Chapter III
Effect of Gallery Applied at Different Growth Stages to Dwarf Burning Bush (*Euonymus alatus* 'Compacta')[5]

[5] Format for this chapter is based on the Journal of Environmental Horticulture.

Chapter III
Effect of Gallery Applied at Different Growth Stages to Dwarf Burning Bush (*Euonymus alatus* 'Compacta')

Abstract

The effect of Gallery application timing on dwarf burning bush tolerance was determined in field trials. Gallery was applied foliarly at 0.84, 1.69 and 3.39 kg ai/ha (0.75, 1.5 and 3 lb ai/A) to dwarf burning bush. Data collected included injury ratings at three different time periods, height and width measurements and percent premature defoliation. Gallery applied at the dormant stage and two months after the bud-break stage did not injure dwarf burning bush. Plants treated one month after bud-break were injured approximately 30 to 45% at one and three months after treatment at all three rates. Gallery applied at all growth stages did not affect the growth index of dwarf burning bush when compared to untreated plants in August. Shoot-dieback was observed in plants treated with Gallery one month after bud-break. Gallery applied one month after bud-break caused 60 to 75% of the leaves to defoliate six weeks earlier than untreated plants. Casoron did not cause injury or affect the growth of dwarf burning bush at any stage of application.

Index words: herbicide tolerance, growth stages
Species used in this study: Dwarf burning bush (*Euonymus alatus* (Thunb.) Sieb. 'Compacta').
Herbicides used in this study: Casoron (Casoron), 2,6 dichlorobenzonitrile; Gallery (Gallery), N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl] 2,6-dimethoxybenzamide.

Significance to the nursery industry

The timing of herbicide application relative to the growth stage of the nursery crop can affect plant tolerance. Gallery applied at the leaf emergence stage caused injury to dwarf burning bush. The dormant and leaf maturation stages were tolerant to Gallery applications. Nurserymen should use caution when using Gallery on areas adjacent to actively-growing dwarf burning bush.

Introduction

Nurseries commonly use herbicides for weed control. Plant tolerance to herbicides is an important factor in the selection of herbicides by nurserymen. Gallery (isoxaben), a selective preemergence herbicide, has been evaluated for broadleaf weed control in ornamentals, turf, landscape plantings, small grains and in orchard crops (1, 6). Gallery is commercially available as a 75 percent dry flowable formulation and in combination with Treflan as Snapshot 2.5TG in the United States. It was also formerly marketed in combination with Surflan as Snapshot 80DF. Gallery is an alternative preemergent herbicide to Princep (simazine) use in nursery crops because of their similar weed spectrum (10).

Herbicides must be used with caution because improper application can cause economic loss due to plant damage. A desirable characteristic of Gallery is the high degree of safety it exhibits for most nursery species (9, 12).

Neal and Senesac (12) reported that Gallery at rates of 0.56 or 1.1 kg ai/ha (0.5 or 1 lb ai/A) did not injure a number of field-grown woody ornamentals and controlled most broadleaf weeds except velvetleaf (*Abutilon theophrasti* Medic.), common mallow (*Malva neglecta* L.) and smooth pigweed (*Amaranthus hybridus* L.).

Mervosh and Ahrens (9) reported that Gallery at 0.84 kg ai/ha (0.75 lb ai/A) did not injure actively growing Japanese yew (*Taxus cuspidata* Sieb. & Zucc.), globe arborvitae (*Thuja occidentalis* L.), Eastern hemlock (*Thuja canadensis* L.), creeping juniper (*Juniperus horizontalis* Moench.) and rhododendron (*Rhododendron catawbiense* Michx.). Gallery did not injure field grown sawara false cypress (*Chamaecyparis pisifera* (Siebold & Zucc.) Endl. 'Plumosa'), honey locust (*Gleditsia triacanthos* L.), Japanese Yew, holly (*Ilex x auipernyi* Gable ex W. Clarke 'San Jose'), white pine (*Pinus stribus* L.), Douglas fir (*Psuedotsuga menziesii* (Mirb.) Franco) and white fir [*Abies concolor* (Gord.) Lindl. ex Hildebr.] (12). However, the growth and vigor of common lilac (*Syringa vulgaris* L.) was reduced by Gallery applied at 0.56 kg/ha (0.5 lb/A). Gallery was not phytotoxic to strawberry and raspberry, was relatively persistent over the winter months and was very effective on *Brassica* weed species (8). Gilliam et al. (4) concluded that Gallery can be safely used on field-grown boxwood (*Buxus microphylla*), holly (I*lex x 'Nellie* R. Stevens') and nandina (*Nandina domestica)*.

Over-the-top spray applications of Gallery may be phytotoxic to certain nursery crops (3, 5, 7). Jacobsen and Walls (7) reported that foliar injury was induced on iceplant (*Mesembryanthemum crystallinum* L.), gazania (*Gazania rigen*s L.) and English ivy (*Hedera helix* L.). Root symptoms included root nubbing, root stunting and root discoloration (7). Derr and Salihu (3) reported that Gallery at 1.12 kg/ha (1 lb/A) reduced new root growth of Japanese holly (*Ilex crenata* Thunb.'Helleri') after one application and the shoot growth of azalea (*Rhododendron obtusum* Planch. 'Tradition') after three applications. Staats and Klett (14) found that Gallery stunted stachys (*Stachys byzantina).*

Gallery applied at 0.8 kg ai/ha (0.75 lb ai/A) caused slight necrosis, and discoloration in common lilac and red oak [*Quercus ruba* (Marsh.) Ashe.] (13). Stem diameter reduction in common lilac, and trunk diameter reduction in red oak were attributed by the authors to inadequate annual grass control resulting from Gallery alone application.

Dwarf burning bush is injured by Gallery applications (1). This experiment was conducted to determine the effect of Gallery application timing on dwarf burning bush tolerance. By adjusting the timing and rate of application, one may be able to alter the sensitivity of certain ornamentals to Gallery.

Materials and methods

General conditions: This field experiment was conducted at a commercial nursery in Waynesboro, Virginia. The soil pH was 6.0 and and the organic matter level was 2.6%. The soil type was Frederick silt loam (clayey, kaolinitic, mesic, Typic Paleudults). The experimental design was a randomized complete block with four replications and five plants per plot. Treatments were applied at three growth stages of dwarf burning bush: at the dormant stage, one month after bud-break (leaves were 2.5 to 3 cm long and actively growing) and two months after bud-break (leaves were 7 to 8 cm long). Casoron, labeled for use on dwarf burning bush, was included for comparison. A 75% dry flowable formulation of Gallery was applied at 0.84, 1.69 and 3.39 kg ai/ha (0.75, 1.5 and 3 lb ai/A) and a 50% wettable powder formulation of Casoron was applied at 4.48 kg ai/ha (4 lb ai/A). Herbicides were applied over the top of plants using a CO_2-pressurized backpack sprayer delivering 230 L/ha (25 gal/A). Data collected include injury ratings at three different time periods, height and width measurements and percent premature defoliation. Growth index was calculated by taking the average of the height and width measurements for a plant. Data were subjected to analysis of variance with mean separation by the Least Significant Difference (LSD) test at P=0.05. The experiment was repeated in 1996 and the results were combined. The treated plants in 1995 were observed for injury symptoms in 1996.

Experiment 1: The first herbicide application (dormant stage application) was made on March 14, 1995. The first rainfall after application was on March 21, and measured 0.33 cm (0.13 inches). Air temperature was 21 C (70 F), wind speed was 0 to 8 kmph (0 to 5 mph), and cloud cover was 0 to 3%. There were 5 plants per treatment. The plant spacing was 120 by 90 cm (3 by 4 feet). The second application (one month after bud-break) was made on April 26, 1995. The first rainfall after application was on April 30, and measured 0.3 cm (0.1”). Air temperature was 24 C (75 F), wind speed was 0 to 8 kmph (0 to 5 mph), cloud cover was 0%, and the soil surface was dry. The third application (two months after bud-break) was made on June 4, 1995. The first rainfall after application was on June 11, and measured 3.3 cm (1.3”). Air temperature was 27 C (78 F), wind speed 0 to 8 kmph (0 to 5 mph) and cloud cover was 50%.

Experiment 2: The first application (dormant stage application) was made on March 14, 1996. The first rainfall after application was on March 16, and measured 0.28 cm (0.11”). Air temperature was 16 C (60 F), wind speed was 0 to 8 kmph (0 to 5 mph), and cloud cover was 80%. There were 3 plants per treatment. The plant spacing was 120 by 90 cm (3 by 4 feet). The second application (one month after bud-break) was made on April 25, 1996. The first rainfall after application was on April 27, and

measured 0.1 cm (0.04”). Air temperature was 21 C (70 F), wind speed was 8 to 16 kmph (5 to 10 mph) and cloud cover was 50%. The third application (two months after bud-break) was made on June 25, 1996. The first rainfall after application was on July 3, and measured 0.53 cm (0.21”). Air temperature was 31 C (87 F), wind speed was 0 to 8 kmph (0 to 5 mph) and cloud cover was 5%.

Results and Discussion

Injury ratings were taken in June, July and August. All rates of Gallery applied at the dormant and at two months after bud-break did not injure dwarf burning bush when compared to untreated plants (Table 1). Gallery did not inhibit the initiation of new leaves after the dormant stage application nor were the emerging leaves affected (data not shown).

Plants treated one month after bud-break were injured about 35% in June regardless of the Gallery rate. Injury symptoms were observed even at four months after treatment from this application. Casoron did not cause any injury to dwarf burning bush at any stage of application.

The injury symptoms observed following Gallery application at the leaf emergence stage were curling of the leaves and smaller leaf size compared to untreated plants. Downward bending of the stems and shoot dieback were also noticed in these injured plants at five months after treatment (Figure 1). These injury symptoms were similar to that reported by Jacobsen and Walls in certain ornamentals (7). They observed bronzing of leaves, curled leaves and meristematic shoot death in actively growing plants injured by Gallery. Growth stage appears to be important in the tolerance of dwarf burning bush to Gallery. Setyowati et al. (13) reported that Gallery applied in combination with trifluralin or oryzalin at 4.2 kg ai/ha caused slight necrosis and discoloration to dwarf burning bush in the first year of application.

Gallery applied at any rate or growth stage did not reduce plant size when compared to the untreated plant (Table 2). However, at the time of measurements, shoot dieback following applications made at one month after bud-break was not as severe as in September. Casoron did not cause reductions in growth of dwarf burning bush at any stage of application.

Premature defoliation of dwarf burning bush plants was observed in plants treated with Gallery one month after bud-break (Figure 2). Gallery applied at the dormant and two months after bud-break stages did not cause premature defoliation. Casoron did not cause premature defoliation when applied at any growth stage. After one year, no injury was observed in the plants treated with Gallery one month after bud-break stage (data not shown).

Dwarf burning bush is more tolerant to Gallery applications at the dormant stage and at two months after bud-break. Although plants are damaged from applications made one month after bud-

break, the injury is only apparent in the year of application. The plants outgrow the damage in the following year as the emerging leaves were unaffected. However, nurserymen could not afford to lose one year’s worth of growth when producing this species.

Literature Review

1. Anonymous. 1994. Gallery, Experimental herbicide for use in turf and ornamentals. Tech. Bulletin, DowElanco, Indianapolis, Indiana. 12p.

2. Colbert, F. O. and D. H. Ford. 1987. Gallery for broadleaf weed control in ornamental, turf and on bearing vines and trees. Proc. West. Weed Sci. Soc. 40:155-163.

3. Derr, J. F. and S. Salihu. 1996. Preemergence herbicide effects on nursery crop root and shoot growth. J. Environ. Hort. 14:210-213.

4. Gilliam, C. H., G. Wehtje, J. E. Eason, T. V. Hicks and D. C. Fare. 1989. Weed control with Gallery and other herbicides in field grown nursery crops. J. Environ. Hort. 7:69-72.

5. Hood, L. R. and J. E. Klett. 1992. Preemergence weed control in container grown herbaceous and woody plants. J. Environ. Hort. 10:8-11.

6. Huggenberger, F., Jennings E. A., P. J. Ryan and K. W. Burrow. 1982. EL-107 a new selective herbicide for use in cereals. Proc. British Crop Prot. Conf. Weeds. 1:47-52.

7. Jacobsen, S. and K. M. Walls. 1987. The phytotoxicity effects of Gallery on ornamental plants and non-bearing trees and vines. Proc. Calif. Weed Conf. 39:20-21.

