CHARACTERIZATION OF THE MECHANISM OF RESISTANCE OF A JOHNSONGRASS (*Sorghum halepense*) BIOTYPE TO SELECTED GRAMINICIDES IN VIRGINIA AND RESPONSE OF MUGWORT (*Artemisia vulgaris*) TO SPECIFIC HERBICIDAL AND CULTURAL CONTROL STRATEGIES

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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

Plant Pathology, Physiology, and Weed Science

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26 April 2000
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(ABSTRACT)

Johnsongrass [*Sorghum halepense* (L.) Pers.] and mugwort (*Artemisia vulgaris* L.) are both rhizomatous perennial weeds that are capable of rapidly colonizing a variety of different environments. Separate experiments were conducted throughout Virginia from 1996 to 1999 to determine more effective methods for reducing infestations of these perennial weeds in the future. Field and greenhouse experiments conducted on a resistant johnsongrass population discovered in New Kent County, Virginia revealed that this biotype exhibits low levels of resistance to the aryloxyphenoxypropionate (APP) herbicides quizalofop-P and fluazifop-P and the cyclohexanedione (CHD) herbicide sethoxydim. Additional laboratory experiments revealed that resistance is not due to differential absorption, translocation, or metabolism of the APP and CHD herbicides in the resistant vs. the susceptible biotype. However, acetyl-coenzyme A carboxylase (ACCase) assays revealed that resistance to the APP and CHD herbicides is conferred by an overproduction of the ACCase enzyme in the resistant compared to the susceptible johnsongrass biotype. In field experiments conducted on mugwort infestations

discovered in several counties throughout Virginia, 100% mugwort control was achieved with standard application rates of picloram at 4 months after treatment (MAT), and also greater than 70% mugwort control was achieved with the higher application rates of clopyralid, glyphosate, and dicamba at 4 MAT. However, all other herbicides evaluated in these experiments provided less than 65% mugwort control at 4 MAT, even at exceptionally high use rates. Additionally, the results from these trials revealed that sequential herbicide applications and sequential mowings prior to herbicide application are both effective mugwort control strategies.

ACKNOWLEDGMENTS

I would first like to thank Dr. Scott Hagood for the financial support, guidance, friendship, and perhaps most of all, for the training and instruction in all of the many different areas that are involved in extension weed science. I would also like to thank all of the members of my committee, Dr. Dan Brann, Paul Davis, Dr. Chester Foy, and Dr. Kriton Hatzios, for their support and input over the past several years. I especially extend appreciation to Paul Davis for 'pushing' me toward weed science and Dr. Hagood's program. I would also like to thank Claude Kenley, who has helped me with various research projects over the past couple of years, and at the same time put up with the skipped meals or drive-through food that comes along with riding with me around the state. I also appreciate the friendship and assistance that Dan Poston, David Langston, Ivan Morozov, and Steve King have provided me over the years. I would especially like to thank my parents for their continued encouragement throughout college and graduate school, and for always supporting me in the choices I have made. Most of all I would like to thank my fiancé, Megan LaRue, for her never-ending love and support through all of the classes, research, and field work that have accompanied me over the past five years.

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Chapter I

Introduction

Johnsongrass [*Sorghum halepense* (L.) Pers.] and mugwort (*Artemisia vulgaris* L.) are two perennial weeds that spread primarily via rhizomes, and are both capable of rapidly colonizing a variety of different environments. Johnsongrass is among the 10 most troublesome weeds in Virginia corn and soybean production (Dowler 1991; Dowler 1992), and is also one of the world's worst weeds in reducing crop yields (Holm et al. 1991). Similarly, mugwort has developed into one of the 10 most problematic weeds of nurseries in the eastern United States (Holm et al. 1997). Perennial weeds like johnsongrass and mugwort seem to be increasing problems in many Virginia's crops, which is likely a result of the widespread adoption of conservation tillage by farmers over the past several years. For example, from 1989 to 1994, the percentage of hectares planted with conservation tillage in the United States rose from 25.6 to 35.7%, and similar increases were observed in Virginia (Bull and Sandretto 1996). These conservation tillage systems usually favor the survival and spread of perennial weeds because their underground rhizomes are rarely, if ever, disturbed (Coffman and Frank 1991; Koskinen and McWhorter 1986). This research investigates the mechanisms of resistance in a johnsongrass biotype from New Kent County, VA and evaluates several perennial weed control strategies for the control of mugwort. Therefore, the results from these experiments should provide insight into the potential mechanisms of resistance that may occur in weed biotypes and should also be useful in preventing the spread of these troublesome perennial weeds in the future.

The selective postemergence control of johnsongrass in soybean [*Glycine max* L. Merr.] first became feasible in 1983 with the registration of the cyclohexanedione (CHD) herbicide sethoxydim {2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydorxy-2-cyclohexen-1-one} and the aryloxyphenoxypropionate (APP) herbicide fluazifop-P {(*R*)-2-[4-[[5-(trifluromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid}. Several years later, the APP herbicide quizalofop {(*R*)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy] propanoic acid and the CHD herbicide clethodim {(*E*, *E*)-2-(±)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} were also registered for use in soybean. These APP and CHD herbicides were immediately adopted by Virginia soybean growers, especially in fields where severe johnsongrass infestations occurred. In fact, in an effort to reduce these severe johnsongrass infestations, many soybean growers applied these herbicides repeatedly from one year to the next, often without crop rotation. Unfortunately, practices of this nature have led to the development of a number of resistant weed biotypes in this country and in others around the world. The development of johnsongrass biotypes that are resistant to the APP and CHD herbicides represents a significant threat to soybean growers throughout Virginia, and may result in the loss of the APP and CHD herbicides as a tool for the management of johnsongrass in this crop. For this reason, experiments were conducted on a suspected resistant-johnsongrass population located in New Kent County with the following research objectives:

1.) To evaluate the response of the New Kent johnsongrass biotype to applications of sethoxydim, clethodim, fluazifop-P, and quizalofop-P at twice

their recommended use rates at the field site where resistance was initially suspected.

2.) To evaluate the response of the New Kent johnsongrass biotype to applications of sethoxydim, clethodim, and quizalofop-P at rates ranging from ½ to 16 times their recommended use rates in greenhouse dose-response experiments.

3.) To determine the precise biochemical mechanism responsible for resistance in the New Kent johnsongrass biotype through acetyl-coenzyme A carboxylase (ACCase) assays and herbicide absorption, translocation, and metabolism experiments.

In the past, the majority of mugwort infestations in Virginia seem to have been confined to orchards and nurseries, where mugwort rhizomes were often transported in the soil around burlap-bound nursery plants (Holm et al. 1997; Rogerson and Bingham 1971; Uva et al. 1997). Recently, however, severe mugwort infestations have been identified in Virginia corn (*Zea mays* L.), soybean, and cotton (*Gossypium hirsutum* L.) fields, and also in many hay and pasture fields. As discussed previously, this may be due to the increasing adoption of conservation tillage by Virginia farmers. Very little research has been conducted on the chemical control of mugwort, and only a few of the available studies have identified herbicides that will provide acceptable mugwort control. However, many of these herbicides like fenac (2, 3, 6-trichlorobenzeneacetic acid) and silvex [2-(2, 4, 5-trichlorophenoxy) propanoic acid] are no longer commercially available for application in Virginia. Additionally, few studies have sufficiently evaluated the

response of mugwort to any of the various cultural and herbicidal control strategies that have been identified in experiments with other perennial weeds. Therefore, field experiments were conducted on severe mugwort infestations discovered in several Virginia counties with the following research objectives:

1.) To evaluate the response of mugwort to a range of rates of several growth regulator and nonselective herbicides, and metsulfuron.
2.) To evaluate mugwort control following one, two, and three applications of each of these herbicides.
3.) To evaluate the effect of applying each of these herbicides to mugwort in the vegetative vs. the flowering stage of growth.
4.) To evaluate the effect of mowing mugwort either once or twice prior to the application of these herbicides.

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Chapter II

Identification of a Johnsongrass (*Sorghum halepense*) Biotype Resistant to Aryloxyphenoxypropionate and Cyclohexanedione Herbicides in Virginia

Abstract: Field and greenhouse dose-response experiments were conducted to investigate the potential for graminicide resistance in a johnsongrass population from New Kent County, VA that survived repeated applications of quizalofop and quizalofop-P. During 1996 and 1997, significant foliar injury (30 to 60%) was initially observed on the johnsongrass at the New Kent field site, but this biotype eventually recovered and survived applications of fluazifop-P, quizalofop-P, and sethoxydim at twice the recommended field use rates. However, applications of clethodim at twice the recommended field use rates during 1997 provided essentially complete control of the New Kent johnsongrass biotype. In greenhouse dose-response experiments, the amount of quizalofop-P required to inhibit shoot growth by 50% (GR_{50}) was 0.05 kg/ha in the New Kent johnsongrass biotype and 0.02 kg/ha in the susceptible johnsongrass biotype. In response to sethoxydim, the GR_{50} for the New Kent biotype was 0.31 kg/ha while that of the susceptible biotype was 0.11.kg/ha. As in the field trials, the New Kent biotype was sensitive to applications of clethodim, which provided a GR_{50} value of 0.09 kg/ha for the New Kent biotype and 0.11 kg/ha for the susceptible biotype. These values indicate that the New Kent biotype displays 2.5-fold more tolerance to quizalofop-P and 2.8-fold more tolerance to sethoxydim than the susceptible biotype, and that the New Kent and susceptible johnsongrass biotypes are equally sensitive to clethodim. These relatively low and essentially equivalent levels of resistance to quizalofop-P and sethoxydim are

inconsistent with the high levels of resistance most commonly observed in graminicide-resistant weed biotypes, and suggests a mechanism of resistance other than an insensitive ACCase in the New Kent johnsongrass biotype.

Nomenclature: Clethodim, (*E, E*)-2-(±)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; fluazifop-P, (*R*)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid; quizalofop-P, (*R*)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propanoic acid; sethoxydim, 2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; johnsongrass, *Sorghum halepense* (L.) Pers. #[1] SORHA; soybean, *Glycine max* (L.) Merr.

Additional index words: Cross-resistance, herbicide resistance, ACCase inhibitors, graminicides.

Abbreviations: ACCase, acetyl coenzyme-A carboxylase; APP, aryloxyphenoxypropionate; CHD, cyclohexanedione; DAT, days after treatment; GR_{50}, dosage giving 50% reduction in shoot dry weight; WAT, weeks after treatment.

INTRODUCTION AND LITERATURE REVIEW

Johnsongrass is one of the world's worst weeds in reducing crop yields (Holm et al. 1991) and is among the ten most common and troublesome weeds in Virginia soybean [*Glycine max* (L.) Merr.] production (Dowler 1992). Earlier studies have demonstrated that full-season johnsongrass competition may reduce soybean yields by as much as 50%

[1] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

(Williams and Hayes 1984), and may also increase the foreign material, moisture content, and damaged seeds present at harvest (McWhorter and Anderson 1993). These effects are primarily due to the ability of johnsongrass to reproduce from both seeds and rhizomes, with a single plant capable of producing approximately 28,000 seeds and 60 to 90 m of rhizomes per season (Bennett 1973).

Prior to the development of the class of herbicides known as the graminicides, the selective postemergence control of johnsongrass in soybean was an extremely difficult task. Many soybean producers attempted johnsongrass control with mechanical methods such as hand removal, cultivation, and plowing. However, these methods are incompatible with many of the reduced tillage practices commonly practiced today. Selective johnsongrass control was also attempted with rope-wick applications of glyphosate [*N*-(phosphonomethyl)glycine], which is a practice that is completely dependent upon the height differential that develops between johnsongrass and the soybean canopy. Unfortunately, this height differential does not usually occur until later in the growing season, often resulting in early season johnsongrass competition and reduction in soybean yield (Williams and Hayes 1984).

