolerance of ajuga, wintercreeper and dwarf burning bush to isoxaben by:
 - a. Comparing absorption, translocation and metabolism following root and shoot exposure to radiolabeled isoxaben in these three crops.
 - b. Comparing absorption, translocation and metabolism during two growth stages of dwarf burning bush following foliar application of radiolabeled isoxaben.
 - c. Determining the effect of isoxaben on the incorporation of radiolabeled glucose into the cell wall of these three species.

² Personal communication with Dr. Ray Cooper, DowElanco.

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Table 1. Ornamental species injured by isoxaben applications.

Common name	Scientific name
Slight to moderate injury	
Dwarf burning bush	<i>Euonymus alatus</i> (Thunb) Sieb. 'Compacta'
Ligustrum	<i>Ligustrum japonicum</i> Thunberg.
Indian azalea	<i>Rhododendron indicum</i> Sweet.
Common lilac	<i>Syringa vulgaris</i> L.
Red oak	<i>Quercus rubra</i> (Marsh.) Ashe
Iceplant	<i>Mesembryanthemum crystallinum</i> L
Gazania	<i>Gazania rigens</i> L..
English ivy	<i>Hedera helix</i> L
Stachys	<i>Stachys byzantina</i> L.
Severe injury	
Shasta daisy	<i>Chrysanthemum maximum</i> Ramond.
Gloriosa daisy	<i>Rudbeckia hirta</i> L.
Ox-eye daisy	<i>Chrysanthemum leucanthemum</i> L.
Lanceleaf coreopsis	<i>Coreopsis lanceolata</i> L.
Yarrow	<i>Achillea millifolium</i> L.
Blanket flower	<i>Gaillardia aristata</i> Pursh.
Purple coneflower	<i>Echinacea purpurea</i> (L.) Moench.
Impatiens	<i>Impatiens wallerana</i> Hook.
Begonia	<i>Begonia</i> spp.
Ajuga	<i>Ajuga reptans</i> L.

Chapter II
Differential Response of Ajuga, Wintercreeper and Dwarf
Burning Bush to Root and Shoot-Applied Isoxaben³

³ Format followed for this chapter is based on Weed Technology.

Chapter II

Differential Response of Ajuga, Wintercreeper and Dwarf Burning Bush to Root and Shoot-Applied Isoxaben

Abstract. Greenhouse and lathhouse studies evaluated the effects of isoxaben rate and application type on the growth of ajuga (*Ajuga reptans* L. 'Alba'), wintercreeper [*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Colorata'] and dwarf burning bush [*Euonymus alatus* (Thunb) Sieb. 'Compacta']. Isoxaben applied in nutrient solution caused root weight reductions to all three species. Isoxaben foliarly applied to plants in nutrient solution caused approximately 30% shoot injury to ajuga and dwarf burning bush, but no visible injury in wintercreeper at 6 weeks after treatment (WAT). Isoxaben at 3.39 kg ai/ha applied to the roots and shoots reduced shoot and root weights of ajuga in sand culture. Isoxaben applied only to the roots caused less injury to ajuga than foliar or root plus foliar application. Isoxaben caused approximately 20 to 30% injury to dwarf burning bush in sand culture from root, foliar and root plus foliar application. Isoxaben applied to roots or the shoot did not visually injure wintercreeper in any study. Both root and shoot tissues of dwarf burning bush and ajuga were affected by root and foliar application. Since shoot application results in root injury in ajuga, there may be an indirect effect on the roots. At the typical application rate of 0.84 kg/ha, no visible injury or weight reductions were noticed in wintercreeper. However, slight root and shoot reductions were noticed in wintercreeper at 3.39 kg/ha of isoxaben, three times the maximum use rate. These results suggest that the site of action for isoxaben is in both root and shoot tissues for these three species.