8. Lawson, L. B. and J. S. Wiseman. 1987. Crop tolerance to trifluralin and Gallery applied alone or in mixture with napropamide as late winter herbicide treatments in established strawberry and raspberry. Proc. British Crop Prot. Conf. Weeds. 6:657-663.

9. Mervosh, T. L. and J. F. Ahrens. 1997. Herbicidal activity of and woody ornamental tolerance to sulfentrazone and halosulfuron. Weed Sci. Soc. Am. Abstracts. 37:29.

10. Neal, J. C. 1993. Alternatives to Princep (simazine) for weed control in field nurseries. Proc. Weed Control In Ornamentals Symp. Dept. of Plant Path. Physiol., and Weed Sci. Info. Note 171. Virginia Polytech. Inst. and State Univ. Edited by J.F. Derr. p:3-6.

11. Neal, J. C. and A. F. Senesac. 1988. Broadleaved weed control in woody ornamentals with Gallery. Proc. Northeast. Weed Sci. Soc. 42:124-125.

12. Neal, J. C. and A. F. Senesac. 1990. Preemergent weed control in container and field grown woody ornamentals with Gallery (Gallery). J. Environ. Hort. 8:103-107.

13. Setyowati, N., L. A. Weston and R .E. McNiel. 1995. Evaluation of selected preemergence herbicides in field grown landscape crops in Kentucky. J. Environ. Hort. 13:196-202.

14. Staats, D. and J. E. Klett. 1993. Evaluation of weed control and phytotoxicity of preemergence herbicides applied to container-grown herbaceous and woody plants. J. Environ. Hort. 11:78-80.

Table 1. Effect of herbicides applied at three different growth stages of field-grown dwarf burning bush, averaged over two years.

Herbicide	Rate (kg ai/ha)	Application timing[1]	Percent Injury[2] June	July	August
Untreated					
Gallery	0.84	March	5	5	6
Gallery	1.69	March	8	4	5
Gallery	3.39	March	1	2	2
Casoron	4.48	March	4	2	5
Gallery	0.84	April	32	37	37
Gallery	1.69	April	34	41	42
Gallery	3.39	April	36	43	45
Casoron	4.48	April	6	5	5
Gallery	0.84	June	-[3]	7	5
Gallery	1.69	June	-	5	9
Gallery	3.39	June	-	6	4
Casoron	4.48	June	-	7	12
LSD (0.05)			6	9	13

[1]Dormant applications were made in March, the second growth stage was treated one month after bud-break in April and the third growth stage was treated two months after bud-break in June.
[2]Percent injury was rated on a scale of 0 to 100 (0 = no injury, 100 = complete kill).
[3]Plants were not rated as plants were treated in June.

Table 2. Effect of herbicides applied at three different growth stages on the growth of field-grown dwarf burning bush, averaged over two years.

Herbicide	Rate (kg ai/ha)	Application timing[1]	Growth index[2] July	Growth index[2] August
			-----cm-----	
Untreated			90	88
Gallery	0.84	March	78	80
Gallery	1.69	March	83	88
Gallery	3.39	March	80	83
Casoron	4.48	March	85	80
Gallery	0.84	April	73	75
Gallery	1.69	April	73	75
Gallery	3.39	April	80	80
Casoron	4.48	April	83	90
Gallery	0.84	June	88	90
Gallery	1.69	June	85	88
Gallery	3.39	June	85	88
Casoron	4.48	June	85	88
LSD (0.05)			NS	NS

[1]Dormant applications were made in March, the second growth stage was treated one month after bud-break in April and the third growth stage was treated two months after bud-break in June.
[2]Growth index was calculated by taking the average of height and width of a plant.

Figure 1. Injury in dwarf burning bush from Gallery applied one month after bud-break.

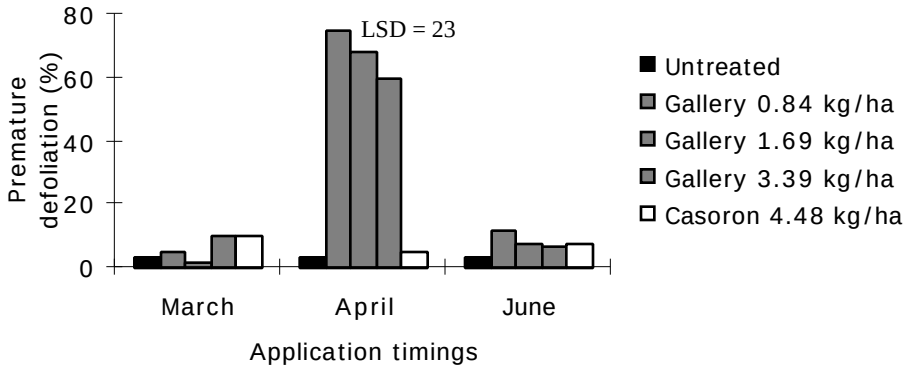

Figure 2. Effect of herbicides applied at three different timings, March, (dormant), April (one month after bud-break) and June (two months after bud-break) on premature defoliation (%) in dwarf burning bush as observed in September. LSDs are listed where significant differences were present for premature defoliation among herbicides at an application timing.

Chapter IV
Uptake, Translocation and Metabolism of Root-applied Isoxaben in Ajuga (*Ajuga reptans*), Wintercreeper (*Euonymus fortunei*) and Dwarf Burning Bush (*Euonymus alatus*)[6]

[6] Format followed is for the journal Weed Science.

Chapter IV
Uptake, Translocation and Metabolism of Root-applied Isoxaben in Ajuga (*Ajuga reptans*), Wintercreeper (*Euonymus fortunei*) and Dwarf Burning Bush (*Euonymus alatus*)

Abstract. The ornamental species ajuga (*Ajuga reptans* L. 'Alba'), wintercreeper [*Euonymus fortunei* (Turcz.) Hand.-Mazz. 'Colorata'] and dwarf burning bush [*Euonymus alatus* (Thunb) Sieb. 'Compacta'] differ in their response to isoxaben. To determine the basis for this selectivity, the uptake, translocation and metabolism of root-applied isoxaben was studied in these three species. Plants treated with radiolabeled isoxaben were harvested 1, 3, 7, and 14 days after treatment (DAT). Uptake of radioactivity increased with time in all three species. Dwarf burning bush absorbed the most radioactivity at 14 DAT compared to ajuga and wintercreeper. Translocation of absorbed radioactivity from roots to shoot at 1 and 3 DAT was greatest in ajuga, the most sensitive species, intermediate in wintercreeper and least in dwarf burning bush. Thin-layer chromatographic analysis of root extracts of wintercreeper and dwarf burning bush showed that most of the recovered radioactivity at all time intervals corresponded to unmetabolized isoxaben. The ^{14}C recovered from ajuga roots at 7 and 14 DAT appeared to be primarily metabolites of isoxaben. Most of the radioactivity recovered from shoots at 14 DAT in the three species appeared to be polar metabolites of isoxaben, possibly conjugates. Greater absorption by ajuga and dwarf burning bush than wintercreeper may explain the greater sensitivity of these species to root applications of isoxaben. Translocation of radioactivity to the foliage may explain the shoot weight reductions seen in ajuga, wintercreeper and dwarf burning bush. Metabolic detoxification of isoxaben did not explain the differential selectivity of isoxaben observed in the sensitive species ajuga and the tolerant species wintercreeper. Metabolism of isoxaben appeared to be greater in wintercreeper than dwarf burning bush. The effect of isoxaben on glucose incorporation into cell wall material was examined using roots of the three species. Isoxaben inhibited glucose incorporation by approximately 10% in roots of ajuga, but did not inhibit glucose incorporation in roots of wintercreeper or dwarf burning bush. Nomenclature: Isoxaben, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl] 2,6-dimethoxybenzamide.
Additional index words: woody ornamentals, glucose incorporation, herbicide selectivity, nursery crops, radiolabeled herbicide.

INTRODUCTION

Isoxaben is a preemergence herbicide for broadleaf weed control in established turf and ornamentals (Anonymous 1994;

Colbert and Ford 1987). Although isoxaben may be used safely on a variety of container and field-grown ornamentals (Neal and Senesac 1990), it can injure certain species (Jacobsen and Walls 1987; Derr 1993; Derr and Salihu 1996). Isoxaben applied at 0.56 kg ha^{-1} injured field-grown common lilac (*Syringa vulgaris* L.) and certain container-grown herbaceous perennials (Neal and Senesac 1990; Porter 1996). Fuller (12) reported that many ornamentals may be injured when multiple applications of isoxaben are made. Isoxaben applied to soil at 0.56 and 1.1 kg ha^{-1} induced root nubbing, root stunting, and root discoloration in iceplant (*Mesembryanthemum crystallinum* L.), gazania (*Gazania rigens* L.) and English ivy (*Hedera helix* L.) (Jacobsen and Walls 1987). Corio-Costet et al. (1991) reported that the cytological symptoms seen in the sensitive tissue cultured cells of soybean [*Glycine max* (L.) Merr.] were a detachment of the plasma membrane from the cell wall and the deposition of fibrillar material in the extracytoplasmic space. These symptoms, which suggested some abnormality of the cell wall, were absent in tolerant cell cultures.

Dicots are generally more sensitive to isoxaben than monocots (Cabanne et al. 1987). Rape (*Brassica napus* L.) a sensitive species, absorbed more isoxaben into the roots and translocated less into the shoots compared to *wheat (Triticum aestivum* L.). The mode of action of isoxaben is thought to be inhibition of synthesis of acid-insoluble material (cellulose) in the cell wall of sensitive species (Heim et al. 1990). Lefebvre et al. (1987) concluded from their study using tissue-cultured maple (*Acer pseudoplatanus* L.) and soybean that isoxaben inhibited the incorporation of glucose into cell wall materials. Heim et al. (1990) concluded that low concentrations of isoxaben inhibited glucose incorporation into acid-insoluble materials of mouse-ear cress (*Arabidopsis thaliana* L.). Isoxaben and dichlobenil are the only herbicides reported to act on cellulose biosynthesis, but they differ in their effects on cell plate formation (1997). Tobacco (*Nicotiana tabacum* L.) cells affected by isoxaben had thin cell plates with little callose and xyloglucan while cells damaged by dichlobenil had thicker cell plates and higher amounts of callose and xyloglucan.

Ajuga, wintercreeper and dwarf burning bush differ in their response to isoxaben (Chapter II). Dwarf burning bush and ajuga are sensitive to isoxaben applications, while wintercreeper is tolerant up to two times the typical use rate. Dwarf burning bush is sensitive to isoxaben when applied one month after bud-break, but tolerant at the dormant stage and three months after bud-break (Chapter III).

This research was conducted to examine possible causes for this differential response. A plant species can be tolerant to herbicide application because of: 1) decreased herbicide uptake or translocation, 2) compartmentation, 3) increased rate of metabolism or 4) modification of the site of herbicide action, among other mechanisms. Therefore the objectives of this study

were: 1) to determine the absorption, translocation and metabolism of isoxaben following root application in these three species and 2) to study the effect of isoxaben on glucose incorporation into the cell walls of root tissues of these three species.

MATERIALS AND METHODS

Chemicals. Analytical-grade isoxaben[7], radiolabeled isoxaben and radiolabeled glucose[8] were used. The radiochemical purity of isoxaben (uniformly phenyl ^{14}C labeled) was 97.8% with a specific activity of 30.2 mCi/mmol. The radiochemical purity of glucose (uniformly ^{14}C labeled) was more than 98% and its specific activity was 276 mCi/mmol.

General conditions. Divisions of ajuga and rooted cuttings of wintercreeper and dwarf burning bush were used for absorption, translocation, metabolism and glucose incorporation studies. To break dormancy and to attain the desired growth stage, dormant dwarf burning bush was moved to the greenhouse from cold storage two weeks before treatment. All plants were kept in a greenhouse and fertilized with a 17N-2.6P-9.9K slow release fertilizer[9] containing micronutrients and watered daily. After the roots were thoroughly washed free of soil, plants were transferred to aluminum foil-covered glass jars filled with 180 ml of full-strength Hoagland's solution (pH = 6.3). After 7 days, each plant was exposed to 0.16 µCi of root-applied ^{14}C isoxaben for 1, 3, 7 or 14 days for combustion and 3, 7 and 14 days for metabolism studies.