In the early 1980s, the selective postemergence control of johnsongrass in soybean first became feasible with the registration of several graminicides, or grass herbicides. The graminicides are divided into two chemically distinct classes of herbicides, the cyclohexanediones (CHD) and the aryloxyphenoxypropionates (APP). Both of these classes of herbicides act by inhibiting the enzyme acetyl coenzyme-A carboxylase (ACCase) in susceptible grass species. ACCase catalyzes the first committed step of fatty acid biosynthesis, which is the ATP-dependent carboxylation of acetyl-coA to

malonyl-coA. Dicotyledonous species, however, possess an insensitive ACCase that is not inhibited by the CHD and APP herbicides (Gronwald 1991). This differential inhibition of grass rather than dicot ACCase has afforded many soybean growers with the ability to control johnsongrass selectively with a postemergence herbicide treatment. For example, Vidrine et al. (1995) observed 83 to 99% johnsongrass control in soybean with the graminicides clethodim, fenoxaprop-ethyl [(±)-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid], fluazifop-P, quizalofop-P, and sethoxydim. Similarly, Carter and Keeley (1987) observed essentially complete control of johnsongrass with sethoxydim, fluazifop-P, or haloxyfop [(±)-2-[4-[[3-chloro-5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid] when applied at 4 and 9 wk after cotton (*Gossypium hirsutum* L.) planting. Cudney and Orloff (1987) also observed good to excellent control of johnsongrass in alfalfa (*Medicago sativa* L.) with two applications of clethodim, fluazifop-P, or sethoxydim.

Several grass crops are also tolerant to certain members of the APP or CHD herbicides, despite the sensitivity of most grass species to these herbicides. Perhaps the most relevant example of this phenomenon is the ability of wheat (*Triticum aestivum* L.) to metabolize the APP herbicide diclofop to a herbicidally-inactive product (Dusky et al. 1980; Shimabukuro et al. 1979). Tissue culture breeding techniques have also selected for corn (*Zea mays* L.) cultivars with an altered ACCase that are tolerant to high rates of the CHD herbicide sethoxydim (Parker et al. 1990). These cultivars first became commercially available in 1996 and since that time have provided growers with a new option for effectively suppressing perennial grass weeds like johnsongrass, bermudagrass (*Cynodon dactylon* L.), and wirestem muhly [*Muhlenbergia frondosa* (Poir.) Fernald] in

sethoxydim-tolerant corn (Ashley and Hagood 1997; Bean and Salisbury 1998; Gallaher et al. 1996; Lingenfelter and Curran, 1999).

The wide variety of crops in which the graminicides are currently registered often leads to the continual use of these herbicides in both time and location (Devine and Shimabukuro 1994). However, the practice of repeatedly applying a herbicide or herbicides with the same mode of action is one of the most common factors leading to the selection of a resistant weed biotype (Shaner 1995). Consequently, at least 21 grass weed biotypes have been identified that display resistance to one or more of the graminicides (Heap 2000). Some of the more threatening grass weeds included in this list are johnsongrass biotypes from Mississippi (Smeda et al. 1997) and Kentucky (Obermeier et al. 1997), and several Italian ryegrass (*Lolium multiflorum* Lam.) biotypes that continue to plague small grain growers throughout Virginia and the southern United States (Barber et al. 1999; Morozov et al. 1999).

In 1995, a soybean grower in New Kent County, VA observed poor johnsongrass control following consecutive applications of the APP herbicide quizalofop-P. Field records indicated that fluazifop-P had been applied at this location for a period of at least five consecutive years followed by consecutive applications of either quizalofop or quizalofop-P for at least three years. Additionally, the available records indicated that crop rotation did not occur throughout this period. The objectives of this research were to investigate the potential for graminicide resistance in the New Kent johnsongrass biotype, and to quantify the levels of resistance to the APP herbicides fluazifop-P and quizalofop-P and the CHD herbicides sethoxydim and clethodim.

MATERIALS AND METHODS

Field Experiments. Field plots were established during the summers of 1996 and 1997 at the site in New Kent County, VA where resistance was originally suspected. In 1996, the experiments were established on fallow land that had been in soybean during the previous year, and in an area of heavy johnsongrass infestation that survived repeated applications of quizalofop-P. In 1997, the experiment was established in a separate area of the same field, in soybean spaced 38 cm apart. Individual plots measured 1.8 by 7.6 m in 1996 and 3 by 122 m in 1997. In both years, the field plots were established on a Pamunkey sandy loam (fine-loamy, mixed, thermic, Ultic Hapludalfs). All graminicides were applied at rates equivalent to twice the normal field use rate with a crop oil concentrate[2] at 1% (v/v). Herbicides and dosages applied were sethoxydim at 0.42 kg/ha, clethodim at 0.35kg/ha, fluazifop-P at 0.42 kg/ha, and quizalofop-P at 0.15 kg/ha. Clethodim was not included in the 1996 experiments due to a limited johnsongrass infestation in the area where the plots were originally established. At the time of the herbicide applications, johnsongrass seedlings and sprouts from rhizomes were in the three- to five-leaf stage and ranged from 30 to 60 cm in height. All treatments were arranged in a randomized complete block design with four replications and were applied with a CO_2-powered backpack sprayer that delivered 210 L/ha of spray solution at 234 kPa. Visual control ratings were taken at 2, 4, 6, and 8 weeks after treatment (WAT) during both years and were based on a scale of 0 to 100, with 0 equal to the johnsongrass vigor and ground cover observed in the untreated control plots, and 100 equal to

[2] Crop Surf® (83% paraffin base petroleum oil), Universal Cooperatives, Inc., Minneapolis, MN 55440.

complete johnsongrass control. Seeds were harvested from all johnsongrass plants that survived graminicide treatment for use in subsequent greenhouse dose-response experiments. Data were subjected to analysis of variance and treatment means were separated with Duncan's new multiple range test at the 5% level. Due to significant treatment-by-year interactions, the results from each year are presented separately in Table 1.

Graminicide Dose-Response Experiments. Johnsongrass seeds harvested from the New Kent field site were planted in greenhouse flats for use in an initial screening to ensure graminicide tolerance. Seedlings of the New Kent biotype were then treated with 0.08 kg quizalofop-P, which corresponds to twice the normal field use rate for seedling johnsongrass less than 20 cm in height. These treatments were applied with a CO_2-powered backpack sprayer that delivered 210 L/ha of spray solution at 234 kPa. Johnsongrass seedlings that survived these treatments were transplanted into additional greenhouse flats and allowed to grow undisturbed for several months. Rhizomes from these johnsongrass plants were then harvested as needed for use in the greenhouse herbicide dose-response experiments. Johnsongrass rhizomes were also harvested from a field in Montgomery County, VA that had no previous history of graminicide application for use as the susceptible biotype. All rhizomes were cut into 5-cm segments and two rhizome segments from either the resistant or susceptible biotype were planted approximately 2 cm deep in separate 10 cm-diameter plastic pots containing a commercial potting medium[3]. After emergence, young plants were thinned to one per pot, maintained in a greenhouse, and watered as needed. Both biotypes were sprayed

[3] Premier Pro Mix BX. Premier Horticulture Lt.'ee, Rivière-du-Loup, Quèbec, Canada 65R4C9.

when the plants reached the three to four-leaf stage and were no more than 40 cm in height. Applications were made with a stationary track sprayer[4] containing a single 8001 flat-fan nozzle tip[5] that delivered 234 L/ha of spray solution at 234 kPa. Each graminicide was applied at rates equivalent to 0.5, 1, 2, 4, 8, and 16 times the normal field use rate for rhizomatous johnsongrass ranging from 20 to 40 cm in height. Sethoxydim was applied at 0, 0.16, 0.31, 0.63, 1.25, 2.5, and 5.0 kg/ha, clethodim at 0, 0.07, 0.14, 0.28, 0.56, 1.1, and 2.2 kg/ha, and quizalofop-P at 0, 0.039, 0.08, 0.16, 0.32, 0.64, and 1.28 kg/ha. All treatments were applied in water with 1% (v/v) crop oil concentrate. Percent visual control ratings were recorded at 1, 2, 3, and 4 WAT and were based on the scale discussed previously. At 4 WAT, treated shoots were cut at the soil surface, dried for 72 h at 60 C, and weighed. From these shoot dry weights, the dosages giving 50% reduction in shoot weight (GR_{50}) were calculated for each herbicide. The experimental design was a randomized complete block with five replications of six herbicide dosages and an untreated control. Each herbicide was evaluated in a separate experiment and all experiments were repeated. All data were subjected to analysis of variance and treatment means were separated with Duncan's new multiple range test at the 5% level.

RESULTS AND DISCUSSION

Field Experiments. During both years, some initial injury was observed at 14 and 42

[4] Allen Machine Works, 607 E. Miller Road, Midland, MI 48640.
[5] Spraying Systems Co.®, P. O. Box 7900, Wheaton, IL 60189.

days after treatment (DAT) in the New Kent johnsongrass biotype, but by 70 DAT the New Kent biotype had almost completely recovered from the fluazifop-P, quizalofop-P, and sethoxydim treatments (Table 1). In 1996, fluazifop-P, quizalofop-P, and sethoxydim provided similar levels of johnsongrass control at each evaluation, but in 1997 quizalofop-P provided a significantly higher level of johnsongrass control than either fluazifop-P or sethoxydim at 14 and 42 DAT. In 1997, however, there was no significant difference in the level of johnsongrass control achieved with fluazifop-P, quizalofop-P, and sethoxydim at 70 DAT. As discussed previously, clethodim was not evaluated in 1996 but provided 83% control of the New Kent johnsongrass biotype during 1997 at 42 and 70 DAT. This level of control was significantly greater than the 17, 34, and 20% control afforded by fluazifop-P, quizalofop-P, and sethoxydim, respectively. The unique ability of clethodim to control otherwise graminicide-resistant grass biotypes has also been reported with giant foxtail (*Setaria faberi* Herrm.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and johnsongrass biotypes (Barrentine et al. 1992; Hidayat and Preston 1997; Smeda et al. 1997; Stoltenberg and Wiederholt 1995). Similarly, other studies have identified goosegrass (*Eleusine indica* L. Gaertn.), annual ryegrass (*Lolium rigidum* Gaud.), green foxtail (*Setaria viridis* L. Beauv.), giant foxtail, large crabgrass, and johnsongrass biotypes that exhibit high levels of cross-resistance to several APP and CHD herbicides, but much lower levels of resistance to the CHD herbicide clethodim (Leach et al. 1995; Obermeier et al. 1997; Shukla et al. 1997; Tardif et al. 1993; Wiederholt and Stoltenberg 1995).

Graminicide Dose-Response Experiments. When compared to the susceptible biotype, the New Kent johnsongrass biotype exhibited low levels of resistance to quizalofop-P and

sethoxydim, but was susceptible to standard use rates of clethodim (Figures 1 through 3). By two weeks after treatment with either quizalofop-P, sethoxydim, or clethodim, similar levels of injury were observed on the foliage of both the New Kent and susceptible biotypes. Visible signs of recovery were eventually observed in the New Kent biotype at rates corresponding to 0.5, 1, and 2 times the normal field use rate of quizalofop-P or sethoxydim, but the remainder of the treatments provided essentially complete control of the New Kent biotype. In contrast to the New Kent biotype, foliar injury on the susceptible biotype progressively worsened with time, until essentially complete control was achieved with all graminicides and rates evaluated.

The calculated GR_{50} values for the New Kent and susceptible biotypes in response to quizalofop-P were 0.05 and 0.02 kg/ha, respectively, indicating that the NK biotype is 2.5 times more tolerant of quizalofop-P than the susceptible biotype (Figure 1). However, this GR_{50} of the NK biotype is slightly lower than the recommended quizalofop-P use rate of 0.64 kg/ha, which provided essentially complete control of the susceptible johnsongrass biotype. Similarly, the calculated GR_{50} value of the New Kent biotype in response to sethoxydim was 0.31 kg/ha, while that of the susceptible biotype was 0.11 kg/ha (Figure 2). This indicates that the New Kent biotype is 2.8 times more tolerant of sethoxydim than the susceptible biotype, and once again that the GR_{50} of this biotype is slightly lower than the recommended sethoxydim use rate of 0.32 kg/ha. In response to clethodim, however, the calculated GR_{50} values for the New Kent and susceptible biotypes were 0.09 and 0.11 kg/ha, respectively, revealing that the two biotypes are equally sensitive to clethodim (Figure 3). These values also indicate that the New Kent and susceptible biotypes are more sensitive to clethodim than to either quizalofop-P or

sethoxydim, since the calculated GR_{50} values for both biotypes are only slightly higher than one-half the recommended clethodim use rate of 0.07 kg/ha. As discussed above, the sensitivity of this otherwise graminicide-resistant johnsongrass biotype to clethodim is similar to the response observed in a number of other studies with graminicide-resistant weed biotypes.