INTRODUCTION

Isoxaben controls annual broadleaf weeds with acceptable crop tolerance in many container and field-grown landscape plants (Skroch et al. 1990). Isoxaben did not injure field-grown boxwood (*Buxus microphylla*), holly (*Ilex* x 'Nellie R. Stevens'), nandina (*Nandina domestica* L.), Japanese yew (*Taxus cuspidata* Sieb. & Zucc.), globe arborvitae (*Thuja occidentalis* L.), Eastern hemlock (*Thuja canadensis* L.), creeping juniper (*Juniperus horizontalis* Moench.) and catawba rhododendron (*Rhododendron catawbiense* Mich.) (Gilliam et al. 1989; Mervosh and Ahrens 1997). Neal and Senesac (1990) reported that isoxaben at 0.56 and 1.1 kg/ha did not injure container-grown 'Coral Bells' azalea [*Rhododendron obtusum* (Lindl.) Planch.], 'Roseum Elegans' rhododendron, rockspray cotoneaster (*Cotoneaster horizontalis* Deene), forsythia (*Forsythia* x *intermedia* Zab.), 'Nana' Japanese dwarf garden juniper [*Juniperus procumbens* (Engl.) Miq.], 'Old Gold' juniper (*Juniperus* x *media* Van Melle), 'Albo-marginata' plaintain lily (*Hosta lancifolia* Engl.) and 'Tangerine' bush cinquefoil (*Potentilla fruticosa* L.).

Generally gymnosperms and grasses are tolerant to isoxaben applications. Although many nursery crops tolerate isoxaben, certain herbaceous perennials and woody ornamentals are injured by this herbicide (Skroch et al. 1990.). Fuller (1990) reported that ligustrum (*Ligustrum japonicum* Thunberg.) and 'Judge Solomon' indian azalea (*Rhododendron indicum* Sweet.) were injured when multiple applications of isoxaben were made. Plants belonging to the family Compositae are injured by isoxaben. Isoxaben at 1.12 kg/ha caused moderate to severe injury to 'Alaska' shasta daisy (*Chrysanthemum maximum* Ramond), gloriosa daisy (*Rudbeckia hirta* L.), 'Summer Carnival' hollyhock (*Althaea rosea* L.), and 'Summer Pastels' yarrow (*Achillea millifolium* L.) (Porter 1996). Isoxaben caused unacceptable injury in field-grown and container-grown lanceleaf coreopsis (*Coreopsis lanceolata* L.), oxeye daisy (*Chrysanthemum leucanthemum* L.), blanket flower (*Gaillardia aristata* Pursh.) and purple coneflower (*Echinacea purpurea* (L.) Moench.) (Derr 1993; Derr 1994a) and stunted Stachys (*Stachys byzantina*) (Staats and Klett 1993). Ajuga and hydrangea were injured by isoxaben in a container study (Tables A.1 and A.2)

Little is known on the importance of root versus shoot uptake in the sensitivity of ornamentals to isoxaben. This research evaluated the effect of root, shoot, and root plus shoot exposure of ajuga, wintercreeper and dwarf burning bush to isoxaben. These species were chosen because ajuga and dwarf burning bush are sensitive to isoxaben applications, while wintercreeper, in the same genus as dwarf burning bush, is listed as tolerant (Anonymous 1994).

MATERIALS AND METHODS

General conditions. Rooted cuttings of wintercreeper and dwarf burning bush and divisions of ajuga were maintained in a greenhouse. Average day/night temperatures in the greenhouse were 35/25 C and ambient light level at noon was approximately 650 $\mu\text{mol}/\text{m}^2/\text{sec}$. Plants were fertilized with 17N-2.6P-9.9K slow release fertilizer containing micronutrients⁴ and watered daily. All plants were actively growing at the time of treatment. Foliar applications of isoxaben were made using a CO₂-pressurized backpack sprayer delivering 230 L/ha. The jars were refilled alternately with water and Hoagland's solution every other day. Injury ratings were taken at 3 weeks and 6 weeks after treatment (WAT). Shoot and root weights were recorded 6 WAT. Data collected included visual ratings of root and shoot injury, shoot fresh weight and root fresh weight. The shoot injury ratings were rated on a scale of 0 to 100 (with 0 = no injury and 100 = complete kill), while the root ratings were on a scale of 1 to 10 (with 1 = healthy white roots and 10 = dead brown roots). The

⁴Osmocote 17-6-12; The Scotts Co., Marysville, OH 43040.

best plant regardless of treatment was given a rating of 0% shoot injury, and other plants were compared to it. Each experiment was repeated and the data presented is the mean of both experiments. Data were subjected to analysis of variance, followed by regression analyses and mean separation. For mean separation, Fisher's Protected Least Significant Difference (LSD) test at $P = 0.05$ level was used.