A randomized complete block design was used and each experiment was duplicated. Three plants per species were used for combustion and metabolism, and two were used for autoradiography. Data were subjected to analysis of variance and means were separated by Fisher's Protected Least Significance Difference (LSD) test at the 0.05 probability level.

Uptake and translocation studies. Absorption and translocation of radiolabeled isoxaben was studied qualitatively by autoradiography and quantitatively by tissue combustion. Harvested plants were autoradiogaphed following the procedures of Crafts and Yamaguchi (1964). Treated plants were mounted on paper, pressed, air dried and exposed to X-ray films[10] for 4 weeks before developing.

For the combustion studies, the roots of harvested plants were washed with deionized water to remove any unadsorbed herbicide and then separated into roots and shoots (stems plus leaves). Plant tissue was weighed and dried in an oven at 60C

---

[7] DowElanco, Indianapolis, IN 46268.

[8] Sigma Chemical Co., St. Louis, MO 63178.

[9] Osmocote 17-6-12, The Scotts Company, Marysville, OH 43040.

[10] Kodak Scientific Imaging Films, Eastman Kodak Com., Rochester, NY 14560.

for 24 h. Absorption and translocation of radioactivity into plant tissue was measured by combustion in a biological sample oxidizer[11] and counting of the radioactivity, trapped as $^{14}CO_2$ in a scintillation solution[12], using a liquid scintillation spectrometer[13]. Radioactivity in the water rinses and in the nutrient solution was monitored by liquid scintillation spectrometry. Absorbed radioactivity is expressed as a percentage of applied radioactivity. Distribution of ^{14}C in plant tissue is expressed as percent of absorbed radioactivity.

Metabolism studies. Both the roots and shoots of all species were analyzed for isoxaben metabolism. Isoxaben and its metabolites were extracted following the methods of Corio-Costet et al. (1991a) with slight modifications. Plant roots and shoots were frozen in liquid nitrogen, pulverized with a mortar and pestle and homogenized with 10 ml of 80% methanol. The homogenates were centrifuged at 2000 x g for 10 min and the supernatant was removed and saved. The pellets were extracted two more times in 80% methanol. The combined supernatants (30 ml) were air dried to 5 ml and then concentrated by rotoevaporation to 0.5 ml. The concentrated extracts were then cleaned by filtration through 0.25 µm nylon disposable filters[14] and further cleaned using a C18[15] cartridge. The cartridge was activated by passing about 2 ml of acetonitrile through it, followed by about 2 ml of water. The extract was transferred to the C18 and the effluent was saved. This was followed by passing 3 ml of 1% acetic acid through the cartridge to remove highly water-soluble materials. Finally, 2 ml of acetonitrile, acetic acid and water (80:1:19) was passed through the cartridge and the effluent was saved. This contained both isoxaben and its metabolite. This eluate was used for thin layer chromatography (TLC). A 50 µl sample from each eluate was loaded on to the TLC plates[16]. Standard ^{14}C isoxaben and a metabolite of isoxaben (hydroxylated on the 2 carbon of the propyl side chain) was co-chromatographed with the plant extracts and the plates were developed in a cholorform:acetone (9:3, v/v) solvent system. Developed TLC plates were then examined under UV light and fractionated. Each fraction was scraped into the scintillation cocktail and the radioactivity was determined by liquid scintillation spectrometry. Metabolites were separated by their Rf values and the distribution of radioactivity detected in each

---

[11] Tri-Carb sample oxidizer Model B306, Packard Instrument Co., Inc. Downers Grove, IL 60515.

[12] Carbosorb E and Permafluor E+, Packard Instrument Company, Mildred, CT 06450.

[13] Beckman model LS 5000TA, Beckman Instruments, Inc. Schaumburg, IL 60173.

[14] Acrodisc Syringe Filters 25 mm, Gelman Sciences, Ann Arbor, MI 48106.

[15] Sep-Pak Cartridges, Millipore Corporation, Milford, MA 01757.

[16] Silica Gel 60F_{254} precoated TLC plates, EM Sciences, 480 Democrat Rd., Gibbstown, NJ 08027.

metabolite was expressed as percent of the total radioactivity recovered during the TLC analysis of the plant extracts.

Glucose incorporation studies. Incorporation of ^{14}C glucose into root cell walls was assayed according to the methods described by Heim et al. (1990) and Schneegurt et al. (1994) with slight modifications. The roots of ajuga, wintercreeper and dwarf burning bush were washed thoroughly with water and 0.5 cm of root tips was excised just before use, blotted dry and incubated in vials containing 5 ml of ^{14}C glucose (0.5 µCi/ml) and 0.025% dimethylsulfoxide (DMSO) with or without isoxaben in a shaker for 3 hours. The isoxaben concentration used was 1 mM in acetone. After incubation, the root tips were washed thoroughly with water, blotted dry and then resuspended in 10 ml of acetic acid, nitric acid and water (8:1:2 by vol), and digested for 2 hours in a boiling water bath. After digestion, the samples were vacuum filtered and the acid insoluble material was collected on preweighed 2.1 cm glass microfibre filters. The acid-insoluble material thus collected was dried in an oven at 60 C and then weighed. Radioactivity was determined by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Uptake and Translocation Studies. The amount of radioactivity recovered in the Hoaglands solution was comparable in the three species and decreased with time (Table 1). The amount of radioactivity recovered from the root wash was also comparable over time (1 to 2%) for all species.

Autoradiographs indicated that all three species had absorbed radioactivity 1 day after treatment (DAT) (Figures 1, 2 and 3). Autoradiography showed that ajuga had apparently translocated more radioactivity into the shoot portion than wintercreeper or dwarf burning bush at 1, 7 and 14 DAT. At 1 DAT, there was more translocation in wintercreeper when compared to dwarf burning bush. However at 7 and 14 DAT, the translocation of radioactivity from roots to shoots appeared to be similar in wintercreeper and dwarf burning bush (Figures 2 and 3). The translocated radioactivity in ajuga was confined to the veins, while in the other two species it was present both in the veins and in the interveinal areas (Figures 1, 2 and 3).

The levels of radioactivity absorbed by all three species increased with time (Table 1). In general, levels of uptake were highest in dwarf burning bush at all harvest timings, ranging from 17% at 1 DAT to 41% at 14 DAT. At 14 DAT, the sensitive species ajuga and dwarf burning bush absorbed about 34 and 41% respectively, of the total applied radioactivity, compared to 21% absorption in wintercreeper, the more tolerant species.

Translocation of the root-absorbed radioactivity to stem and leaves seemed to be greater in ajuga and wintercreeper than in dwarf burning bush after 7 and 14 days of exposure to

radiolabeled isoxaben (Table 2). In all species, the translocation of radioactivity from roots to shoots increased with time. At 7 and 14 DAT, the translocation was similar in ajuga and wintercreeper. By 14 days, dwarf burning bush had translocated only 28% of the applied radioactivity as compared to 58% and 50% in ajuga and wintercreeper, respectively.

Heim et al. (1991) reported that there were no differences in the uptake of isoxaben in isoxaben-resistant mutants of mouse-ear cress when compared to the sensitive wild type. Corio-Costet et al. (1991a) reported that the differential effect of isoxaben on selected soybean cell and wheat cell cultures was not due to reduced absorption of isoxaben. Our results show that ajuga, the more sensitive species, absorbed more radioactivity than wintercreeper, a more tolerant species. Although more radioactivity is absorbed by ajuga than wintercreeper, they seem to have similar patterns of translocation.

Metabolism studies. Standard isoxaben and its known metabolite (hydroxy metabolite of isoxaben) migrated to an Rf value of 0.9 and 0.65, respectively. Two more unknown metabolites with an Rf value of 0 (the origin) and 0.3 were detected.

In root extracts at 3, 7, and 14 DAT, ajuga metabolized isoxaben faster than the other two species (Figure 4). Isoxaben metabolism was similar in wintercreeper and dwarf burning bush at 3, 7, and 14 DAT. Levels of metabolites at the Rf values 0.3 and 0.65 were low in all three species for all levels of exposure. A polar metabolite of isoxaben (possibly conjugates) with Rf equal to 0 (origin) was found in greater amounts in ajuga.

Isoxaben and its metabolites were also recovered from shoot extracts of the three species. Most of the radioactivity recovered from shoots of the three species at 14 DAT appeared to be polar metabolites of isoxaben, since the majority of radioactivity remained at the origin (Figure 5). At 3 DAT, ajuga had the highest amount of radioactivity recovered at the origin and dwarf burning bush had the lowest. Although ajuga metabolized more isoxaben than wintercreeper at 3 DAT, isoxaben metabolism in ajuga and wintercreeper were similar at 7 and 14 DAT. At 14 DAT, ajuga and wintercreeper had similar amounts of radioactivity at the origin. At 3, 7, and 14 DAT, dwarf burning bush had the highest amount of unmetabolized isoxaben. In roots and shoots, dwarf burning bush metabolized isoxaben the least compared to ajuga and wintercreeper. However, greater amounts of metabolites at the origin were found in all species in the shoots compared to the roots. These results are similar to that of Cabanne et al. (1987) who observed that metabolism of isoxaben in wheat occurred predominantly in the shoot. Heim et al. (1993) concluded from their study that differential metabolism of isoxaben did not explain the differences in tolerance of bentgrass (*Agrostis palustris* (L.) Huds.var Penncross), and mouse-ear cress, a dicot, to this herbicide.

Glucose incorporation studies. There was no increase in glucose incorporation in the roots of these species when the

period of exposure to ^{14}C glucose was increased from 3 to 6 hours (data not shown). Therefore, the roots of all three species were exposed to radiolabeled glucose for 3 hours. Isoxaben did not inhibit radiolabeled glucose incorporation in the root tissues of wintercreeper and dwarf burning bush (Figure 6). There seemed to be more incorporation of glucose in the presence of isoxaben in the root tips of wintercreeper and dwarf burning bush, for unknown reasons. Isoxaben inhibited glucose incorporation by approximately 10% in ajuga, but this effect was not significant statistically. Our results are in agreement with other reports (Corio Costet et al. 1991b; Heim et al. 1991; Heim et al. 1993) in that less incorporation of ^{14}C glucose is obtained in sensitive species treated with isoxaben. Heim et al. (1991) reported that isoxaben was unable to inhibit the synthesis of acid-insoluble cell wall material in mutants of mouse-ear cress which are resistant to isoxaben. The mutants may contain an altered binding site for isoxaben. Isoxaben caused less inhibition of synthesis of acid insoluble cell wall material in wheat cells as compared to soybean cells and this could explain the tolerance noticed in wheat (Corio-Costet et al. 1991b). Heim et al. (1993) reported that the monocot bentgrass is more tolerant to inhibition of glucose incorporation by isoxaben than is mouse-ear cress, a dicot.

Although dwarf burning bush is sensitive to applications of isoxaben made one month after bud-break (Chapter III), no inhibition of glucose incorporation into the cell wall in root tissue was observed. This observation was different than that reported by Heim et al. (1991). Isoxaben inhibited cellulose biosynthesis in the sensitive wild type of mouse-ear cress but not in the tolerant mutant, indicating that the differential response to isoxaben is brought about by differential effect on the binding site. This may be due to the lower amounts (dpm mg^{-1}) of glucose absorbed by dwarf burning bush root tips as opposed to mouse-ear cress[17]. Also, more inhibition of glucose incorporation was obtained when seedling roots were exposed to radiolabeled glucose as opposed to excised root tips (21). There also may be differences in the rate of cellulose biosynthesis between woody and herbaceous plants.

Uptake and translocation of isoxaben from root application (Tables 1 and 2) can explain the root and shoot injury in ajuga and dwarf burning bush (Chapter II). Uptake of isoxaben by roots of wintercreeper resulted in root weight reductions at the highest applied rate. The relative sensitivity of these species to isoxaben may be due to uptake and translocation differences, as more metabolism was observed in the sensitive species ajuga compared to the tolerant species wintercreeper. Inhibition of glucose incorporation was observed in the sensitive species ajuga, but not in dwarf burning bush or wintercreeper. Future

[17] Personal communication with Dr. Mark Schneegurt, Dept. of Biological Sciences, Purdue University.

work is needed on the incorporation of ^{14}C glucose into the cell walls of the leaves of these three species, which may help explain the differences in sensitivity to isoxaben.

LITERATURE CITED

Anonymous. 1994. Gallery. Experimental herbicide for use in turf and ornamentals. Technical Bulletin, DowElanco, Indianapolis, Indiana. 12p.