Collectively, the results from the greenhouse dose-response experiments are consistent with the response of the New Kent biotype observed in the field. In both the field and greenhouse dose-response experiments, the New Kent johnsongrass biotype exhibited low levels of resistance to quizalofop-P and sethoxydim, but was sensitive to clethodim. These low levels of resistance are inconsistent with the relatively high levels of resistance most commonly reported in graminicide-resistant weed biotypes that possess an insensitive ACCase as the mechanism of resistance. For example, the johnsongrass biotypes characterized by Smeda et al. (1997) exhibited > 388-fold resistance to fluazifop-P, > 16-fold resistance to quizalofop-P, and from 2.8- to 8.5-fold resistance to sethoxydim. This suggests a mechanism of resistance other than an insensitive ACCase in the New Kent johnsongrass biotype. However, additional laboratory experiments will be required to determine the precise mechanism responsible for resistance in the New Kent johnsongrass biotype.

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Table 1 . Percent visual control of the New Kent johnsongrass biotype with fluazifop-P, quizalofop-P, clethodim, and sethoxydim at 14, 42, and 70 days after treatment (DAT) in 1996 and 1997.[a]

		Johnsongrass control					
		1996			1997		
Herbicide[b]	Rate	14 DAT	42 DAT	70 DAT	14 DAT	42 DAT	70 DAT
	kg/ha	---------- (%) ----------					
Fluazifop-P	0.42	68 a	67 ab	13 a	34 b	30 c	17 bc
Quizalofop-P	0.15	67 a	33 b	9 b	47 a	50 b	34 b
Clethodim	0.35	---	---	---	54 a	83 a	83 a
Sethoxydim	0.42	68 a	54 ab	11 ab	34 b	32 c	20 b
Untreated	----	0 b	0 c	0 c	0 c	0 d	0 c

[a] Means in a column followed by the same letter(s) are not significantly different at the 5% level according to Duncan's multiple range test.

[b] All treatments contained 1.0% (v/v) crop oil concentrate.

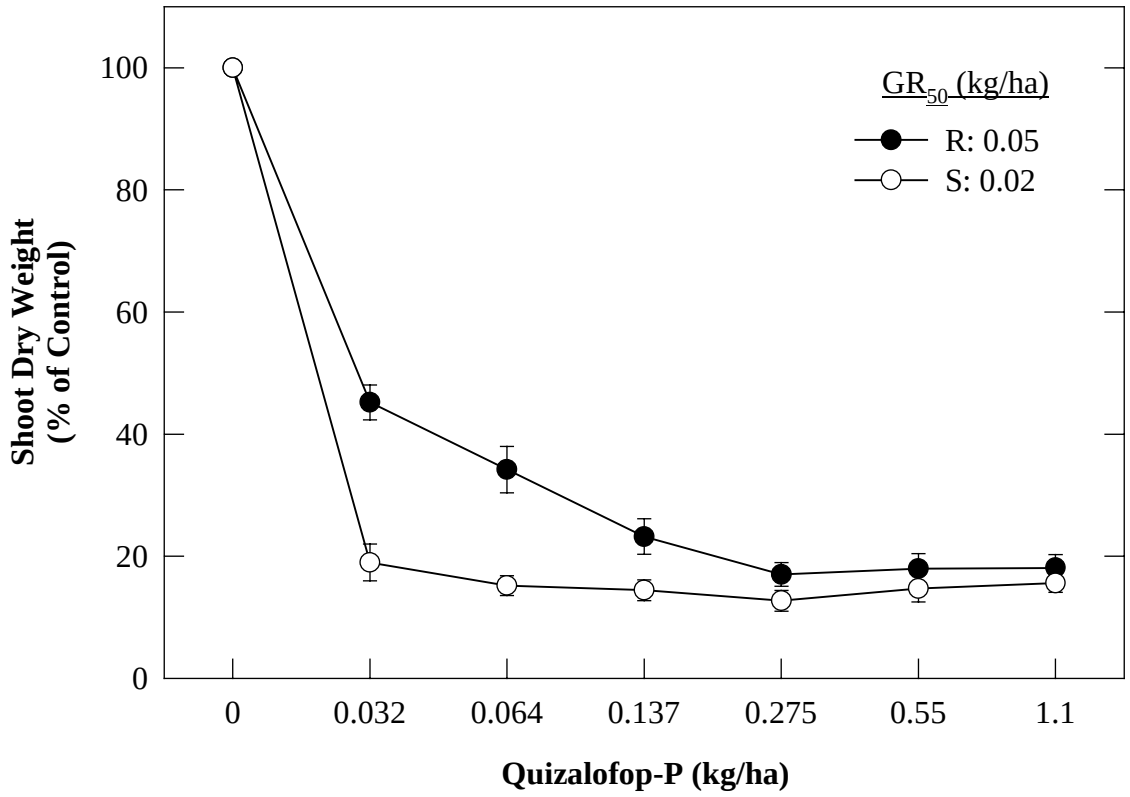

Figure 1. Influence of quizalofop-P on the shoot dry weight of resistant (R) and susceptible (S) johnsongrass biotypes at 4 wk after treatment. Error bars represent the standard error of the mean.

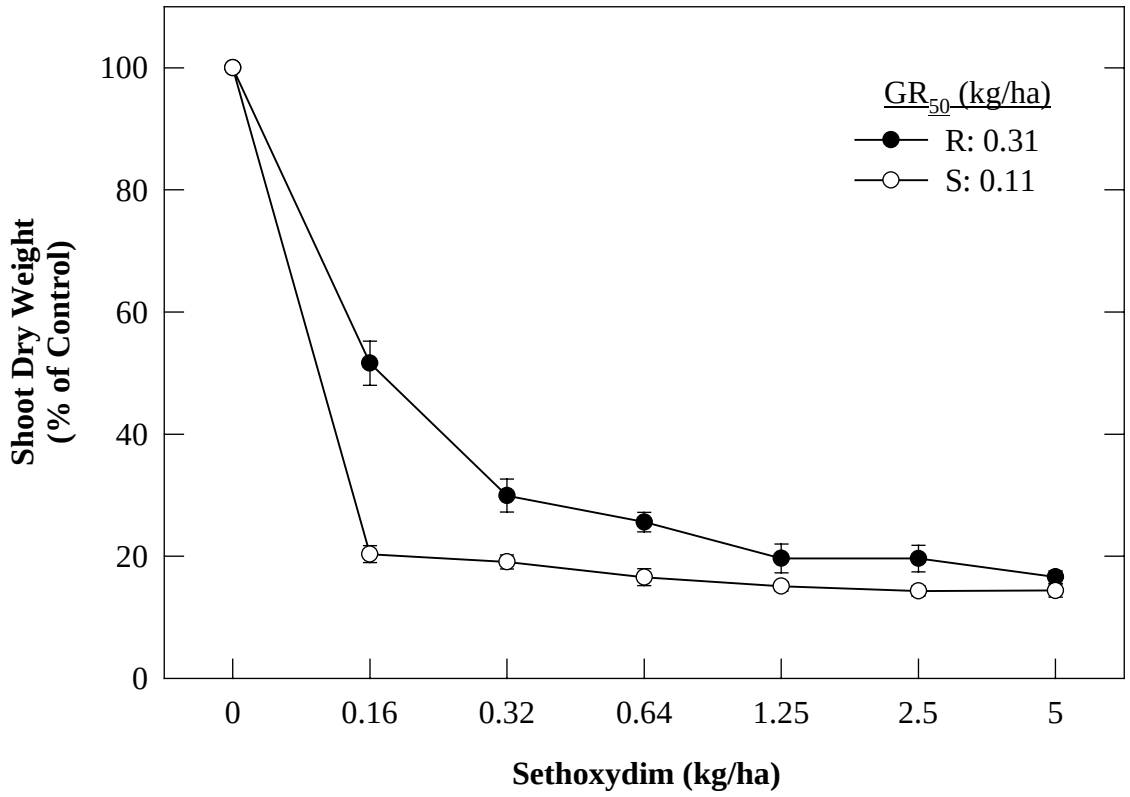

Figure 2. Influence of sethoxydim on the shoot dry weight of resistant (R) and susceptible (S) johnsongrass biotypes at 4 wk after treatment. Error bars represent the standard error of the mean.

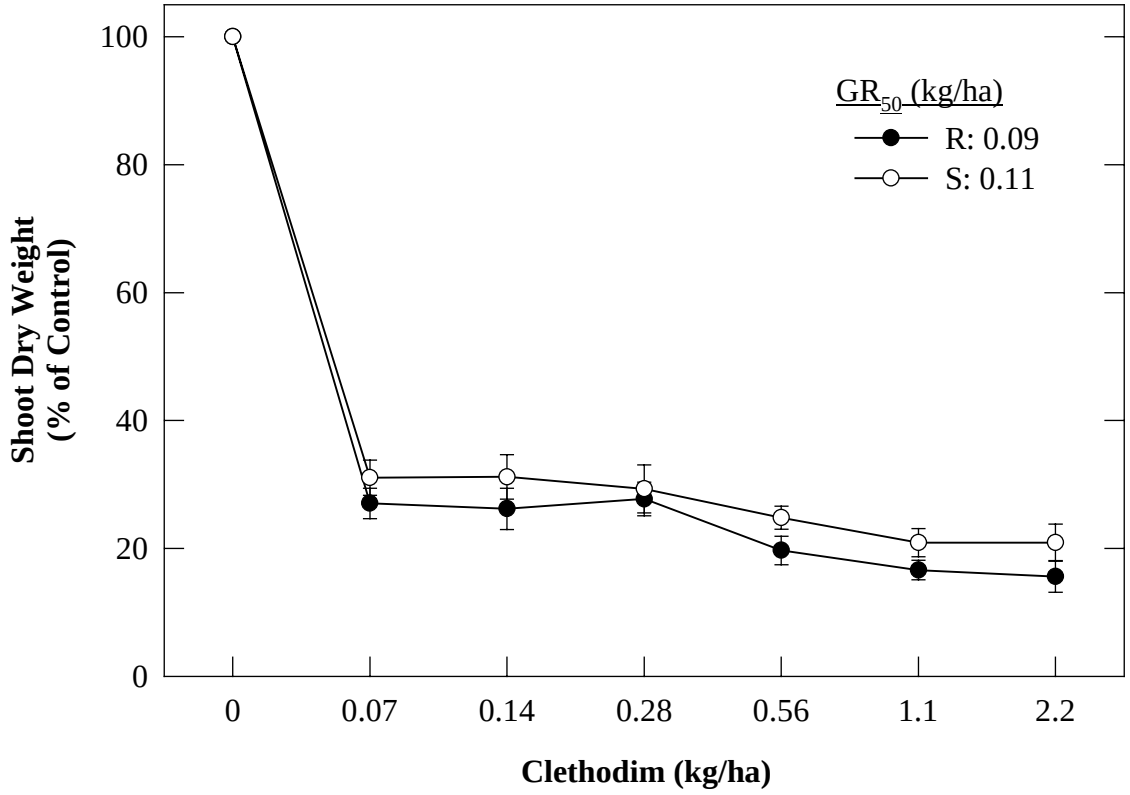

Figure 3. Influence of clethodim on the shoot dry weight of resistant (R) and susceptible (S) johnsongrass biotypes at 4 wk after treatment. Error bars respresent the standard error of the mean.