Nutrient solution study. The experiment was a randomized complete block design with 4 replications. After the roots were thoroughly washed free of pine bark, plants were transferred to aluminum foil-covered glass jars filled with 180 ml of full-strength Hoagland's solution. After a week of acclimatization to the Hoagland's solution, plants were treated with isoxaben. For the root only applications, the nutrient solution was replaced with fresh Hoagland's solution containing isoxaben concentrations of 0, 0.5, 1, 2 and 4 parts per million (ppm). The 0.5 ppm rate corresponds to a field application of approximately 1.1 kg/ha.

Another experiment containing the shoot only applications was conducted. To prevent root exposure to isoxaben, jars were covered with aluminum foil prior to herbicide application.

Isoxaben was applied at 0.84, 1.69 and 3.39 kg/ha to the foliage.

Sand culture study. The experiment was a randomized complete block with a factorial arrangement of treatments. The three factors were species, isoxaben rates and application types. Plants were transplanted into 417 ml styrofoam cups containing river-type silica sand and drain holes. Isoxaben at 0.84, 1.69 and 3.39 kg/ha was applied to the roots, shoots and roots plus shoots. The shoot only treatments were achieved by shielding the sand surface with aluminum foil and then removing the foil after application. The root only treatments were made by spraying the sand in the styrofoam cups before transplanting. For the root plus shoot treatments, applications of isoxaben were made over-the-top of plants. The data were subjected to factorial analysis of variance.

RESULTS AND DISCUSSION

Nutrient solution study. *Root application:* An increase in isoxaben rate did not increase shoot or root injury nor shoot or root weight reduction for ajuga, wintercreeper and dwarf burning bush (data not shown). At 3 and 6 WAT, isoxaben applied to roots injured shoots of ajuga but not wintercreeper or dwarf burning bush (Table 1). Greater injury was observed in ajuga at 6 WAT than at 3 WAT. Ajuga roots were visually injured by isoxaben, but no injury was seen in wintercreeper or dwarf burning bush roots. Isoxaben caused shoot fresh weight reduction in ajuga but not in wintercreeper or dwarf burning bush following root application. This injury following root exposure to ajuga is consistent with Derr (1994b) who observed a 57% reduction in the shoot fresh weight of ajuga with a granular formulation containing isoxaben and trifluralin. Isoxaben caused greater

root weight reduction in ajuga as compared to wintercreeper and dwarf burning bush.

Foliar application: Shoot and root injury did not increase as isoxaben rate increased (data not shown). Isoxaben caused similar amounts of shoot injury to ajuga and dwarf burning bush at 3 and 6 WAT (Table 2). However, isoxaben caused more root injury to ajuga than to dwarf burning bush. Ajuga roots injured by isoxaben were stunted and brown in color, while the untreated plants had healthy white roots. Roberts (1991) noticed swelling of the seedling root tip in redroot pigweed (*Amaranthus retroflexus* L.) due to isoxaben treatments.

The shoot injury symptoms observed in ajuga included chlorotic new leaves and general stunting of the plant. Injury symptoms for dwarf burning bush included puckered and chlorotic leaf appearance, downward curling of leaves and bending of petioles. Setyowati et al. (1994) reported slight necrosis and discoloration of leaves in dwarf burning bush from foliar applications of isoxaben. Swollen and split stems and petioles were noticed following foliar application in redroot pigweed (Schneegurt et al. 1994). Derr (1994b) also observed that isoxaben applied foliarly reduced shoot fresh weight and caused visual injury in ajuga.

Isoxaben applied to ajuga roots caused a 40% reduction in root weight, while foliar applications reduced root weights by 32% (Tables 1 and 3). Greater shoot reduction was observed with foliar application than with root application for ajuga. The highest rate of isoxaben caused a 10% reduction in shoot weight in wintercreeper, but the lower rates did not decrease shoot weight. Isoxaben foliarly-applied at 3.39 kg/ha reduced root weight of dwarf burning bush by 20% with no reductions seen at the lowest rate.