Cabanne, F., A. Lefebvre and R. Scalla. 1987. Behavior of herbicide EL-107 in wheat and rape grown under controlled conditions. Weed Res. 27:135-142.

Colbert, F. O. and D. H. Ford. 1987. Isoxaben for broadleaf weed control in ornamental, turf and on bearing vines and trees. Proc. Western Weed Sci. Soc. 40:155-163.

Corio-Costet, M. F., M. Dall'Agnese and R. Scalla. 1991a. Effects of isoxaben on sensitive and tolerant plant cell cultures:I. Metabolic fate of isoxaben. Pestic. Biochem. Physiol. 40:246-254.

Corio-Costet, M. F., J. Lherminier, and R. Scalla. 1991b. Effects of isoxaben on sensitive and tolerant plant cell cultures:II. Cellular alterations and inhibition of the synthesis of acid insoluble cell wall material. Pestic. Biochem. Physiol. 40:255-265.

Craft, A. S. and S. Yamaguchi. 1964. The autoradiography of plant materials. California Agr. Exp. Sta. Manual 35. Berkeley, California. 143p.

Derr, J. F. 1994. Tolerance of groundcovers to preemergence herbicides. Proc. South. Nur. Assoc. Res. Conf. 39:303-304.

Derr, J. F. and S. Salihu. 1996. Preemergence herbicide effects on nursery crop root and shoot growth. J. Environ. Hort. 14:210-213.

Derr, J. F. 1993. Wildflower tolerance to metolachlor and metolachlor combined with other broadleaf herbicides. HortScience. 28:1023-1026.

Heim, D. R., L. A. Bjelk., J. James., M. A. Schneegurt and I. M. Larrinua. 1993. Mechanisms of isoxaben tolerance in *Agrostis palustris* var. Penncross. J. Exp. Bot. 44:1185-1189.

Heim, D. R., J. R. Skomp, C. Waldion and I. M. Larrinua. 1991. Differential response to isoxaben of cellulose biosynthesis by wild type and resistant strains of *Arabidopsis thaliana*. Pestic. Biochem. Physiol. 39:93-99.

Heim, D. R., J. R. Skomp, E. E. Tschabold and I. M. Larrinua. 1990. Isoxaben inhibits the synthesis of acid insoluble cell wall materials in *Arabidopsis thaliana*. Plant Physiol. 93:695-700.

Jacobsen, S and K. M. Walls. 1987. The phytotoxic effect of isoxaben on ornamental plants and nonbearing trees and vines. Proc. Calif. Weed Conf. 39:20-21

Lefebvre, A., D. Maizonnier, J. C. Gaudry, D. Clair and R. Scalla. 1987. Some effects of the herbicide EL-107 on cellular growth and metabolism. Weed Res. 27:125-134.
Neal, J. C. and A. F. Senesac. 1990. Preemergent weed control in container and field grown woody ornamental crops with isoxaben. J. Environ. Hort. 8:103-107.
Porter, W. C. 1996. Isoxaben and isoxaben combinations for weed control in container grown herbaceous flowering perennials. J. Environ Hort. 14:27-30.
Salihu, S., J. F. Derr and K. K. Hatzios. 1996. Tolerance of dwarf burning bush at different growth stages to isoxaben. Proc. Northeast. Weed Sci. Soc. 50:66.
Schneegurt, M. A., D. R. Heim and I. M. Larrinua. 1994. Investigation into the mechanism of isoxaben tolerance in dicot weeds. Weed Sci. 42:163-167.
Vaughn, K. C. 1997. Isoxaben and dichlobenil affect two different steps in cell plate formation. Weed Sci. Soc. Am. Abst. 37:68.

Table 1. Radioactivity recovered in Hoaglands solution, root wash and the plants at 1, 3, 7 and 14 days after root application of ^{14}C isoxaben.

Species	Time after application (days)	^{14}C recovered in Hoaglands	rootwash	plants	
		------% of applied-------			dpm
Ajuga	1	83	0.8	3	12207
	3	82	0.9	11	33513
	7	74	1.6	21	68598
	14	57	0.8	34	112624
LSD (0.05)		15	NS	20	71638
Winter-creeper	1	86	0.7	7	23385
	3	80	0.8	9	32013
	7	63	1.1	26	84505
	14	61	1.1	21	69765
LSD (0.05)		22	NS	NS	NS
Dwarf burning bush	1	73	2.2	17	55504
	3	70	1.5	20	61717
	7	54	1.6	36	117660
	14	48	1.3	41	139468
LSD (0.05)		22	NS	19	67614

Table 2. Distribution of radioactivity in roots and shoots of ajuga, wintercreeper and dwarf burning bush after 1, 3, 7 and 14 days of exposure to root-applied ^{14}C isoxaben.

Species	Time after application (days)	^{14}C recovered in plants			
		Roots		Shoots	
		dpm	%	dpm	%
Ajuga	1	7196	71	3540	29
	3	17520	53	15993	47
	7	28421	43	40176	57
	14	44634	40	67990	60
LSD (0.05)		24554	11	57502	11
Winter-creeper	1	16980	80	4677	20
	3	18722	71	9284	29
	7	36049	47	48456	53
	14	35234	51	34530	49
LSD(0.05)		15567	14	21748	14
Dwarf burning bush	1	45033	91	5948	9
	3	50072	81	11644	19
	7	90381	77	27279	23
	14	100003	72	39465	28
LSD (0.05)		47956	8	21801	8

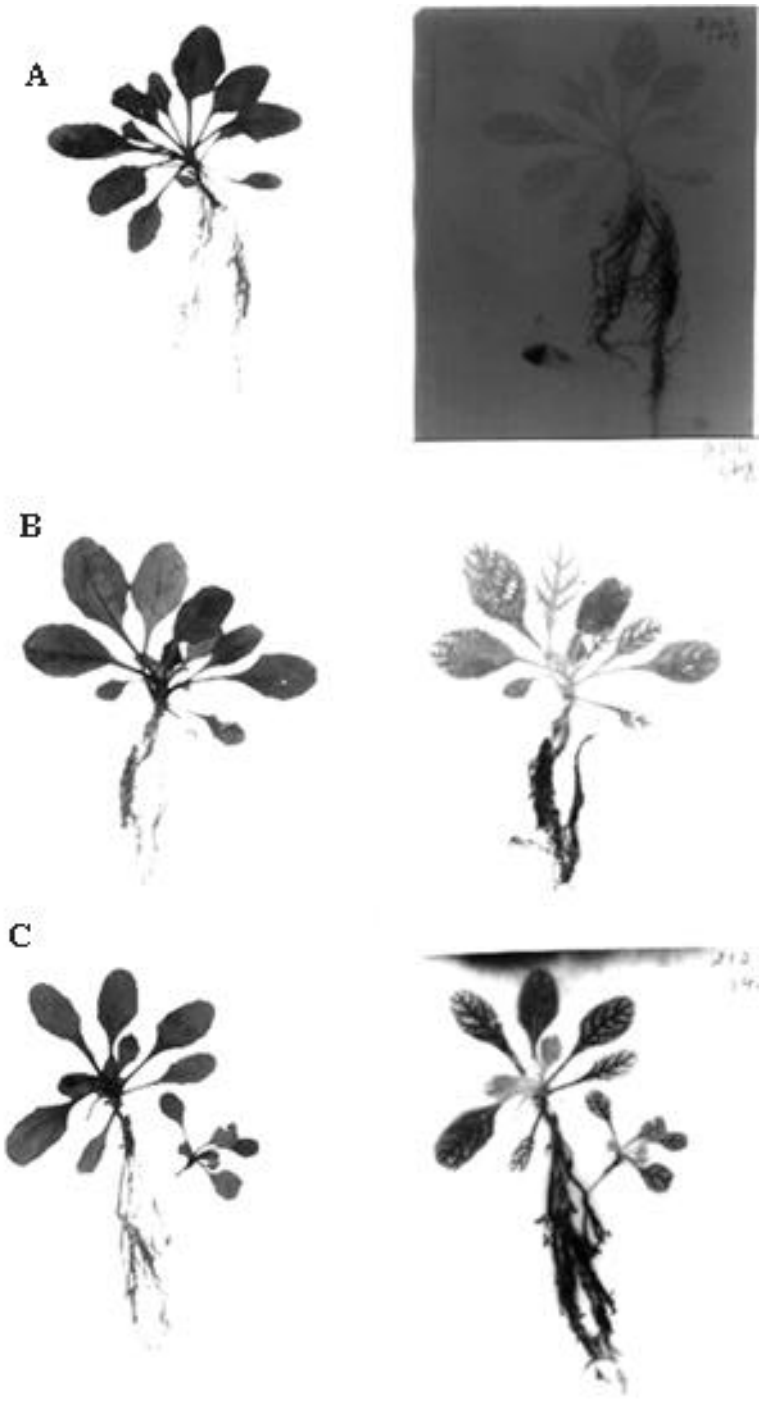

Figure 1. Autoradiograph of ajuga at A) 1, B) 7 and C) 14 days after exposure to root-applied ^{14}C isoxaben. On the left is the mounted plant and on the right is the autoradiograph.

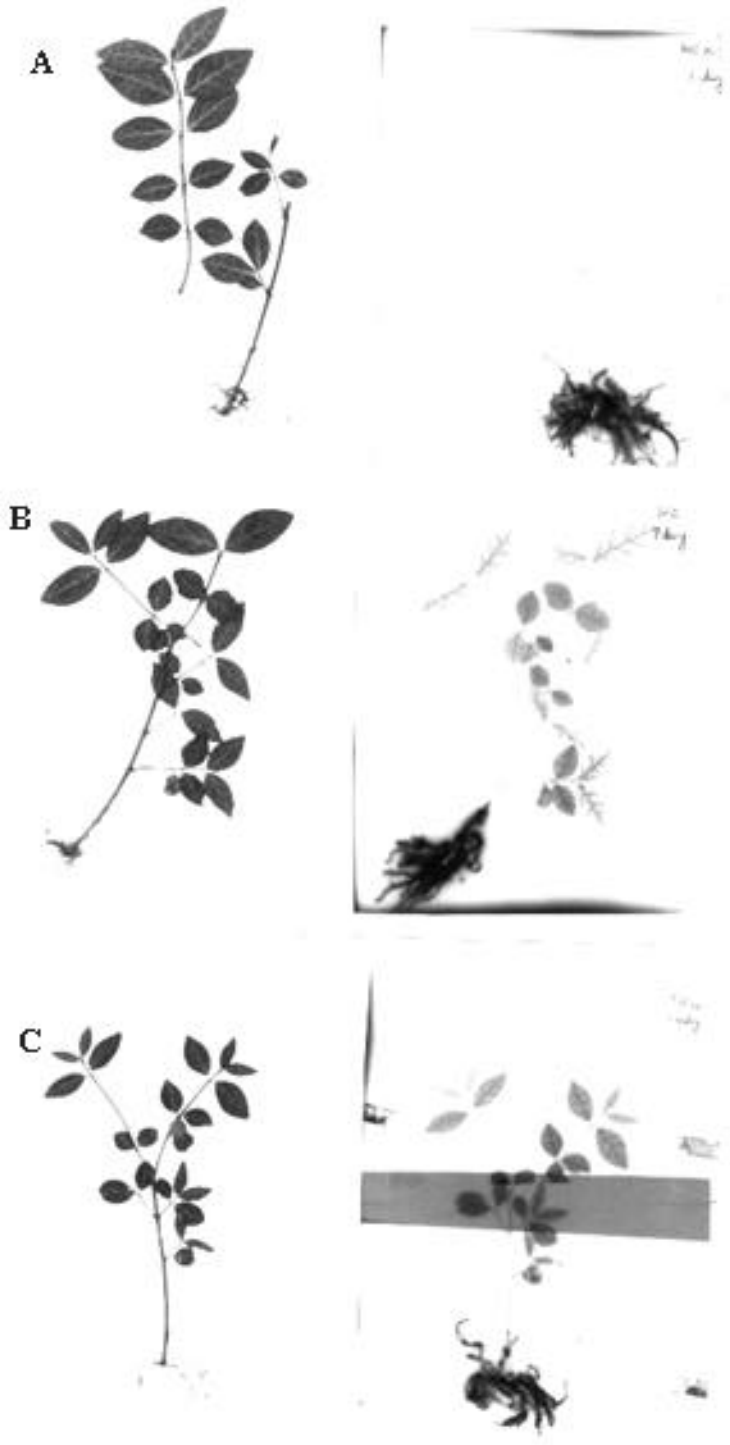

Figure 2. Autoradiograph of wintercreeper at A) 1, B) 7 and C) 14 days after exposure to root-applied ^{14}C isoxaben. On the left is the mounted plant and on the right is the autoradiograph.