Chapter III

Investigations of the Mechanism of Resistance to Aryloxyphenoxypropionate and Cyclohexanedione Herbicides in a Johnsongrass (*Sorghum halepense*) Biotype from Virginia

Abstract: Acetyl-coenzyme A carboxylase (ACCase) assays and absorption, translocation, and metabolism experiments were conducted to investigate the biochemical mechanism responsible for resistance in a johnsongrass biotype that exhibits low levels of resistance to the cyclohexanedione (CHD) herbicide sethoxydim and the aryloxyphenoxypropionate (APP) herbicides quizalofop-P and fluazifop-P. The rate of [^{14}C]quizalofop-ethyl absorption was significantly higher in the resistant compared to the susceptible biotype at 8, 24, and 48 hours after treatment (HAT), but by 72 HAT there was no significant difference in the amount of [^{14}C]quizalofop-ethyl detected in either biotype. Additionally, little or no differences in the translocation of [^{14}C]quizalofop-ethyl were observed in the resistant and susceptible biotypes at any time interval after application. Therefore, it is unlikely that differential absorption or translocation confers tolerance to the APP and CHD herbicides in the resistant johnsongrass biotype. In [^{14}C]quizalofop-ethyl metabolism experiments, similar levels of quizalofop-ethyl and quizalofop conjugates were observed in the resistant and susceptible biotypes at 8, 24, 48, and 72 HAT, but slightly higher levels of quizalofop acid were detected in the resistant biotype at 48 and 72 HAT. However, it is unlikely that these differences can sufficiently explain resistance in the johnsongrass biotype. In ACCase assays, the concentrations of quizalofop-P, clethodim, and sethoxydim that inhibited ACCase activity by 50% (I_{50})

were statistically similar in the two biotypes, indicating that the resistant johnsongrass biotype contains an ACCase that is sensitive to the APP and CHD herbicides. In the absence of APP or CHD herbicides, however, the specific activity of ACCase in the resistant biotype ranged from 145.8 to 282.6 pmol mg^{-1} min^{-1}, while that of the susceptible biotype ranged from 71.4 to 94.9 pmol mg^{-1} min^{-1}. These results indicate that the specific activity of ACCase in the resistant biotype is 2 to 3 times greater than that of the susceptible biotype in the absence of ACCase-inhibiting herbicides. The specific activity of ACCase in the resistant biotype was also significantly greater than that of the susceptible biotype in the presence of all concentrations of quizalofop-P and sethoxydim, and in the presence of 0.1, 1, and 10 μM clethodim. These results suggest that resistance to quizalofop-P and sethoxydim is conferred by an overproduction of ACCase in the resistant johnsongrass biotype. Overproduction of ACCase appears to be a unique mechanism that explains the evolved resistance of the New Kent johnsongrass biotype to the APP and CHD herbicides.

Nomenclature: Clethodim, (*E, E*)-2-(±)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; fluazifop-P, (*R*)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoic acid; quizalofop-P, (*R*)-2-[4-[(6-chloro-2-quinoxalinyl)oxy]phenoxy]propanoic acid; sethoxydim, 2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one; johnsongrass, *Sorghum halepense* (L.) Pers. #[1] SORHA.

Additional index words: Absorption, enzyme amplification, enzyme overproduction

[1] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence, KS 66044-8897.

Abbreviations: ACCase, acetyl-coenzyme A carboxylase; APP, aryloxyphenoxypropionate; BSA, bovine serum albumin; CHD, cyclohexanedione; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; HAT, hours after treatment; I_{50}, herbicide concentration providing 50% inhibition of ACCase; PMSF, phenylmethylsulfonyl fluoride; PVP, polyvinylpyrrolidone; TLC, thin-layer chromatography.

INTRODUCTION AND LITERATURE REVIEW

The selective postemergence control of johnsongrass in soybean first become feasible in the early 1980s with the registration of several graminicides that belong to the CHD and APP classes of herbicides. Although chemically dissimilar, the target site of both the APP and CHD herbicides is the enzyme ACCase (Gronwald 1991). For this reason, members of either of these herbicide classes are commonly referred to as ACCase inhibitors. ACCase catalyzes the first committed step of fatty acid biosynthesis, which is the ATP-dependent carboxylation of acetyl-coA to malonyl-coA (Harwood 1988). Generally, ACCases from grasses are sensitive to inhibition by the APP and CHD herbicides, while dicot ACCases are not (Burton et al. 1991). This phenomenon is partly explained by the presence of a herbicide-insensitive (prokaryotic) form of ACCase in the plastids of dicots, compared to a herbicide-sensitive (eukaryotic) form of ACCase in the plastids of grasses (Konishi and Sasaki 1994).

The selectivity of the APP and CHD herbicides, coupled with the excellent efficacy of these herbicides on many grass weed species, has led to the widespread use of these

herbicides on a variety of crops, often resulting in repeated applications of these herbicides in both time and location (Devine and Shimabukuro 1994). Unfortunately, practices of this nature have led to the selection of at least 21 grass weed biotypes in 17 countries that are resistant to one or more of the APP and CHD herbicides (Heap 2000). To date, four principal biochemical mechanisms have been proposed to explain resistance to the APP or CHD herbicides in weed biotypes (Devine and Shimabukuro 1994). First, graminicide resistance in a weed biotype may be due to the presence of an insensitive form of ACCase. This is the mechanism conferring resistance in the vast majority of graminicide-resistant weeds identified thus far (Devine 1997). For example, an insensitive ACCase has been identified as the mechanism of resistance in biotypes of *Alopecurus myosuroides* Huds. (Cocker et al. 1999), *Avena fatua* L. (Seefeldt et al. 1996), *Avena sterilis* ssp. *ludoviciana* Malzew. (Maneechote et al. 1994, 1997; Shukla et al. 1997a), *Digitaria ischaemum* (Schreb.) Muhl. (Kuk et al. 1999), *Eleusine indica* (L.) Gaertn. (Leach et al. 1995), *Lolium multiflorum* Lam. (Gronwald et al. 1992), *Lolium rigidum* Gaud. (Tardif et al. 1993; Tardif and Powles 1994), *Phalaris minor* Retz. (Tal et al. 1996), *Setaria faberi* Herrm. (Shukla et al. 1997b), *Setaria viridis* (L.) Beauv. (Marles et al. 1993a; Shukla et al. 1997b), and *Sorghum halepense* (L.) Pers. (Marles et al. 1993b). Increased herbicide metabolism has also been proposed as a mechanism of resistance to the APP and CHD herbicides, and was determined to be the mechanism responsible for resistance in a biotype of *Digitaria sanguinalis* (L.) Scop. (Hidayat and Preston 1997) and two biotypes of *Alopecurus myosuroides* (Hall et al. 1997; Menendez and DePrado 1996). Further investigations into the mechanism of tolerance in these *Alopecurus myosuroides* biotypes revealed elevated levels of the enzyme glutathione

transferase, which was determined to be a mechanism that contributed, at least partially, to the overall tolerance of these biotypes to the APP herbicide fenoxaprop-ethyl [(±)-2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoic acid] (Cummins et al. 1997). Increased herbicide metabolism was also identified as a mechanism that played some role in the overall tolerance of *Avena sterilis* (Maneechote et al. 1997) and *Lolium rigidum* (Holtum et al. 1991) biotypes. Another resistance mechanism involving the repolarization of the plasma membrane electrogenic potential (E_m) in resistant but not susceptible biotypes has been proposed for *Avena fatua* (Devine and Shimabukuro 1994) and *Lolium rigidum* (Häusler 1991) biotypes, but additional alterations of the ACCase extraction buffer used in the *Avena fatua* experiments revealed that this resistant biotype does in fact possess an insensitive ACCase (Shukla et al. 1997a). Conflicting results of this nature have been the subject of some debate within the literature and have led several authors to question the validity of the membrane response as a mechanism that confers resistance to the ACCase-inhibiting herbicides (Devine 1997; DiTomaso 1994). In a recent experiment conducted by DePrado et al. (1999), however, repolarization of the plasma membrane E_m occurred upon removal of 25 μM diclofop [(±)-2-[4-(2, 4-dichlorophenoxy)phenoxy]propanoic acid] in resistant, but not susceptible, biotypes of *Lolium rigidum*, *Lolium multiflorum*, and *Alopecurus myosuroides*. Additionally, differences in the depolarization of the plasma membrane E_m were observed between resistant and susceptible biotypes of all three weed species. These results reveal an obvious correlation between the resistant phenotype and the plasma membrane response to diclofop, and also reveal the need for further evaluations of this response as a mechanism that contributes in some manner to the resistance observed in these weed

biotypes. Lastly, decreased absorption and/or translocation has been proposed as a potential mechanism of resistance to the ACCase-inhibiting herbicides, but currently no resistant weed biotypes have been identified that exhibit differences in the absorption or translocation of APP or CHD herbicides when compared to susceptible biotypes of the same species (Devine 1997; Devine and Shimabukuro 1994).

In 1995, a soybean grower in New Kent County, Virginia reported poor johnsongrass control following repeated applications of the APP herbicide quizalofop-P. Field records indicated that either quizalofop or quizalofop-P had been applied at this location for a period of at least three consecutive years. Field and greenhouse investigations subsequently confirmed that the resistant johnsongrass biotype is 2.5 and 2.8 times more tolerant of quizalofop-P and sethoxydim than a susceptible biotype, and also that the resistant johnsongrass biotype is susceptible to standard application rates of clethodim. The objective of this study was to determine the biochemical mechanism responsible for resistance in this johnsongrass biotype through examinations of the absorption, translocation, and metabolism of the APP herbicide quizalofop-P in the resistant and susceptible johnsongrass biotypes, and through the quantification of the specific activity of ACCase in both biotypes in the presence of increasing concentrations of quizalofop-P, clethodim, and sethoxydim.

MATERIALS AND METHODS

Plant Material. In the fall of 1996 and 1997, seeds were harvested from johnsongrass that survived treatment with 0.42 kg/ha sethoxydim, 0.35 kg/ha clethodim, 0.42 kg/ha

fluazifop-P, and 0.15 kg/ha quizalofop-P at the New Kent field site where resistance was originally suspected. Seeds from a susceptible johnsongrass biotype with no previous history of APP or CHD applications were also harvested for use as a control. For ACCase assays, approximately 50 seeds of each biotype were sown in separate 26- by 18-cm greenhouse flats containing commercial potting soil. Seedlings for all experiments were grown to the two to three leaf-stage in a greenhouse, watered as needed, and provided with supplemental lighting from metal halide lamps (400 μmol photon m^{-2} s^{-1}) for a 12-h photoperiod. For herbicide absorption, translocation, and metabolism experiments, johnsongrass seedlings from each biotype were transferred to glass jars covered with aluminum foil and filled with 100 ml of quarter-strength Hoagland's nutrient solution (pH 6.3). The seedlings were then acclimated in this medium for 4 days prior to the start of these experiments.

Herbicide Absorption and Translocation. Resistant and susceptible johnsongrass seedlings were grown in the greenhouse and transferred to aluminum foil-covered glass jars as described above. At the four-leaf stage, hydroponically-grown seedlings were sprayed with 0.39 kg ha^{-1} of quizalofop-P, which represented approximately 50% of the field use rate. This application was made with a stationary track sprayer[2] containing a single flat-fan nozzle tip[3] that delivered 234 L ha^{-1} of spray solution at 269 kPa. Approximately 3 h after spraying, 10 μl of quizalofop-ethyl containing an approximate radioactivity content of 3,700 Bq/μl was applied to the second leaf of resistant and susceptible johnsongrass seedlings. [^{14}C]quizalofop-ethyl was diluted to 5% acetone in

[2] Allen Machine Works, 607 E. Miller Road, Midland, MI 48640.
[3] Spraying Systems Co.®, P. O. Box 7900, Wheaton, IL 60189.

an aqueous solution containing 0.5% (v/v) Tween 20[4]. Thirty-two plants of each biotype were spotted, and eight plants of each biotype were randomly selected for harvesting at 8, 24, 48, and 72 HAT. At each harvest time, treated seedlings were divided into the treated leaf, the remaining shoot and leaves, and the roots. Unabsorbed [^{14}C]quizalofop-ethyl was removed at each harvest time by agitating the treated leaf in a 10 ml wash solution (10% ethanol and 0.1% Tween 20) for 30 s. A 1-ml aliquot from each leaf rinse was mixed with 10 ml scintillation cocktail[5] and the total unabsorbed radioactivity was determined by liquid scintillation spectrometry[6]. Three seedlings of each biotype were then retained for use in the metabolism studies. For the remaining seedlings, the total absorption and translocation of the applied radioactivity was determined by combustion of the seedling tissues in a biological sample oxidizer[7] and liquid scintillation spectrometry. Absorption of [^{14}C]quizalofop-ethyl was expressed as a percentage of the applied radioactivity and was calculated by dividing the amount of ^{14}C from the respective plant section by the sum of the ^{14}C from the leaf wash of each seedling and the ^{14}C recovered from all oxidized plant sections. Distribution of [^{14}C]quizalofop-ethyl was expressed as a percentage of the absorbed radioactivity and was calculated by dividing the amount of ^{14}C from the respective plant section by the total amount of ^{14}C recovered from all oxidized plant sections. Absorption and translocation experiments were repeated three times and each treatment was replicated three times in an experiment.