Sand culture study. Species responded differently to isoxaben rate and application type as the three way interaction for shoot injury was significant (Table 4). Isoxaben at 1.69 and 3.39 kg/ha caused more injury to ajuga than dwarf burning bush or wintercreeper following all application types at 2 MAT (Table 5). The injury to ajuga from root and shoot application is consistent with reports that sprayable and granular formulations of isoxaben caused visual injury to ajuga (Derr 1994b).

In ajuga, isoxaben applied only to roots caused 12 to 18% shoot injury, which was less than the injury observed with the foliar and root plus foliar applications (Table 5). At 1 MAT, ajuga injury did not increase significantly with increasing rate of isoxaben. However, at 2 MAT, injury increased with increasing rate from the root application and root plus foliar applications. No visual injury was observed in wintercreeper with any application type or rate. Isoxaben caused approximately 20% injury to dwarf burning bush 1 MAT at all rates shoot applied. However, at 2 MAT, more injury was noticed with the root plus foliar application as compared to root only or foliar only application.

Averaged over species, application type had an effect on root weight reductions, but not shoot weight reductions (Table 4). Root applications resulted in lower root weight reduction than shoot or root plus shoot application (Table 6). Greater root weight reductions were seen in ajuga than wintercreeper or dwarf burning bush averaged over application type. Also, generally greater shoot weight reductions, were seen in ajuga than the other two species. Derr (1994b) reported that isoxaben at 1.1 and 2.2 kg/ha caused 30 and 75% shoot fresh weight reductions, respectively, to ajuga six weeks after treatment. In wintercreeper, root applications caused greater root weight reductions than shoot weight reductions. In dwarf burning bush, shoot applications caused greater root weight reductions than shoot weight reductions. Averaged over species, approximately 20% shoot weight reductions was seen with all three application types.

Species differed in their response to varying isoxaben rate for both root and shoot weight (Table 4). Isoxaben at all rates caused greater reduction of root and shoot fresh weight in ajuga as compared to wintercreeper and dwarf burning bush, when averaged over application type (Table 7). Isoxaben at 0.84 kg/ha and 1.69 kg/ha did not reduce shoot or root weight in wintercreeper. Wintercreeper and dwarf burning bush had similar root and shoot weight reductions within an isoxaben application rate. Although no visible root injury was observed in wintercreeper and dwarf burning bush (data not shown), approximately 30% reduction of root weight was noticed at the highest rate. Derr and Salihi (1996) reported that isoxaben at 1.12 kg/ha reduced new root growth of 'Helleri' Japanese holly (*Ilex crenata* Thunb.) and the shoot growth of 'Tradition' azalea (*Rhododendron* spp.). Isoxaben reduced shoot fresh weight of redroot pigweed and wild mustard [*Brassica kaber* (D.C) L.C.Wheeler] following application to roots, foliage, and roots plus foliage (Schneegurt et al. 1994).

Isoxaben caused greater shoot and root weight reductions in ajuga than wintercreeper and dwarf burning bush following both root and foliar application in nutrient solution as well as in sand (Tables 1, 3, and 6). Greater reductions were seen in sand culture when compared to plants in nutrient solution. This may be due to the longer exposure of the plants to the herbicide in the sand culture versus the nutrient solution. In wintercreeper, root uptake of isoxaben from the nutrient solution resulted in root weight reductions but not shoot weight reductions in the nutrient solution. In the sand study, root uptake of isoxaben by wintercreeper resulted in root and shoot weight reductions. Although shoot injury was noticed in dwarf burning bush, very little reduction in shoot weight occurred. This may be because shoot injury was primarily leaf distortions with little effect on plant size. Greater shoot weight reductions would possibly be noticed after longer exposure to isoxaben.

Foliar injury resulted from both root and shoot uptake in ajuga and dwarf burning bush, while no visible injury was observed in wintercreeper. Wintercreeper growing in nutrient solution tolerated isoxaben applied foliarly at 0.84 and 1.69 kg/ha, but shoot weight reduction occurred at 3.39 kg/ha. However, root and shoot weight reductions occurred when isoxaben was applied foliarly at 3.39 kg/ha to wintercreeper growing in sand. Unlike ajuga and dwarf burning bush, foliar uptake did not result in root weight reductions in wintercreeper. The roots and shoots of ajuga and dwarf burning bush appear to be sensitive to isoxaben.

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