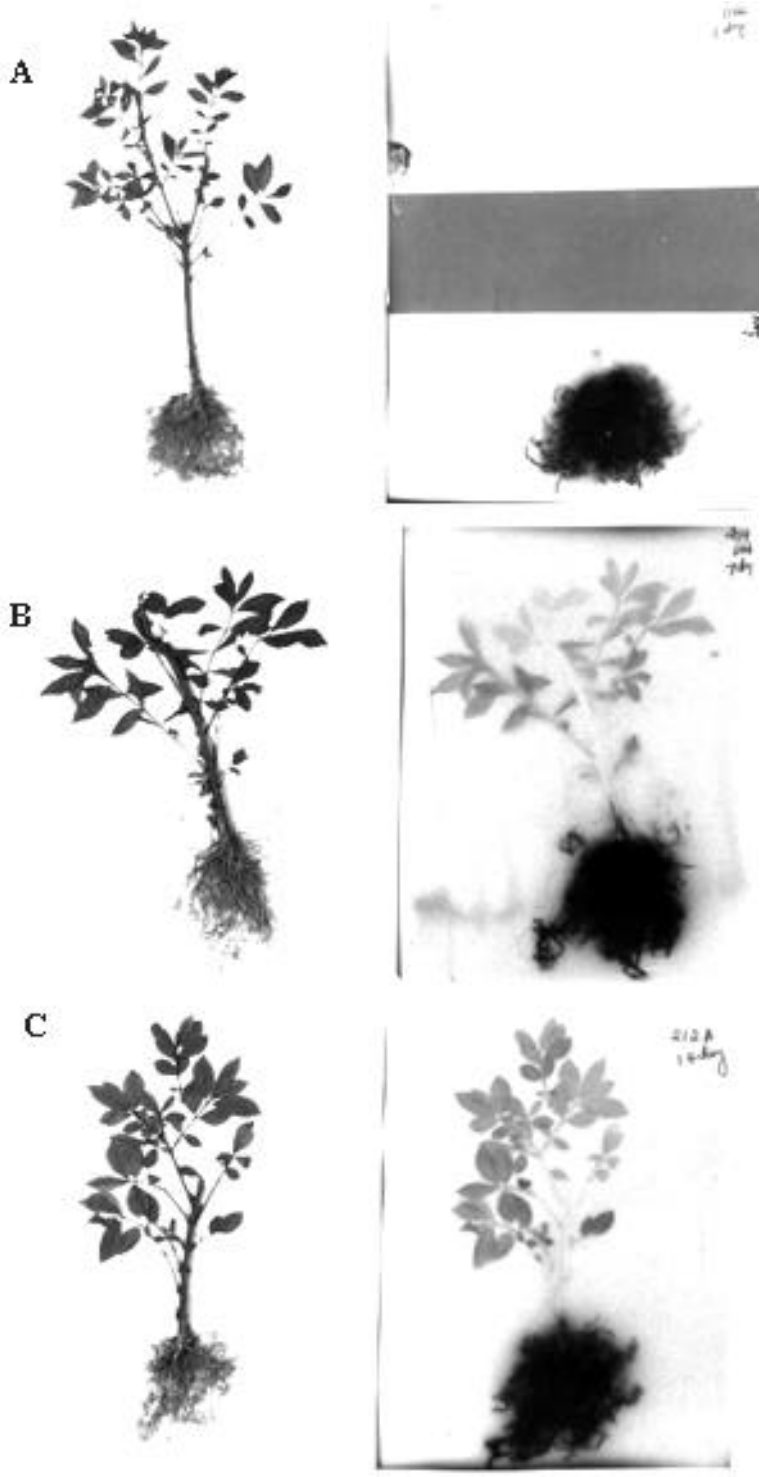

Figure 3. Autoradiograph of dwarf burning bush at A) 1, B) 7 and C) 14 days after exposure to root-applied ^{14}C isoxaben. On the left is the mounted plant and on the right is the autoradiograph.

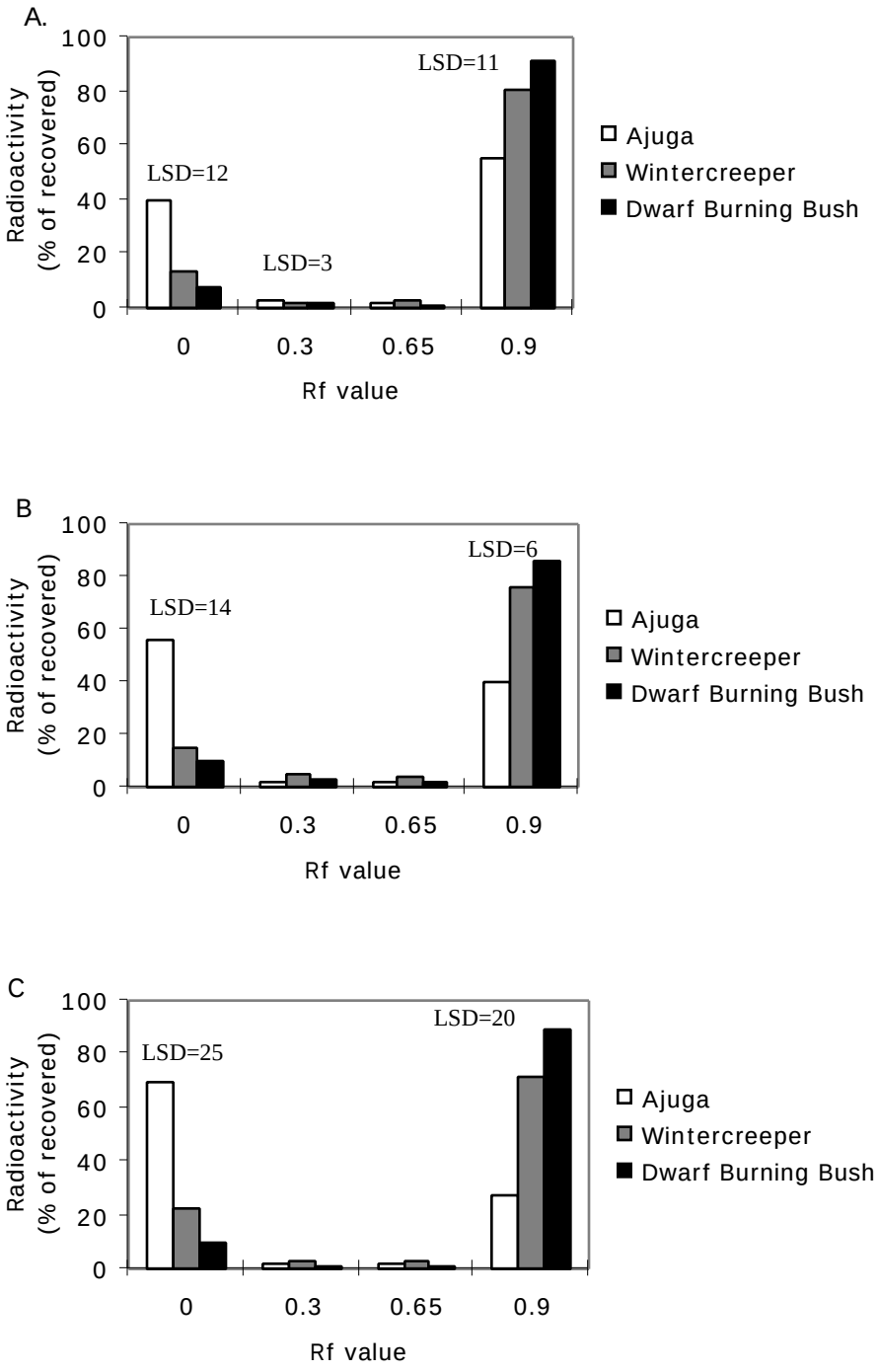

Figure 4. Thin layer chromatographic analysis of isoxaben metabolism in root extracts of ajuga, wintercreeper and dwarf burning bush after 3 (A), 7 (B) and 14 (C) days of exposure to root-applied ^{14}C isoxaben. LSDs are listed where significant differences among species were present within an Rf value. Standard isoxaben migrated to an Rf value of 0.9.

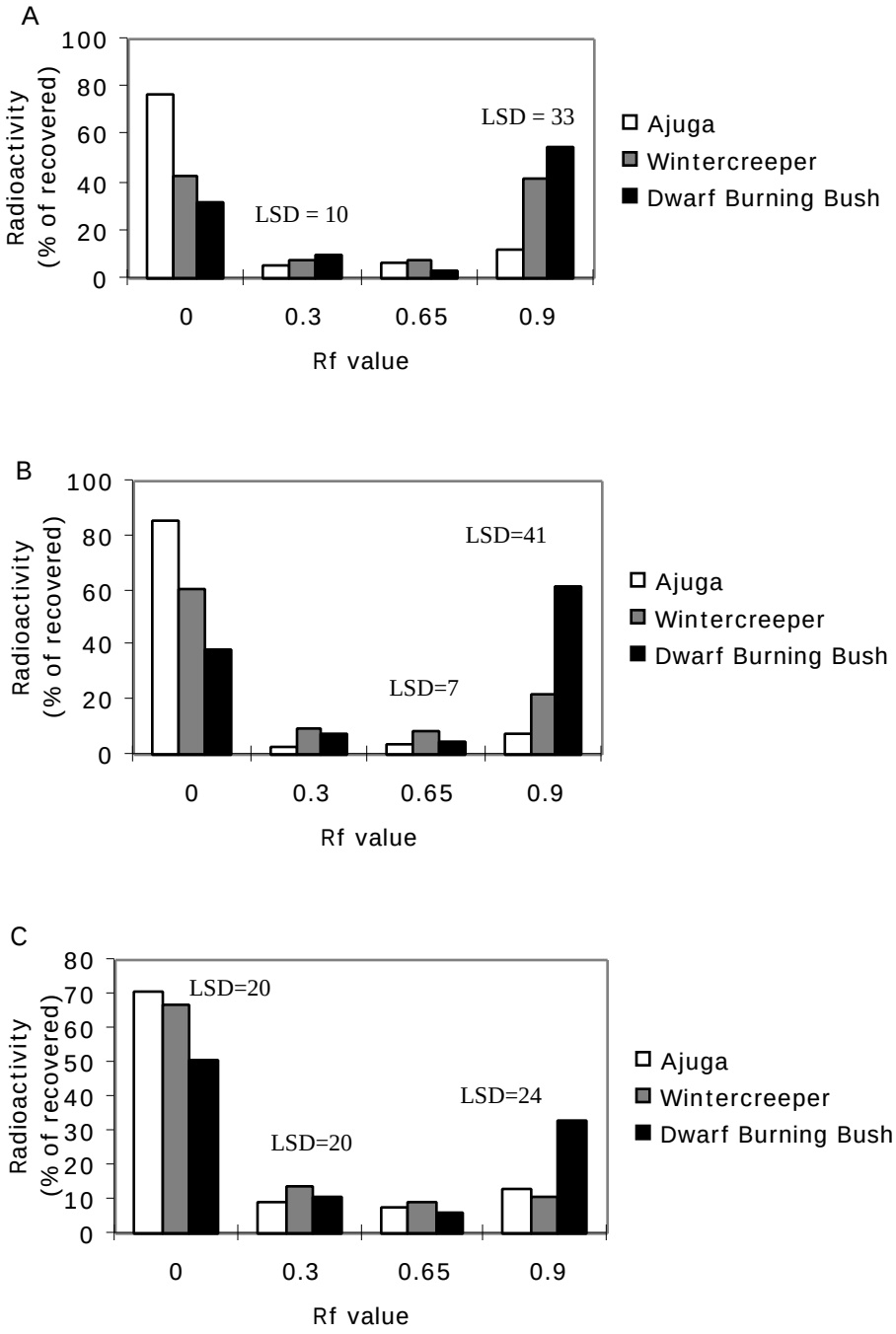

Figure 5. Thin layer chromatographic analysis of isoxaben metabolism in shoot extracts of ajuga, wintercreeper and dwarf burning bush after 3 (A), 7 (B) and 14 (C) days of exposure to root-applied ^{14}C isoxaben. LSDs are listed where significant differences among species were present within an Rf value. Standard isoxaben migrated to an Rf value of 0.9.