Herbicide Metabolism. Resistant and susceptible johnsongrass seedlings were treated

[4] Polysorbate 20 [polyoxyethylene (20) sorbitan monolaurate], ICI America, Inc., Wilmington, DE 19899

[5] ScintiVerse® BD, Fisher Scientific, Fair Lawn, NJ 07410.

[6] Liquid scintillation counter, Beckman LS 5000TA Model, Beckman Instruments, 4300 N. Harbor Blvd., Fullerton, CA 92634.

[7] Biological Oxidizer Model 307, Packard Instrument Co., 2200 Warrenville Road, Downers Grove, IL 60515.

and harvested as described above in the absorption and translocation experiments. Following the leaf rinse, treated leaves were homogenized in 3 ml of 80% acetone and centrifuged at 13,000 *g* for 10 min. Supernatants were pooled, evaporated to dryness under N_2 and redissolved in 200 μl absolute ethanol. These extracts were then spotted on silica gel thin-layer chromatography (TLC) plates[8], which had been prepared by baking for 1 h at 110° C. Standards of [^{14}C]quizalofop-ethyl and nonlabeled quizalofop-P were cochromatographed with the plant extracts and the TLC plates were developed in a solvent system previously utilized by Ruizzo and Gorski (1988) containing toluene, acetone, methanol, glacial acetic acid, and water (40:25:15:10:15 by volume) . After development, TLC plates were dried and observed under ultraviolet light. Each detected band was scraped from the TLC plates and the radioactivity was quantified by liquid scintillation spectrometry. Metabolites were separated by their Rf values and the distribution of radioactivity in each metabolite fraction was expressed as a percentage of the absorbed radioactivity recovered in the TLC analysis of all of the plant sections. Metabolism experiments were repeated three times with two replications of the pooled extracts per experiment.

ACCase Extraction and Assay. All enzyme extraction and assay procedures were performed according to Shukla et al. (1997a) with only slight modifications. ACCase from the susceptible biotype was extracted and assayed in the same buffers as the resistant biotype throughout all of the experiments. Enzyme extraction procedures were conducted at 4°C. Three grams of leaf tissue were ground in liquid nitrogen with a mortar and pestle and extracted with 15 ml of extraction buffer [100 mM Tris (pH 8.0),

[8] Thin layer chromatography plates, Silica Gel 60 F_{254} TLC Plates, EM Science, 480 Democrat Road, Gibbstown, NJ 08027.

1 mM ethylenediaminetetraacetic acid (EDTA), 10% (v/v) glycerol, 2 mM isoascorbic acid, 0.5% insoluble polyvinylpyrrolidone (PVP), 0.5% (w/v) PVP-40, 20 mM dithiothreitol (DTT), and 0.2 mM phenylmethysulfonyl fluoride (PMSF)]. The homogenate was filtered through two layers of Miracloth and centrifuged at 27,000 *g* for 15 min. The supernatant was subjected to a 40% ammonium sulfate cut, stirred for 30 min, and centrifuged at 27,000 *g* for 30 min. The resulting pellet was resuspended in 1 ml of elution buffer [50 mM Tricine (pH 8.0), 2.5 mM $MgCl_2 \cdot 6H_20$, 50 mM KCl, and 1.0 mM DTT] and desalted on a Sephadex G-25 column previously equilibrated with 5 ml of the same buffer. Partially purified enzyme extracts were immediately assayed for ACCase activity. Protein concentrations in the enzyme extracts were determined by the Bradford method (1976) using Bio-Rad dye reagent and bovine serum albumin (BSA) as a standard.

ACCase activity was quantified by measuring the rate of incorporation of ^{14}C from $NaH^{14}CO_3$ into an acid- and heat-stable product (Stoltenberg et al. 1989). All assays were conducted at 32°C in microcentrifuge tubes. Aliquots of the partially purified enzyme extract were added to an assay buffer [20 mM Tricine-KOH (pH 8.3), 10 mM KCl, 5 mM ATP, 2 mM $MgCl_2$, 0.2 mg (w/v) BSA, 2.5 mM DTT, 3.7 m*M* $NaHCO_3$ (including 0.185 Mbq of $NaH^{14}CO_3$)] and to either sethoxydim, clethodim, or quizalofop at the 0, 0.1, 1, 10, 100, and 1000 μM concentration. Assays were initiated after a 3-min incubation period by the addition of 5 μl acetyl-coA and terminated after 10 min by the addition of 20 μl concentrated HCl. A 110-μl aliquot of solution from each assay was transferred to 2.2-cm filter paper discs in liquid scintillation vials and heated until dry. Ten milliliters of scintillation cocktail were added and radioactivity determined by liquid

scintillation spectrometry. Due to the nature of the results obtained, the specific activity of ACCase from the resistant and susceptible johnsongrass biotypes was calculated and expressed in pmole of carbon fixed per mg of protein per min. Each herbicide was assayed three times with at least two replications per herbicide concentration.

RESULTS AND DISCUSSION

Herbicide Absorption and Translocation. [^{14}C]quizalofop-ethyl absorption was significantly higher in the resistant biotype at 8, 24, and 48 HAT, but by 72 HAT similar levels of radioactivity were detected in both the resistant and susceptible johnsongrass biotypes (Figure 1). At 8 HAT, 45% of the recovered radioactivity was detected in the resistant biotype, while only 30% of the recovered radioactivity was detected in the susceptible biotype. The percent absorption of [^{14}C]quizalofop-ethyl increased steadily at 24 and 48 HAT in both biotypes, but remained significantly higher in the resistant biotype during both evaluations. At 72 HAT, however, there was no significant difference in the levels of [^{14}C]quizalofop-ethyl detected in either biotype. The increased rate of [^{14}C]quizalofop-ethyl absorption detected in the resistant biotype at 8, 24, and 48 HAT may be linked with the proposed mechanism of resistance for this biotype discussed below. However, it is unlikely that the initial increase in the absorption of [^{14}C]quizalofop-ethyl is a mechanism that contributes to resistance in this johnsongrass biotype, since the levels of radioactivity in the two biotypes were statistically similar by 72 HAT. Additionally, decreased herbicide absorption has been proposed as a potential mechanism of resistance in a weed biotype, but it seems clear that the increased level of

herbicide absorption observed in the resistant johnsongrass biotype does not confer any particular advantage to the survival of this biotype following treatment with quizalofop-P or sethoxydim.

There were no significant differences between the resistant and susceptible biotypes in the translocation of [^{14}C]quizalofop-ethyl out of the treated leaf at any sampling time (Table 1). The majority of the absorbed [^{14}C]quizalofop-ethyl remained in the treated leaf at all sampling times in both the resistant and susceptible johnsongrass biotypes, and there was a general trend toward decreased translocation out of the treated leaf with time. Overall there was no consistent trend toward differential translocation in the resistant compared to the susceptible biotype at any sampling time, therefore it is unlikely that decreased herbicide translocation contributes to resistance to quizalofop-P or sethoxydim in the resistant johnsongrass biotype.

Herbicide Metabolism. Based on previously reported Rf values (Koeppe et al. 1990) and also on labeled and nonlabeled quizalofop standards, quizalofop-ethyl (Rf = 0.93), quizalofop acid (Rf = 0.5), and a quizalofop conjugate (Rf = 0-0.19) were identified as the primary metabolites in both biotypes in the [^{14}C]quizalofop-ethyl metabolism experiments (Figure 2). There were no significant differences between the resistant and susceptible biotypes in the percentage of ^{14}C recovered as quizalofop-ethyl at any time interval after application (Figure 2A). At 8 HAT, the amount of ^{14}C recovered as quizalofop-ethyl in the resistant biotype was 43% of the total ^{14}C absorbed, which decreased to 16% by 72 HAT. Similarly, the amount of ^{14}C recovered as quizalofop-ethyl in the susceptible biotype was 38% of the total ^{14}C absorbed, and this decreased to 14% by 72 HAT. These results indicate that the rate of quizalofop-ethyl hydrolysis to

quizalofop acid is similar in the resistant and susceptible johnsongrass biotypes. There were also no significant differences between the resistant and susceptible biotypes in the percentage of ^{14}C recovered as quizalofop acid at 8 and 24 HAT, but by 48 and 72 HAT there was a significantly lower percentage of quizalofop acid recovered in the resistant biotype (Figure 2B). For example, the amount of ^{14}C recovered as quizalofop acid at 48 HAT was 30% of the total ^{14}C absorbed in the resistant biotype and 41% of the total ^{14}C absorbed in the susceptible biotype. Similarly, at 72 HAT, 34 and 44% of the total ^{14}C absorbed was recovered as quizalofop acid in the resistant and susceptible biotypes, respectively. Lastly, there were no significant differences between the resistant and susceptible biotypes in the percentage of ^{14}C recovered as quizalofop conjugates at any time interval after treatment (Figure 2C). The percentages of ^{14}C recovered as quizalofop conjugates increased gradually with time in both biotypes, from 21 to 50% of the total ^{14}C absorbed in the resistant biotype, and from 14 to 42% of the total ^{14}C absorbed in the susceptible biotype.

Collectively, the results from the metabolism experiments reveal a slightly higher rate of quizalofop acid metabolism in the resistant johnsongrass biotype at 48 and 72 HAT, but no qualitative differences between the metabolites formed in either biotype. These differences are relatively minor, however, when compared to other weed biotypes that are resistant due to enhanced metabolism of the APP and/or CHD herbicides. For example, in the chlortoluron- [*N*-(3-chloro-4-methylphenyl)-*N*,*N*-dimethylurea] and diclofop-methyl-resistant *Alopecurus myosuroides* biotype characterized by Menendez and DePrado (1996), approximately twice as much ^{14}C was recovered as a diclofop conjugate, and approximately three times less ^{14}C was recovered as diclofop acid when compared to

the susceptible biotype at 72 HAT. Therefore, it seems unlikely that the relatively small difference observed in these experiments contributes any appreciable amount of tolerance to quizalofop-P or sethoxydim in the resistant johnsongrass biotype.

ACCase Extraction and Assay. As illustrated in Figure 3, ACCase from the resistant and the susceptible johnsongrass biotypes was equally sensitive to quizalofop-P, clethodim, and sethoxydim. In the resistant biotype, the concentration of quizalofop-P, clethodim, and sethoxydim that provided 50% inhibition of ACCase (I_{50}) was 1.8, 2.9, and 4 μM respectively. Similarly, the I_{50}s for the susceptible ACCase were 1.8 μM for quizalofop-P, 2.8 μM for clethodim, and 4.2 μM for sethoxydim. In the absence of ACCase-inhibiting herbicides, however, there was a significantly higher level of ACCase specific activity in the resistant compared to the susceptible johnsongrass biotype (Figure 4). The specific activity of ACCase in the resistant biotype ranged from 145.8 to 282.6 pmol mg^{-1} min^{-1}, while specific activity of ACCase in the susceptible biotype ranged from 71.4 to 94.9 pmol mg^{-1} min^{-1}. These figures indicate that the specific activity of ACCase in the resistant biotype is approximately two to three times greater than that found in the susceptible biotype in the absence of ACCase-inhibiting herbicides. ACCase specific activity in the resistant biotype was also significantly greater than that of the susceptible biotype in the presence of all concentrations of quizalofop-P and sethoxydim evaluated, and in the presence of 0.1, 1, and 10 μM clethodim (Figure 4). Approximately three times as much ACCase specific activity was found in the resistant compared to the susceptible biotype in the presence of 0.1 and 1 μM quizalofop-P, and approximately twice as much ACCase specific activity was found in the resistant biotype in the presence of 0.1, 1, 10, and 100 μM sethoxydim and 0.1 and 1 μM clethodim.

However, the specific activities of ACCase from the resistant and susceptible biotypes in 100 and 1,000 μM clethodim were not significantly different, which may be correlated with the sensitivity of the resistant biotype to standard field use-rates of clethodim. This ability of clethodim to control weed biotypes that are otherwise resistant to APP and CHD herbicides has also been reported for biotypes of *Setaria faberi* (Stoltenberg and Wiederholt 1995), *Digitaria sanguinalis* (Hidayat and Preston 1997), and *Sorghum halepense* (Smeda et al. 1997), and also for sethoxydim-resistant corn (*Zea mays* L.) hybrids (VanGessel et al. 1997). Additionally, in recent evaluations of ACCase isozymes from sethoxydim-resistant and susceptible corn hybrids, Incledon and Hall (1999) determined that the extrachloroplastic ACCase isozyme (ACCase 220) was inhibited by clethodim in both resistant and susceptible corn hybrids, but was not inhibited by either sethoxydim or tralkoxydim in the resistant corn hybrid. These results suggest an additional binding site and/or site of action of clethodim. This hypothesis is supported by the results of our studies, where the resistant biotype was completely controlled by standard field use rates of clethodim despite the overproduction of ACCase that was detected in the presence of 0.1, 1, and 10 μM clethodim.