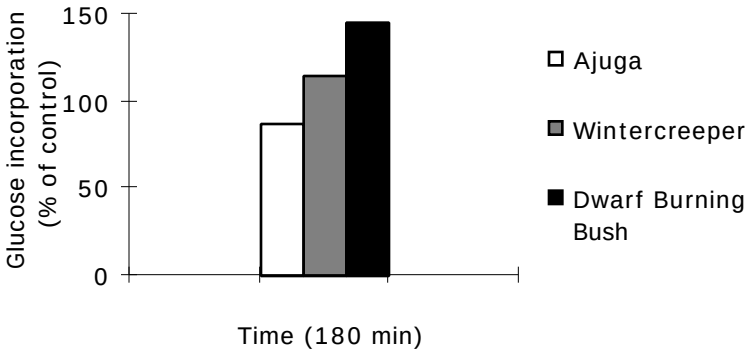

Figure 6. Effect of isoxaben on radiolabeled glucose incorporation in root tips of ajuga, wintercreeper and dwarf burning bush.

Chapter V
Absorption, Translocation, and Metabolism of Shoot-Applied Isoxaben in Ajuga (*Ajuga reptans*), Wintercreeper (*Euonymus fortunei*) and Dwarf Burning Bush (*Euonymus alatus*)[18]

[18] Format followed for this chapter is based on Weed Technology.

Chapter V
Absorption, Translocation, and Metabolism of Shoot-Applied Isoxaben in Ajuga (*Ajuga reptans*), Wintercreeper (*Euonymus fortunei*) and Dwarf Burning Bush (*Euonymus alatus*)

Abstract: Radiolabeled isoxaben (N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide) was foliarly applied to ajuga (*Ajuga reptans* L. 'Alba'), wintercreeper [*Euonymus fortunei* (Turcz.) Hand.-Mazz 'Colorata'] and dwarf burning bush [*Euonymus alatus* (Thunb.) Sieb. `Compacta']. Most of the absorbed radioactivity was recovered in the treated leaf for all three species. In the sensitive species ajuga, the radioactivity absorbed by the treated leaf increased from 46% at 3 DAT to 64% at 14 DAT. In the tolerant species wintercreeper, there was no increase in the amount of radioactivity (approximately 40%) recovered from the treated leaf over time. Dwarf burning bush at the two growth stages showed a time-dependent increase in the amount of radioactivity present in the treated leaf, but did not differ significantly from each other. Ajuga and wintercreeper metabolized isoxaben faster than dwarf burning bush. An unknown metabolite was present at higher levels in wintercreeper extracts than dwarf burning bush or ajuga. There was no difference in metabolism between the two growth stages of dwarf burning bush. Ajuga absorbed more isoxaben than wintercreeper, which may explain its greater sensitivity to the herbicide. Wintercreeper absorbed less isoxaben and metabolized it faster than dwarf burning bush, which may explain its greater tolerance.
Nomenclature: Isoxaben, (N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide); ajuga, *Ajuga reptans* L. 'Alba'; wintercreeper, *Euonymus fortunei* (Turcz.) Hand.-Mazz 'Colorata', dwarf burning bush, *Euonymus alatus* (Thunb.) Sieb. `Compacta'.
Additional index words: woody ornamentals, growth stage, radiolabeled herbicide.

INTRODUCTION

Isoxaben is a preemergence herbicide for broadleaf weed control in ornamentals and turf (Colbert and Ford 1987). The mode of action of isoxaben in plants is thought to be inhibition of cellulose biosynthesis (Ahrens 1994; Heim et al. 1990). Although isoxaben is used mainly as a preemergence herbicide, it can injure certain weed species when applied postemergence (Chandran and Derr 1997; Neal and Senesac 1991; Roberts et al. 1993; Schneegurt et al. 1994b). Neal and Senesac (1991) reported excellent control of ground ivy (*Glechoma hederacea* L.) and good control of healall (*Prunella vulgaris* L.) with postemergence applied isoxaben. Chandran and Derr (1997) reported that isoxaben applied postemergence provided poor to no control of dandelion (*Taraxacum officinale* Weber.), 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 (*Euphorbia maculata* L.). Roberts et al. (1993) reported that foliarly-applied isoxaben caused cell swelling in redroot pigweed (*Amaranthus retroflexus* L.), resulting in swollen and split stems and petioles and formative effects on leaves.

Redroot pigweed treated 7 to 10 days after planting were more sensitive to isoxaben applications than 12 to 14 day old plants (Schneegurt et al. 1994b). The growth stage of the plants at the time of treatment, rather than the isoxaben rate, appeared to affect the magnitude of the response towards isoxaben applications. The herbicidal activity of isoxaben was enhanced by soil and foliar interception, but measurable activity was observed with foliar interception alone. The absorption and translocation of isoxaben in redroot pigweed was poor when compared to that of mobile herbicides like glyphosate, sethoxydim and mefluidide. Their report was in agreement with Roberts et al. (1993) in that the postemergence activity of isoxaben appeared to be limited by poor absorption and translocation.

The ornamental species ajuga, wintercreeper and dwarf burning bush show differential response to the herbicide isoxaben (Chapter II). Dwarf burning bush and ajuga are sensitive to foliar applications of isoxaben, but wintercreeper is tolerant to isoxaben applications at twice the typical use rate of 0.84 kg/ha. Dwarf burning bush is not sensitive to foliar applications of isoxaben later in the year when leaves are more developed (Chapter III). Isoxaben at 1.1 and 2.2 kg/ha caused 30 and 75% shoot fresh weight reduction, respectively, to ajuga six weeks after treatment (Derr 1994). Setyowati et al. (1995) reported that isoxaben applied in combination with trifluralin or oryzalin at 4.2 kg/ha caused slight necrosis and discoloration to dwarf burning bush in the first year of application.

This study was conducted to determine the basis for the differential tolerance of ajuga, wintercreeper and dwarf burning bush to isoxaben applications. Absorption, translocation and metabolism of radiolabeled isoxaben following foliar application were determined in these ornamental species.

MATERIALS AND METHODS

General conditions. Divisions of ajuga and rooted cuttings of wintercreeper and dwarf burning bush were used for absorption, translocation, and metabolism studies. Two growth stages of dwarf burning bush were used in this study. To attain the two growth stages of dwarf burning bush, dormant dwarf burning bush cuttings were taken from the cold room and maintained in the greenhouse for two and four weeks, respectively, before treatment. All plants were kept in a greenhouse and fertilized

with a 17N-2.6P-9.9K slow release fertilizer[19] containing micronutrients and watered daily. After the roots were thoroughly washed free of soil, plants were transferred to aluminum foil covered glass jars filled with 180 ml of full-strength Hoagland's solution. After 7 days, each plant was exposed to 0.11 µCi of shoot-applied radiolabeled isoxaben for 3, 7 or 14 days. The second or third newly-developed leaves of ajuga and the second or third fully-developed leaf and leaflet from the shoot tip for wintercreeper and dwarf burning bush, respectively, were treated. All treatments consisted of foliar spot applications of 5 to 6, 1 µl-droplets of the treatment solution. The treatment solution consisted of the radiolabeled herbicide in 80% ethanol and 0.1% surfactant[20]. Plants were maintained in the greenhouse until harvested. The experimental design was a randomized complete block. Three plants per species were used for combustion, three for metabolism and one for autoradiographs. The experiment was repeated and the data shown are the mean of two experiments. All data were subjected to analysis of variance and the means were separated using the Fishers Protected Least Significant Differences (LSD) test at the 0.10 probability level.

Absorption and translocation of ^{14}C isoxaben. The radiochemical purity of isoxaben (phenyl ring uniformly ^{14}C labeled) was 97.8% and its specific activity was 30.2 mCi/mmol. At each harvest, selected plants of all three species were analyzed by autoradiography following the procedures of Crafts and Yamaguchi (1964). Treated plants were mounted on paper, pressed, dried and exposed to X-ray films[21] for 8 weeks before developing.

Plants were separated into the treated leaf or leaflet, remaining leaves, and roots at 3, 7, and 14 days after treatment (DAT). Unabsorbed isoxaben was removed from the treated leaf by dipping it into a beaker containing 100 ml of methanol for 30 s. One ml samples of the wash solution were added to scintillation cocktail[22] and was quantified in a commercial liquid scintillation spectrometer[23]. After washing, the treated leaves were placed in paper bags and dried in an oven at 60 C for 24 hrs. After drying, the leaves were weighed, cut up into small pieces and combusted in a biological sample oxidizer[24]. The $^{14}CO_2$ evolved was

---

[19] Osmocote 17-6-12, The Scotts Company, Marysville, OH 43040.
[20] X-77 surfactant, Chevron Chemical Company, San Francisco, CA 94120.
[21] Kodak Scientific Imaging Film, Eastman Kodak Co., Rochester,NY 14650.
[22] ScintiVerse, Universal L.S.C. Cocktail, Fisher Scientific, Fairlawn, NJ 07410.
[23] LS-5800TA Model, Beckman Instrument Co., Fullerton, CA 92634.
[24] B0306 Biological Sample Oxidizer, Packard Instruments Co., Downer Grove IL 60515.

trapped in 20 ml of a ^{14}C scintillation solution[25] and quantified by liquid scintillation spectrometry. Recovery in the leaf wash and the treated leaf was more than 97% of applied ^{14}C in all three species. $^{14}CO_2$ trapping efficiency was above 95% for all analyses. Absorption data are expressed as a percent of the total radioactivity applied.

Metabolism of ^{14}C isoxaben: After the methanol wash, the treated leaves were placed in polyethylene bags and immediately frozen at -20C. The procedure followed for metabolism was slightly modified from the one reported by Corio-Costet et al.(5). Leaves were ground with liquid nitrogen, and then isoxaben was extracted with 10 ml of aqueous 80% methanol in a mortar and pestle. Homogenates were centrifuged at 2000 x g for 10 min and the supernatant was removed and saved. Pellets were extracted two more times using 10 ml of 80% methanol. The combined supernatants were concentrated under air to about 5 ml and were cleaned by filtration through 0.25 µm nylon disposable filters[26]. The filtrates were concentrated by rotoevaporation to 0.5 ml and 50 µl of each sample was loaded on thin layer chromatography (TLC) plates[27]. Standard ^{14}C isoxaben as well as a hydroxy metabolite of isoxaben (hydroxylated at the 2 carbon of the propyl side chain) was co-chromatographed with the plant extracts and the plates were developed in a chloroform:acetone (9:3, v/v) solvent system. After development, plates were visualized under UV light to find the position of radioactive spots that cochromatographed with the reference compounds. Plates were separated into four segments from the origin to the solvent front, scraped
and the radioactivity was quantitatively determined by liquid scintillation spectrometry.

RESULTS AND DISCUSSION

Absorption and translocation of ^{14}C isoxaben. The autoradiographs indicated no movement of isoxaben from the treated leaf into other plant parts in all species for all time intervals, even after 14 DAT (Figure 1). Most of the foliarly applied radioactivity was recovered in the methanol leaf wash and the treated leaf of the three species (Figures 2 and 3). Therefore plant parts other than the treated leaf were not analyzed further.

There were no significant differences in the amount absorbed among the three species at 3 and 7 DAT. The amount of radioactivity recovered from the treated leaf of ajuga seemed to

---

[25]Carbo-sorb E and Permafluor E$^+$, Packard Instrument Company, Mildred, CT 06450.

[26]Acrodisc Syringe Filters 25 mm, Gelman Sciences, Ann Arbor, MI 48106.

[27]Silica Gel 60 F_{254} precoated TLC plates, EM Sciences, 480 Democrat Rd, Gibbstown, NJ 08027.

increase from 3 to 14 DAT (Figure 3). At 14 DAT, approximately 60% of the radioactivity applied to ajuga was recovered in the treated leaf.

In wintercreeper, there was no apparent increase in the amount of radioactivity absorbed from 3 to 14 DAT. The amount absorbed by the wintercreeper at 3 DAT was similar to the other two species. However, by 14 DAT, the other species had absorbed more than wintercreeper. The treated leaflet from both growth stages of dwarf burning bush showed an increase in the amount absorbed over time. Although the absorption rate was similar at 3 DAT, the active growth stage of dwarf burning bush seemed to absorb more than the mature growth stage of dwarf burning bush by 14 days. It is possible that greater absorption occurs with time in the active growth stage as compared to the mature leaf growth stage of dwarf burning bush. However, other reasons, including a more sensitive target site at the earlier growth stage, could possibly explain the injury seen with isoxaben applications one month after bud-break, but not at two months after bud-break.