Collectively, these results suggest that an overproduction of ACCase is the mechanism that confers low-levels of resistance to the CHD herbicide sethoxydim and the APP herbicide quizalofop-P in the resistant johnsongrass biotype. As discussed above, the nearly identical I_{50} values of the resistant and susceptible ACCase indicates that both biotypes possess ACCases that are equally sensitive to inhibition by quizalofop-P, clethodim, and sethoxydim. However, due to the two- to three-fold higher level of ACCase specific activity detected in the resistant johnsongrass, this biotype is

presumably able to sustain a level of malonyl-coA production necessary for survival following treatment with these herbicides. The increased rate of [^{14}C]quizalofop-ethyl absorption discussed above also seems to support this proposed mechanism of resistance. For example, the resistant johnsongrass may be able to absorb the herbicide at a faster rate due to the increased number of ACCase binding sites available in the resistant compared to the susceptible biotype. Similarly, the relatively low tolerance of this biotype to the ACCase-inhibiting herbicides observed in the dose-response trials lends additional support to this proposed mechanism of resistance. For example, the resistant johnsongrass biotype was found to be 2.5 and 2.8 times more tolerant of quizalofop-P and sethoxydim than the susceptible biotype in greenhouse dose-response experiments. Additionally, the resistant biotype was as sensitive as the susceptible biotype to clethodim both in field experiments and in the dose-response trials. These levels of resistance correlate well with the two- to three-fold overproduction of ACCase observed in the assays, and suggest that increasing concentrations of quizalofop-P, clethodim, or sethoxydim at the site of action will result in the binding and inhibition of all available ACCase, ultimately resulting in the control of the resistant johnsongrass biotype. This is much different from the levels of resistance that are most commonly observed in weed biotypes that possess an insensitive ACCase as the mechanism of resistance. In the majority of these biotypes, extremely high levels of resistance are often observed due to the inability of the herbicide to bind the target enzyme, regardless of the herbicide concentration at the site of action.

To date, an overproduction of ACCase has not been reported as a mechanism of resistance in any naturally-occurring weed biotype that is resistant to the APP and/or

CHD herbicides. However, target enzyme overproduction has been identified as the mechanism conferring herbicide tolerance in a number of plant cell cultures subject to stepwise selection in the presence of herbicides. For example, *Daucus carota* (Caretto et al. 1994), *Nicotiana tabacum* (Harms et al. 1992; Odell et al. 1990), and *Cichorium intybus* (Dewaele et al. 1997) cell cultures have been identified that contain amplified levels of the enzyme acetolactate synthase (ALS). Similarly, stepwise selection with glyphosate and glufosinate led to the identification of resistant cell cultures of *Daucus carota* and *Medicago sativa* with amplified levels of the enzymes 5-enolpyruvylshikimate-3-phosphate synthase and glutamine synthetase, respectively. Lastly, cell cultures of corn with amplified levels of ACCase were also identified during the initial search for a sethoxydim-tolerant corn hybrid (Parker et al. 1990). In these experiments, Western blots revealed an increased level of ACCase protein in the cell cultures with amplified ACCase specific activity, confirming overproduction of ACCase as the mechanism of resistance in the sethoxydim-resistant cell cultures. This represents a potential area of future research with the resistant johnsongrass biotype identified in these experiments. Although the results from these experiments have revealed an amplified level of ACCase specific activity in the resistant johnsongrass biotype, further Western blots of resistant ACCase extracts will be necessary to confirm overproduction of ACCase as the mechanism of resistance to quizalofop-P, clethodim, and sethoxydim.

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Table 1. Translocation of [^{14}C]Quizalofop-P from the treated leaf in resistant and susceptible johnsongrass biotypes at 8, 24, 48, and 72 hours after treatment (HAT).

	Percentage of [^{14}C]quizalofop-ethyl absorbed[a]					
	Resistant biotype			Susceptible biotype		
Time (HAT)	Treated leaf	Shoots	Roots	Treated leaf	Shoots	Roots
8	88.4 ± 1.5	6.3 ± 1.1	5.3 ± 0.7	88.1 ± 1.5	4.8 ± 1.3	7.1 ± 0.9
24	90.5 ± 1.4	4.8 ± 1.1	4.8 ± 0.6	92.6 ± 0.5	3.3 ± 0.2	4.1 ± 0.4
48	92.7 ± 0.5	2.8 ± 0.2	4.4 ± 0.4	91.3 ± 0.6	3.6 ± 0.4	5.1 ± 0.4
72	92.8 ± 0.3	2.9 ± 0.1	4.3 ± 0.2	91.5 ± 0.4	3.1 ± 0.1	5.4 ± 0.3

[a] Values represent means ± standard errors.

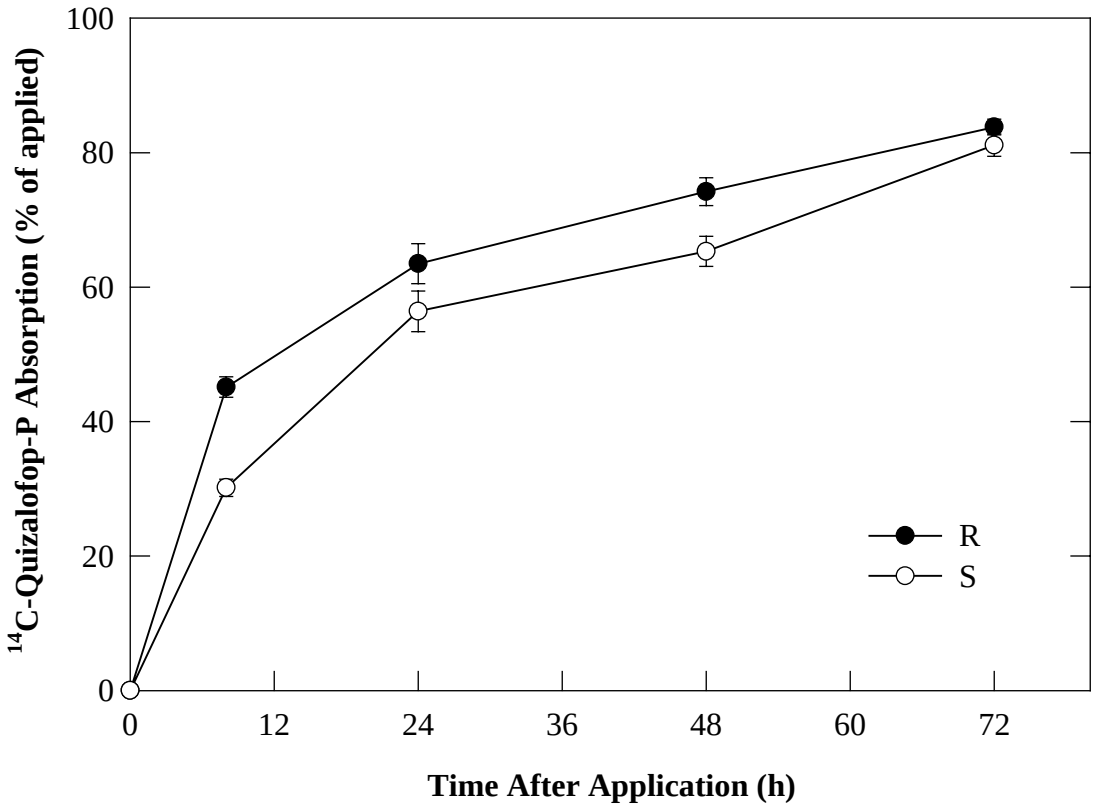

Figure 1. Absorption of [^{14}C]quizalofop-ethyl in resistant (R) and susceptible (S) biotypes of *Sorghum halepense* at 8, 24, 48, and 72 hours after treatment. Error bars represent the standard error of the mean.

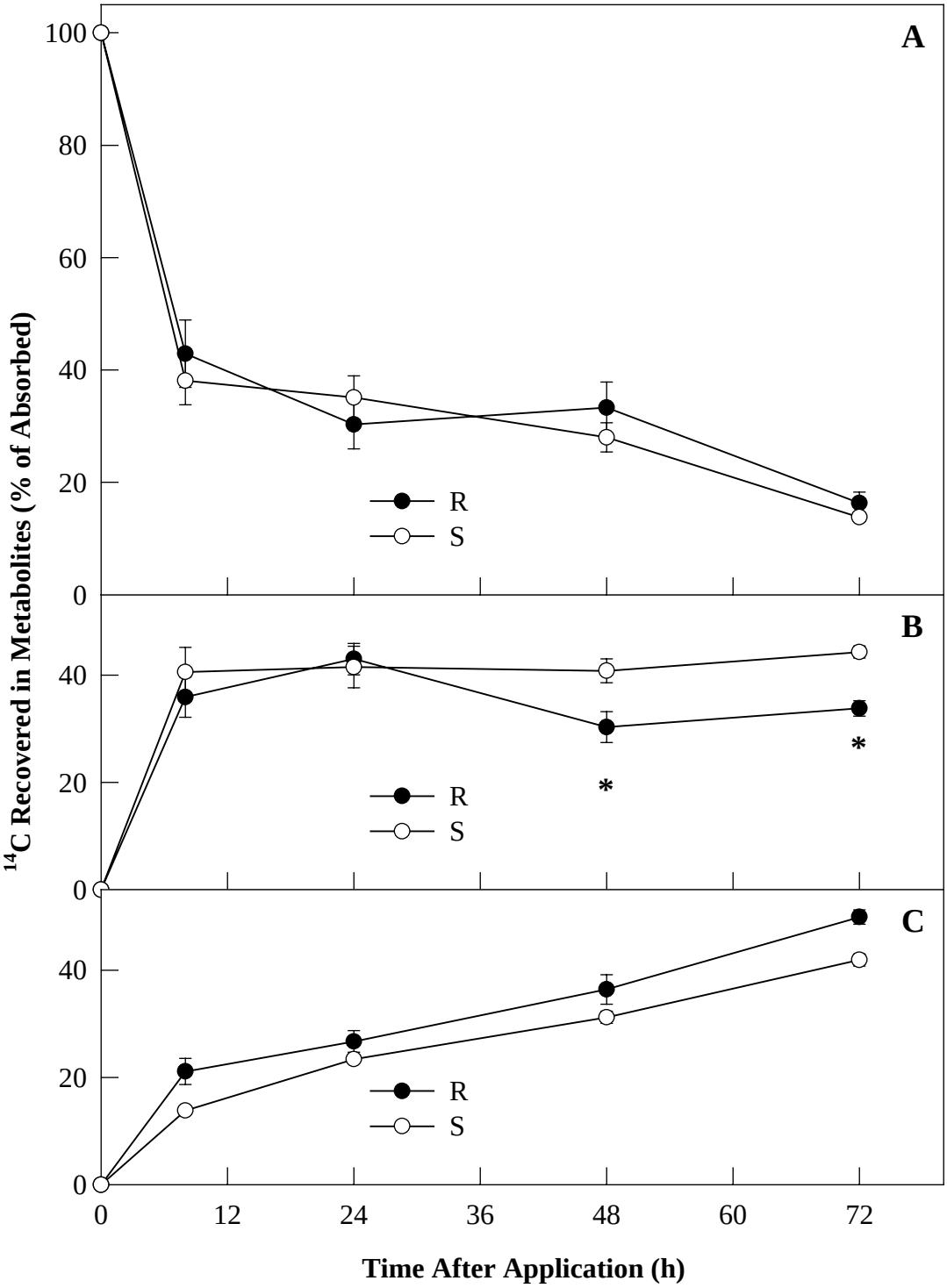

Figure 2. Metabolism of [^{14}C]Quizalofop-ethyl in resistant (R) and susceptible (S) biotypes of *Sorghum halepense*. Percentages of absorbed ^{14}C as quizalofop-ethyl (A), quizalofop acid (B), and quizalofop conjugates (C) are illustrated. Error bars represent the standard error of the mean (n = 6). Asterisk indicates significant difference between the biotypes at the 0.05 significance level.