Schneegurt (1994b) also reported that the majority of applied isoxaben remained in the treated leaf. They concluded that very little of foliarly applied isoxaben (0.08%) was translocated beyond the point of application. However, our results are different to those of Schneegurt et al. (1994b) regarding the absorption of radiolabeled isoxaben. They reported that only 3.3% of applied isoxaben entered the leaf of redroot pigweed while in ajuga and the younger growth stage of dwarf burning bush had absorbed 64 and 71%, respectively, of applied isoxaben by 14 DAT.

Metabolism of ^{14}C isoxaben: Metabolism of isoxaben was studied in the treated leaves of all three species. Standards of isoxaben and a hydroxy metabolite had an Rf value of 0.9 and 0.65, respectively, in the TLC system. For treated samples, radioactivity was also found at an Rf value of 0 (origin) and 0.3, both of which are unknown metabolites.

Metabolism of isoxaben appeared to differ in the three species (Figure 4). Wintercreeper metabolized isoxaben faster than either ajuga or dwarf burning bush at 3 DAT. In ajuga at 3 DAT, 67% of radioactivity migrated to an Rf value of 0.9 suggesting that most of the radioactivity recovered was probably unaltered isoxaben. At 7 DAT, the amount of unmetabolized isoxaben recovered from origin had decreased to 47% and by 14 DAT, it was only 34%. At 7 DAT, both wintercreeper and ajuga had similar isoxaben metabolism. At 14 DAT isoxaben metabolism in ajuga and wintercreeper was significantly greater than that of the two growth stages of dwarf burning bush.

Metabolites found at the origin (Rf = 0) may be polar conjugates, possibly because of no movement in this system. Levels of metabolites found were higher in ajuga than the other species at 14 DAT. Most of the radioactivity was recovered as polar conjugates, which may be reversible. In the treated leaf extracts of wintercreeper, levels of the unknown metabolite (Rf =

0.3) were relatively higher than in ajuga or in both the growth stages of dwarf burning bush.

At 3 DAT, most of the ^{14}C recovered (approximately 70%) from the treated leaflet extracts of both growth stages of dwarf burning bush cochromatographed with unmetabolized isoxaben. At 7 and 14 DAT, approximately 60% of radioactivity recovered, cochromatographed with isoxaben in the two growth stages of dwarf burning bush.

Heim et al. (1993) and Schneegurt et al.(1994a) reported that the selectivity of isoxaben in plant species cannot be explained by differential metabolism. Heim et al. (1993) suggested that the mechanism of isoxaben tolerance in bentgrass (*Agrostis palustris* (L.) Huds. var. Penncross) may be due to differences in the isoxaben binding site. Similarly, Schneegurt et al. (1994a) concluded that the differences in tolerance of dicot weeds like catchweed bedstraw (*Galium aparine* L.), redroot pigweed and velvetleaf (*Abutilon theophrasti* L.) to isoxaben could not be explained by differential metabolism but was due to differences in sensitivity at the site of action.

Ajuga, a species sensitive to isoxaben, does absorb relatively more herbicide through the leaf than the other two species. Wintercreeper, the more tolerant species, absorbs less through the leaf at 14 DAT as compared to the other two species, possibly due to a higher wax content. The absorption, translocation and metabolism of isoxaben seems to be similar for both the growth stages of dwarf burning bush.

Our results suggest that uptake of isoxaben by ajuga leaves may be responsible for the sensitivity of this species to isoxaben applied foliarly. Limited foliar uptake over time by wintercreeper as compared to the other two species may explain the tolerance of this species to isoxaben. It is also possible that wintercreeper could have a more tolerant site of action to isoxaben than ajuga or dwarf burning bush. Although the absorption, translocation and metabolism are similar in the leaves of the two growth stages of dwarf burning bush, isoxaben injury is observed only with actively growing plants in the field. Information is needed about the sensitivity of the target site in these two growth stages of dwarf burning bush as well as ajuga and wintercreeper.

LITERATURE CITED

Ahrens, W. H. (Editor). 1994. Herbicide Handbook of Weed Science Society of America. Weed Sci. Soc. Am. Champaign, IL, USA. p.173-175.

Chandran, R. S. and J. F. Derr. 1997. Effect of isoxaben applied postemergence for broadleaf weed control. Proc. Northeast. Weed Sci. Soc. 51:41.

Colbert, F. O. and D. H. Ford. 1987. Isoxaben for broadleaf weed control in ornamentals, turf and nonbearing trees and vines. Proc. West. Soc. Weed Sci. 40:155-163.

Corio-Costet, M. F., M. Dall'Agnese, and R. Scalla. 1991. Effects of isoxaben on sensitive and tolerant plant cell culture: I. Metabolic fate of isoxaben. Pestic. Biochem. Physiol. 40:246-254.

Craft, A. S. and S. Yamaguchi. 1964. The autoradiography of plant materials. California Agr. Exp. Sta. Manual 35. Berkely, California. 143p.

Derr, J. F. 1994. Tolerance of ground covers to preemergence herbicides. Proc. South. Nur. Assoc. Res. Conf. 39:303-304.

Heim, D. R., J. R Skomp, E. E. Tschabold, and I. M. Larrinua. 1990. Isoxaben inhibits the synthesis of acid insoluble cell wall materials in *Arabidopsis thaliana*. Plant Physiol. 93:695-700.

Heim, D. R., L. A. Bjelk, J. James, M. A. Schneegurt, and I. M. Larrinua. 1993. Mechanism of isoxaben tolerance in *Agrostis palustris* var. Penncross. J. Exp. Bot. 44:1185-1189.

Neal, J. C. and A. F. Senesac. 1991. Ground ivy and healall control in turf. Proc. Northeast. Weed Sci. Soc. 45:120.

Roberts, J. L., L. A. Bjelk, M. A. Schneegurt, and B. C. Gerwick. 1993. Foliar symptoms of isoxaben. Weed Sci. Soc. Am. Abstracts. 33:109.

Schneegurt, M. A., D. R. Heim, and I. M. Larrinua. 1994a. Investigation into the mechanism of isoxaben tolerance in dicot weeds. Weed Sci. 42:163-167.

Schneegurt, M. A., J. L. Roberts, L. A. Bjelk, and B. C. Gerwick. 1994b. Postemergence activity of isoxaben. Weed Technol. 8:183-189.

Setyowati, N., L. A. Weston, and R. E. McNeil. 1995. Evaluation of selected preemergence herbicides in field grown landscape crops in Kentucky. J. Environ. Hort 13:196-202.

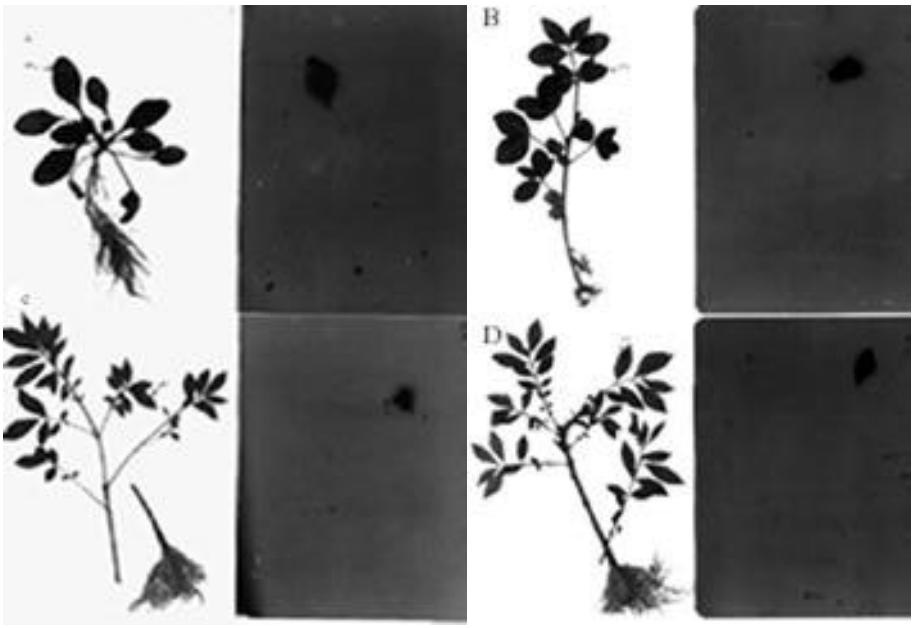

Figure 1. Autoradiographs of A) ajuga, B) wintercreeper, C) dwarf burning bush treated two weeks after bud-break and D) dwarf burning bush treated four weeks after bud-break following 7 days of exposure to shoot-applied ^{14}C isoxaben. On the left is the mounted plant and on the right is the autoradiograph.

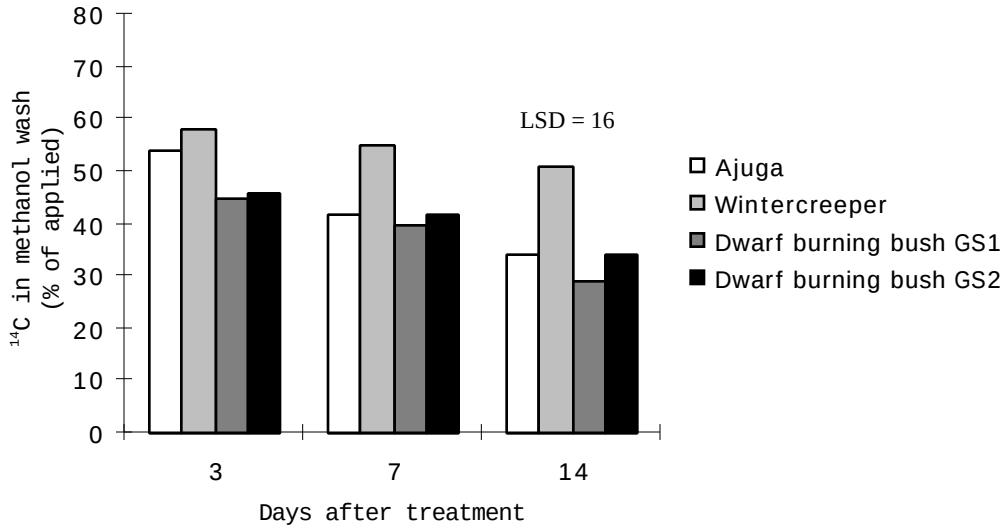

Figure 2. Radioactivity in the methanol wash of the treated leaf or leaflet (as a % of applied ^{14}C isoxaben) for ajuga, wintercreeper, and dwarf burning bush which was treated 2 weeks (GS1) and 4 weeks (GS2) after bud-break. LSDs are listed where differences between species are significant within a date of harvest.

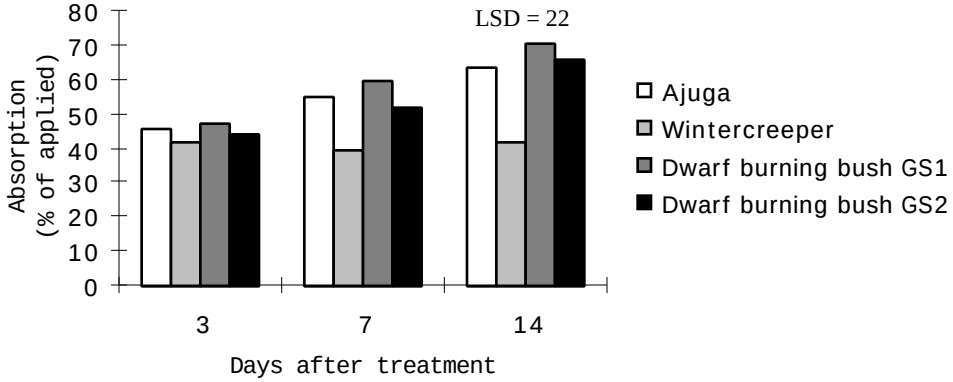

Figure 3. Absorption of ^{14}C (as a % of applied ^{14}C isoxaben) by treated leaves of ajuga, wintercreeper, and dwarf burning bush which was treated 2 weeks (GS1) and 4 weeks (GS2) after bud-break. LSDs are listed when differences among species are significant within a date of harvest.