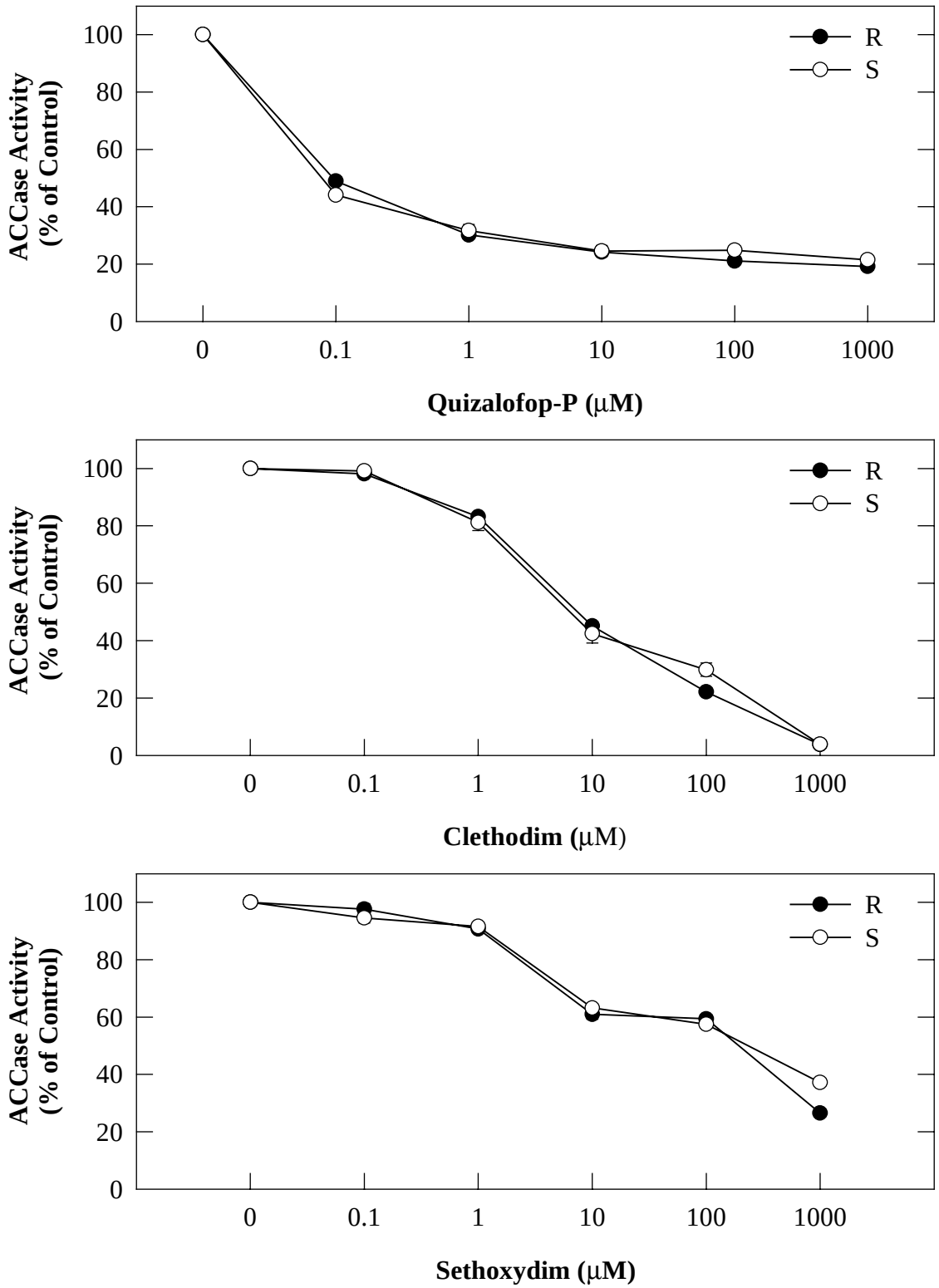

Figure 3. Inhibition of ACCase activity from resistant (R) and susceptible (S) biotypes of *Sorghum halepense* by quizalofop-P, clethodim, and sethoxydim. Error bars represent the standard error of the mean.

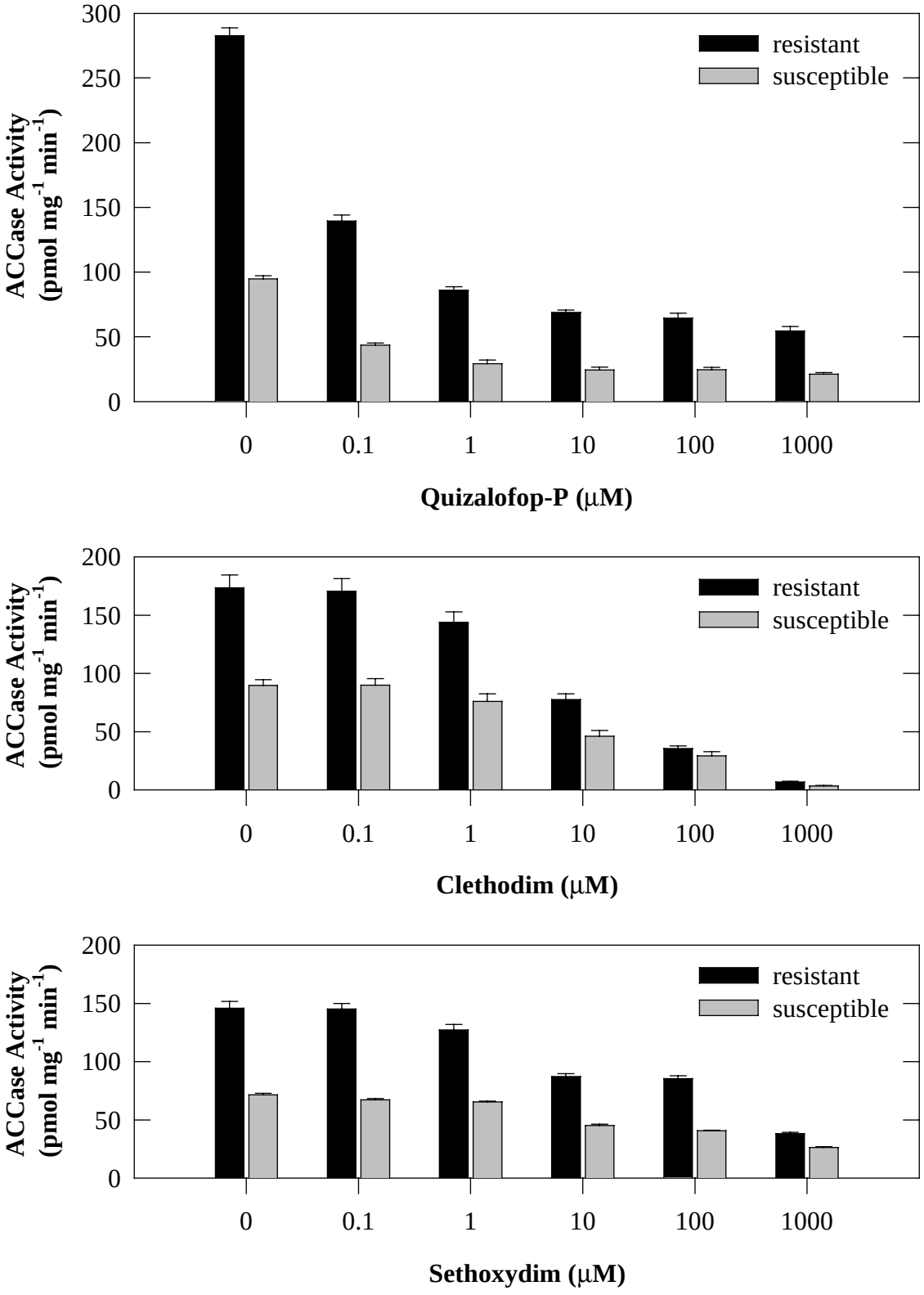

Figure 4. ACCase activity (pmol mg^{-1} min^{-1}) of resistant (R) and susceptible (S) biotypes of *Sorghum halepense* in the presence of quizalofop-P, clethodim, and sethoxydim. Error bars represent the standard error of the mean.

Chapter IV

Influence of Herbicide Rate, Sequential Herbicide Treatment, and Mowing Regimes on Mugwort (*Artemisia vulgaris*) Control

Abstract: Three field studies were conducted in Virginia during 1998 and repeated in 1999 to evaluate the effectiveness of several weed control strategies for the control of mugwort. In the first field trial, a logarithmic backpack sprayer was utilized to evaluate a range of rates of the herbicides dicamba, triclopyr, clopyralid, picloram, metsulfuron, glufosinate, glyphosate, pelargonic acid, and the dimethylamine salt and the isooctyl ester of 2, 4-D. Mugwort control at 4 mo after treatment during both years was 100% with all rates of picloram evaluated and greater than 85% with all rates of clopyralid. Glyphosate and dicamba provided the next highest level of mugwort control during both years when applied at the rates of 4.4 to 8.9 kg ai/ha. However, less than 65% mugwort control was achieved in either year with the remainder of the herbicides evaluated in these experiments. All of these herbicides, except pelargonic acid, were also applied to mugwort at 7-wk intervals in the second field trial to evaluate mugwort control following one, two, and three sequential herbicide applications. All herbicides provided relatively good mugwort control when applied in three sequential applications, but only one application of picloram was required to provide 100% mugwort control in either year. Additionally, 98 to 99% mugwort control was achieved with two sequential applications of clopyralid, and these levels of control were not significantly different from those achieved with three sequential clopyralid applications in either year. Similarly, two sequential application of dicamba and glyphosate provided good to excellent mugwort

control in both years. In the third field trial, the effect of the timing of herbicide application and the effect of mowing followed by herbicide application on mugwort control were evaluated. Generally, there was no significant difference in the level of mugwort control achieved with applications of these herbicides to mugwort in the flowering vs. the vegetative stage of growth. However, when averaged over all of the herbicides included in these trials, two sequential mowings conducted prior to herbicide application significantly enhanced the control of mugwort compared to either unmowed mugwort or mugwort that had been mowed once prior to herbicide application. Collectively, the results from all three field trials indicate that picloram and clopyralid will provide essentially complete mugwort control at relatively low use rates, and also that sequential herbicide treatment and sequential mowing are effective mugwort control strategies that will significantly improve the control of mugwort when combined with the majority of herbicides included in these trials.

Nomenclature: 2, 4-D dimethylamine salt, (2, 4-dichlorophenoxy) acetic acid dimethylamine salt; 2, 4-D isooctyl ester, (2, 4-dichlorophenoxy) acetic acid isooctyl ester; clopyralid, 3, 6-dichloro-2 pyridinecarboxylic acid; dicamba, 3, 6-dichloro-2-methoxybenzoic acid; glufosinate, 2-amino-4-(hydroxymethylphosphinyl)butanoic acid; glyphosate, *N*-(phosphonomethyl)glycine; metsulfuron, methyl 2-[[[[(4-methoxy-6-methyl-1, 3, 5-triazin-2-yl)amino]carbonyl]amino]sulfonyl]benzoate; picloram, 4-amino-3, 5, 6-trichloro-2-pyridinecarboxylic acid; pelargonic acid, nonanoic acid; triclopyr, [(3, 5, 6-trichloro-2-pyridinyl)oxy]acetic acid; mugwort, *Artemisia vul*garis L. #[1] ARTVU.

[1] Letters following this symbol are a WSSA-approved computer code from *Composite List of Weeds*, Revised 1989. Available from WSSA, 810 East 10th Street, Lawrence KS 66044-8897.

Additional index words: Application timing, cultural control, growth stage, logarithmic sprayer, mowing, perennial weed control, regrowth, rhizome, sequential herbicide treatment, growth regulator herbicides, nonselective herbicides.

Abbreviations: MAT, months after treatment; WAT, weeks after treatment.

INTRODUCTION AND LITERATURE REVIEW

Mugwort is a perennial weed that spreads primarily through rhizomes and is capable of rapidly colonizing a variety of different environments (Rogerson and Bingham 1971). Rogerson (1964) observed that a single mugwort plant is capable of producing 23 m of rhizomes in a mere 4-mo time period. Mugwort may also produce as many as 200,000 seeds per plant, but seed viability varies considerably across the world and appears to be dependent on climate (Pawlowski et al. 1968). In the eastern United States, few viable seeds are produced (Holm et al. 1997; Uva et al. 1997), while in other parts of the world the majority of mugwort seeds produced during the growing season will germinate in the following spring (Dorph-Petersen 1925). Worldwide, mugwort has been reported in 25 crops and has been observed in 56 countries (Holm et al. 1997), illustrating its ability to adapt to a wide variety of soils and climates. For example, mugwort has been reported from the extremely cold climates of Siberia and the northern Himalayas to the much warmer environments of Argentina and Hawaii (Holm et al. 1997).