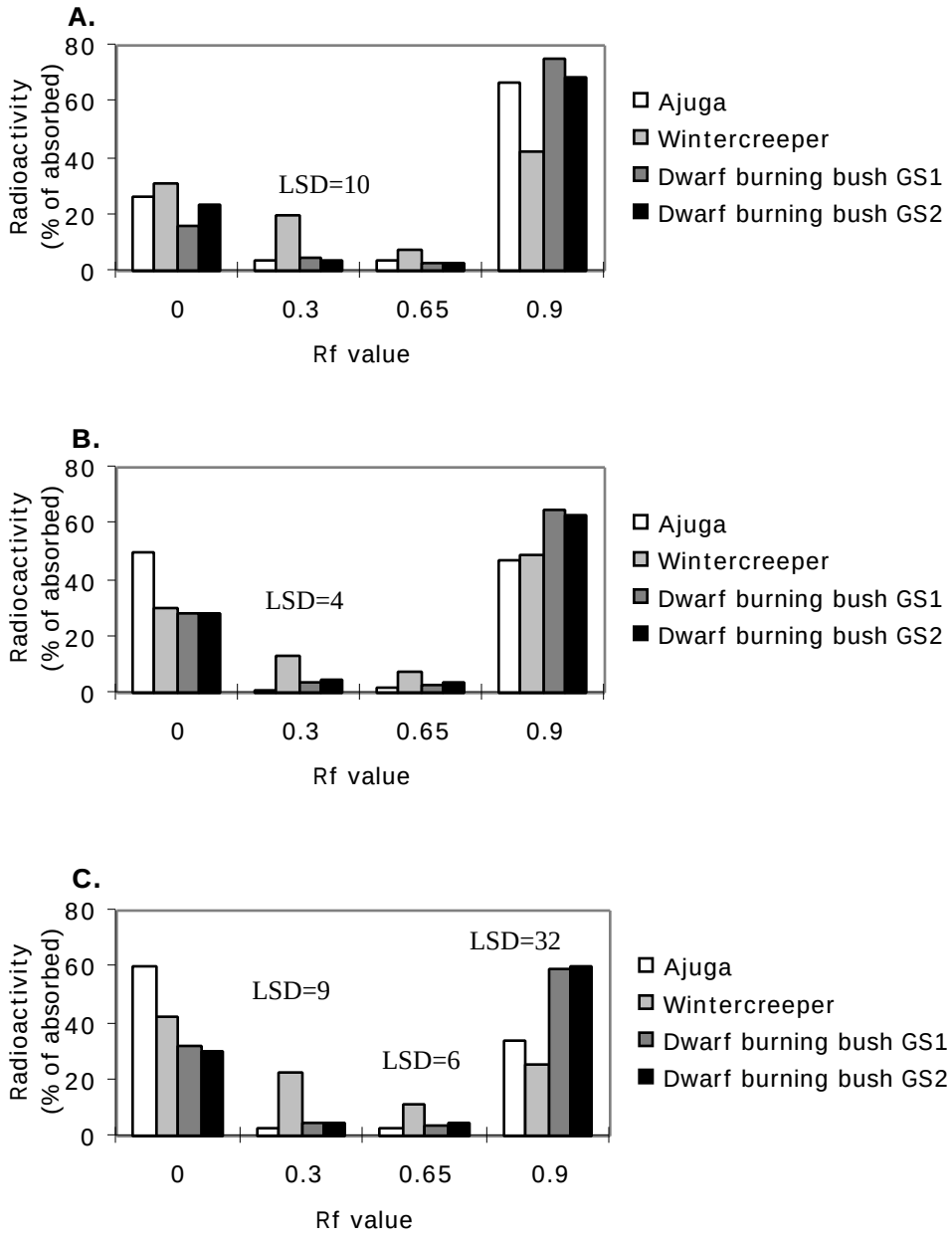

Figure 4. Thin layer chromatographic analysis of isoxaben metabolism in treated leaf or leaflet extracts of ajuga, wintercreeper, and dwarf burning bush which was treated at 2 (GS1) and 4 weeks (GS2) after bud-break, after 3 (A), 7 (B) and 14 (C) days of exposure to shoot applied ^{14}C isoxaben. LSDs are listed when differences among species are significant within an Rf value. Standard isoxaben migrated to an Rf value of 0.9.

Chapter VI
Summary and Conclusions

The three ornamental species ajuga, wintercreeper and dwarf burning bush responded differently to root and shoot applications of isoxaben. Isoxaben caused more injury to ajuga than dwarf burning bush or wintercreeper following root application. In dwarf burning bush, isoxaben applied to shoots caused greater reductions in root weights than shoot weights, in both nutrient solution and sand culture.

Ajuga absorbed more isoxaben by 14 days after treatment following root application and this may explain the greater root injury observed in this species compared to wintercreeper. Although no visible injury was observed in wintercreeper, the highest rate of isoxaben caused root and shoot weight reductions.

The amount of translocated radioactivity to the shoots from the roots was more in ajuga than in wintercreeper or dwarf burning bush. This may explain the greater injury seen in the shoots of ajuga compared to wintercreeper and dwarf burning bush following root application. Similar shoot weight reductions were noticed in wintercreeper and dwarf burning bush following application to the nutrient solution, possibly due to the similar amounts of radioactive material translocated to the shoots.

Ajuga roots metabolized isoxaben faster following root application than wintercreeper and dwarf burning bush at all days after harvest. Metabolism was least in dwarf burning bush roots compared to ajuga and wintercreeper. Metabolism of the translocated material was also greatest in ajuga shoots at 3 DAT. However, by 7 DAT, ajuga and wintercreeper had similar metabolism. As in the roots, dwarf burning bush shoots metabolized isoxaben the slowest compared to ajuga and wintercreeper at all days after harvest following root application. Metabolism does not explain the differential selectivity of isoxaben to ajuga and wintercreeper. Ajuga the most sensitive species, metabolized isoxaben faster than wintercreeper, the most tolerant species, in both roots and shoots. Metabolism may play a role in selectivity between wintercreeper and dwarf burning bush, as wintercreeper had greater metabolism than dwarf burning bush.

Although approximately 40% of the applied radioactivity is absorbed in wintercreeper, shoot weight reduction was observed only at the highest rate of isoxaben applied foliarly. Also, shoot application caused less root weight reductions than root applications. Isoxaben applied to the shoots reduced the root weights of ajuga and dwarf burning bush. Visual injury in shoots did not correlate well with shoot weight reductions in both the sand and the nutrient solution studies for dwarf burning bush. However, these studies were conducted only for a period of 2 months. Longer exposure to the herbicide may have resulted in greater shoot weight reductions, since premature defoliation was

noticed in the plants treated one month after the bud-break stage in the field study.

Wintercreeper absorbed less isoxaben through the shoots compared to ajuga and dwarf burning bush over time. This could explain the less injury seen in wintercreeper from foliar application, compared to ajuga and dwarf burning bush. Since no translocation is evident from shoot-applied isoxaben in the three species, one may assume that isoxaben affects a target site in the shoots of these plants. Since isoxaben is thought to inhibit cellulose biosynthesis, and since actively-growing shoot cells synthesize cellulose, isoxaben may interfere with this process in the shoot cells. Since these three species are injured at different levels by foliar applications of isoxaben, there could be differences in sensitivity of the target site in the three species, in addition to absorption differences. There is an indirect effect on the roots of these species from foliar applications, since root injury is noticed from foliar treatments. One possible reason may be the inhibition of required growth materials for root growth. Absorbed radioactivity remained in the treated leaf for all three species. In wintercreeper, no visible injury is observed even though all the material seems to be contained in the treated leaf itself as in ajuga and dwarf burning bush.

Metabolism of isoxaben was similar in the treated leaves of ajuga and wintercreeper at 14 DAT, and greater than in both growth stages of dwarf burning bush. Wintercreeper can metabolize isoxaben faster than dwarf burning bush and this may explain the greater tolerance of wintercreeper to foliar applications of isoxaben.

Inhibition of radiolabeled-glucose incorporation was observed only in ajuga roots. Although root weight reductions were observed in wintercreeper and dwarf burning bush, no inhibition of glucose incorporation was seen. This indicates that there could be differences in target site sensitivity for these species. It is also possible that there could be differences in glucose absorption between herbaceous and woody plant roots.

Injury was observed in dwarf burning bush from foliar applications of isoxaben made one month after bud-break. However, there were no differences in the uptake, translocation and metabolism following shoot-applied radiolabeled isoxaben between the two growth stages of dwarf burning bush. This could partly be explained by the different nature of leaves emerging in greenhouse conditions versus field conditions. Greenhouse conditions favor less waxy leaves and therefore the differences in the leaves of the two growth stages may not be as significant as in the field. This may have led to the same level of absorption by the leaves of the two growth stages. Another factor was the longer time period between bud-break and time of treatment in the field as compared to the radiolabeled study. Therefore, the leaves would have been relatively more mature in

the field versus the greenhouse. It is also possible that the differential selectivity could be due to differences in the sensitivity of the target site in the leaves of dwarf burning bush during the two growth stages.

The greater sensitivity of ajuga compared to wintercreeper appears to be due to greater absorption from root and shoot application. Dwarf burning bush is more sensitive compared to wintercreeper because of increased absorption and lower metabolism, following both root and shoot application.

Future studies can include identifying the conjugates found at the origin. Sucrose can be used as an alternative to glucose to study effect of isoxaben on cellulose biosynthesis, as plants may take up more sucrose compared to glucose. In addition, whole plants can be used to study incorporation of glucose in plant roots rather than root tips. More information is needed regarding the effect of isoxaben on the target site in these species in both root and shoot tissues.

APPENDIX

Tolerance of Nursery Crops to Isoxaben

Crop tolerance is an important factor in the selection of herbicides by nurserymen. Isoxaben, a preemergence herbicide does not injure most groundcovers, perennial flowers and woody ornamentals. An experiment was conducated to study the response of ajuga (*Ajuga reptans* L. 'Alba'), dwarf burning bush [*Euonymus alatus* (Thunb.) Sieb. 'Compacta'], wintercreeper [*Euonymus fortunei* (Turcz.) Hand.-Mazz 'Coloratus'], hydrangea (*Hydrangea arborscens* L. 'Glory Blue') and azalea (*Rhododendron obtusum* (Lindl.) Planch. 'Hinocrimson') to foliar applications of isoxaben. The nursery crops were planted in 4 L containers using a pine bark:sand (4:1 v/v) mixture. The experimental design was a randomized complete block with four replications and two plants per plot. Isoxaben was applied at 0.84, 1.69 and 3.39 kg ha^{-1}. Dichlobenil was applied at 4.48, 8.96 and 17.92 kg ha^{-1} for comparison purposes. Data collected include ornamental visual injury ratings and ornamental shoot fresh weight. At 2 MAT, the highest rate of isoxaben caused 20% injury to ajuga and 45% injury to hydrangea, but no injury was seen in azalea or wintercreeper when compared to untreated plants (Table A.1.). Chlorosis of newer leaves and necrosis of older leaves was observed. Dichlobenil at all rates caused significant injury to ajuga compared to untreated plants. No isoxaben or dichlobenil treatments injured wintercreeper or azalea at 2 and 4 MAT. At 4 MAT, the injury caused by isoxaben in ajuga increased slightly. The injury was mainly manifested as chlorotic new leaves and a decrease in plant size.

At 4 MAT, the lowest rate of isoxaben caused approximately 25% and 35% reduction of shoot fresh weight for ajuga and hydrangea, respectively, when compared to the untreated plants (Table A.2.). A shoot fresh weight reduction of approximately 60% was observed for ajuga and hydrangea at the high rate of isoxaben. The highest rate of isoxaben reduced root weight in wintercreeper. None of the isoxaben nor dichlobenil treatments caused reduction of shoot fresh weight of azalea. Dichlobenil at the lowest rate caused a severe reduction of shoot fresh weight of ajuga while the other two rates (8.96 and 17.92 kg ha^{-1}) killed ajuga.

The three rates of isoxaben caused a numerical reduction in root dry weight of ajuga. No reduction in root dry weight was noticed with any of the treatments for hydrangea and azalea. Ajuga and hydrangea were more sensitive to isoxaben, while azalea seemed to be the most tolerant among the nursery species tested. In ajuga and hydrangea, isoxaben seems to have a more pronounced effect on shoot weights rather than root weights, while in wintercreeper the root tissues were affected more than the shoots.