Mugwort was introduced into the United States from Europe and Asia (Bailey 1930), and is one of the 10 most problematic weeds of nurseries in the eastern United States (Holm et al. 1997). The spread of mugwort throughout the eastern United States has

frequently been attributed to the nursery stock industry, where mugwort rhizomes are often unintentionally transported in the soil around burlap-bound nursery plants (Holm et al. 1997; Rogerson and Bingham 1971; Uva et al. 1997). In addition to the mugwort infestations observed in many of Virginia's orchards and nurseries, severe mugwort infestations have recently been identified in a number of new settings in Virginia, including flower beds, landscapes, lawns, corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] fields, and most often in hay and pasture fields.

Very little research has been conducted on the chemical control of mugwort, and only a few of the available studies have identified herbicides that will provide acceptable mugwort control. Many of the initial investigations evaluated extremely high rates of the growth regulator herbicides fenac [2, 3, 6-trichlorobenzeneacetic acid], silvex [2-(2, 4, 5-trichlorophenoxy) propanoic acid], 2, 4-D amine, dicamba, and picloram. However, fenac and silvex are no longer commercially available for application in Virginia, and picloram is not currently registered for application to pasture or hay fields in areas east of the Mississippi. In one of the earliest investigations of mugwort control with herbicides, Bingham (1964) observed almost complete control of mugwort rhizomes with applications of fenac at 11.2 kg ai/ha, and 75 and 0% control with applications of 2, 4-D at 9 and 4.5 kg ai/ha, respectively. Other studies have reported similar levels of mugwort control with the lower rates of 2, 4-D (Ahrens 1976; Rabbitt and Cook 1964). Bingham (1964) also observed greater than 90% control of mugwort rhizomes and roots when dicamba was applied at 11.2 kg ai/ha to small mugwort shoots in April. However, this level of control was reduced to approximately 80% when the same rate of dicamba was applied to larger mugwort shoots in May. In a separate investigation conducted by

Rabbitt and Cook (1964), lower application rates of dicamba afforded little to no control of mugwort by the end of the growing season.

Of all the growth regulator herbicides evaluated in these early experiments, perhaps the most promising results were obtained with picloram, which provided essentially complete elimination of mugwort at relatively low use-rates. In the experiments conducted by Bingham (1964), applications of picloram at 0.6, 1.1, 2.2, and 4.5 kg ai/ha in March and at 1.1, 2.2, and 4.5 kg ai/ha in May resulted in complete control of mugwort rhizomes and roots. Additionally, applications of picloram at 0.3 kg ai/ha in either March or May provided greater than 98% mugwort control. This level of mugwort control is more than likely a result of the highly persistent nature of picloram in the soil when compared to the other growth regulator-type herbicides (Ahrens 1994).

In addition to the growth regulator herbicides, several of the initial studies on the chemical control of mugwort included evaluations of the herbicides asulam [methyl [(4-aminophenyl) sulfonyl] carbamate], EPTC [*S*-ethyl dipropyl carbamothioate], amitrole [1 *H*-1, 2, 4-triazol-3-amine], and glyphosate. Both Rabbitt and Cook (1964) and Bing and Pridham (1963) observed regrowth of mugwort following applications of amitrole. This type of response is similar to that observed in many of the growth regulator herbicide evaluations, and suggests that herbicides which only provide initial mugwort suppression do not successfully control mugwort rhizomes. This hypothesis was initially investigated with mugwort in a translocation study involving applications of ^{14}C-amitrole to either mature leaves of primary plants, or to young leaves of secondary plants (Rogerson and Bingham 1971). In this study, Rogerson and Bingham determined that there was little translocation of ^{14}C-amitrole from primary to secondary plants, or from secondary to

primary plants, and that a large majority of the absorbed ^{14}C-amitrole remained in the treated leaf at 48 h after treatment. Therefore, insufficient concentrations of the herbicide were translocated to mugwort rhizomes, ultimately resulting in inadequate control and mugwort regrowth.

In one of the few studies conducted on the control of mugwort with glyphosate, Ahrens (1976) observed less than 80% mugwort control at the rates of 1.7, 3.4, and 5 kg ai/ha. In addition to this study, Ahrens (1976) evaluated the possibility of mugwort eradication with three sequential glyphosate applications of 0.6 kg ai/ha, but observed only 70% control of mugwort with these sequential glyphosate applications by the end of the growing season. In this same study, sequential applications of glyphosate at 1.1 kg ai ha provided only 85% control of mugwort at the end of the growing season.

Similar sequential herbicide treatment strategies have been adopted in attempts to control other troublesome perennial weeds. For example, in a recent investigation of perennial weed control in glyphosate resistant cotton (*Gossypium hirsutum* L.), Keeling et al. (1998) observed season-long suppression of the perennial weeds silverleaf nightshade (*Solanum elaeagnifolium* Cav.), Texas blueweed (*Helianthus ciliaris* DC.), and woollyleaf bursage [*Ambrosia grayi* (A. Nels.) Shinners] with three sequential applications of glyphosate at 0.8 kg ai/ha. Other researchers have observed effective control of the perennial weeds horsenettle (*Solanum carolinense* L.,) honeyvine milkweed [*Ampelamus albidus* (Nutt.) Britt], and hogpotato [*Hoffmanseggia glauca* (Ortega) Eifer], with two or more sequential annual herbicide applications (Gorrell et al. 1981; Moshier et al. 1986; Westerman et al. 1993).

The most recent study involving the chemical control of mugwort was conducted by Day et al. (1997). In this study, the herbicides clopyralid [3, 6-dichloro-2-pyridinecarboxylic acid], flumetsulam [N- (2, 6-difluorophenyl)-5-methyl [1, 2, 4] triazolo [1, 5-α] pyrimidine-2-sulfonamide], and 2, 4-D were applied either alone or in combination and evaluated for their ability to control mugwort in corn. Day et al. (1997) observed approximately 70 and 85% mugwort control with applications of clopyralid at 0.15 and 0.30 kg ai/ha, respectively, and determined that neither the addition of flumetsulam nor 2, 4-D to clopyralid treatments significantly enhanced mugwort control compared to the control achieved with clopyralid alone.

In addition to the use of herbicides as a method of perennial weed control, many studies have investigated the effects of mowing followed by herbicide application, sequential mowing, and/or sequential mowing followed by herbicide application as a method of perennial weed control. One of the most recent examples of a troublesome perennial weed that is effectively controlled with either mowing alone or mowing followed by herbicide applications is tropical soda apple (*Solanum viarum* Dunal.). Mislevy et al. (1999) observed 92% control of tropical soda apple with just three sequential mowings and no herbicide applications, and 100% control of tropical soda apple with two sequential mowings followed by an application of triclopyr at 0.6 kg ai/ha. All of these mowing strategies have been adopted in an attempt to achieve higher levels of weed control through the depletion of carbohydrates in the underground rootstocks of perennial weeds. In their investigations of tropical soda apple, Mislevy et al. (1999) observed a 68% decrease in the total nonstructural carbohydrates in tropical soda apple crowns that were sequentially mowed three times at 60-d intervals. They also

reported a significant reduction in the crown weights of tropical soda plants that were mowed three times compared to unmowed plants. Similar reductions in the weight of mugwort rhizomes have been reported for mowed mugwort plants (Bingham 1964). For example, Bingham (1964) found that the weight of mugwort rhizomes from mowed plants was reduced by 65% compared to the weight of rhizomes from unmowed plants. In addition, generally higher levels of mugwort control were observed with fenac applications of 2.8, 5.6, and 11.2 kg ai ha in mowed compared to unmowed mugwort plots. Other perennial weeds that have responded favorably to sequential mowings and/or sequential mowings followed by herbicide applications include dogfennel [*Eupatorium capillifolium* (Lam.) Small], yellow starthistle (*Centaurea solstitialis* L.) and cogongrass [*Imperata cylindrica* (L.) Beauv.] (Macdonald et al. 1994; Thomsen et al. 1994; Willard et al. 1996).

Another strategy often employed in the management of perennial weeds is the application of herbicides at growth stages when maximum basipetal transport of carbohydrates is occurring. If this strategy is used correctly, more effective control of perennial weeds should be achieved due to the concurrent flow of herbicides and carbohydrates toward perennating rootstocks. Many studies have quantified the seasonal fluctuations of carbohydrates in perennial weed rootstocks in an effort to predict the growth stage and/or time of year when weeds are most susceptible to control measures (Becker and Fawcett 1998; Cyr et al. 1990; Hogg and Lieffers 1991; Katovich et al. 1998; Mullahey and Cornell 1994; Potter et al. 1986; Tworkoski 1992). To date, however, no information is available on seasonal carbohydrate fluctuations in mugwort

rhizomes, although Rogerson et al. (1972) did conduct some initial evaluations on the carbohydrate levels in mugwort rhizomes following applications of fenac.

In addition to the quantification of seasonal carbohydrate fluctuations in perennial weeds, many researchers have evaluated perennial weed control at several different herbicide application timings to determine the optimum growth stage for herbicide application. For example, Canada thistle control with clopyralid was greater when applied to plants in the pre-bud stage compared to plants treated in the full bud to early bloom stage (Bixler et al. 1991). Similar differences in weed control due to application timing have been observed with horsenettle, purple nutsedge (*Cyperus rotundus* L.), quackgrass [*Elytrigia repens* (L.) Beauv.], hemp dogbane (*Apocynum cannabinum* L.), johnsongrass [*Sorghum halepense* (L.) Pers.], and redvine [*Brunnichia ovata* (Walt.) Shinners], and a variety of other perennial weeds (Banks et al. 1977; Edenfield et al. 1998; Mitra and Bhowmik 1999; Orfanedes and Wax 1991; Shaw et al. 1990; Shaw and Mack 1991). Again, however, few studies have sufficiently evaluated the effect of herbicide application timing on mugwort control. For example, Bingham (1964) observed statistically similar levels of mugwort control with applications of picloram in March and May, but no additional timings were evaluated in the study. Additionally, Day et al. (1997) observed the highest level of mugwort control when plants were 20 to 25 cm in height, but the only other timings evaluated in the study were those typically used in corn production.

As a result of the rapid growth and colonizing ability of mugwort, this weed has the potential to continue it's spread into a variety of cultivated and non-cultivated areas throughout Virginia and the southeastern United States. Field trials were conducted on

severe mugwort infestations discovered in several Virginia counties during 1998 and 1999 and were based on a variety of control strategies that have been successfully utilized in the management of other troublesome perennial weeds. The effects of sequential herbicide treatment, herbicide application timing, mowing followed by herbicide application, and sequential mowing followed by herbicide application were evaluated in these field trials for their ability to provide mugwort control. A variety of growth regulator and non-selective herbicides, in addition to the sulfonylurea herbicide metsulfuron, were evaluated in combination with these strategies to determine the most effective herbicides and methods of mugwort control.

MATERIALS AND METHODS

Logarithmic Sprayer Trial. Field trials were established in Westmoreland County, VA during 1998 and in Nelson County, VA during 1999 on farms containing extensive natural infestations of mugwort. The soil at the Westmoreland County site was a Leaf silt loam (clayey, mixed, thermic Typic Abaquults), while that at the Nelson County site was a Lew clay loam (loamy-skeletal, siliceous, mesic Glossic Fragiudults). Severe mugwort infestations were initially observed at both locations, with mugwort covering approximately 90 to 100% of the experimental area in the Westmoreland field trial, and 80 to 90% of the experimental area in the Nelson field trial. All herbicide treatments were applied at a constant speed of 5 km/h with a CO_2-powered logarithmic backpack sprayer[2] set to deliver 336 L/ha. This logarithmic backpack sprayer continuously dilutes

[2] R & D Sprayers, Inc., 790 E. Natchez Boulevard, Opelousas, LA 70570.

a particular herbicide as it is being applied, and in this manner makes it possible to evaluate weed control with a wide range of herbicide rates. In both trials, the isooctyl ester of 2, 4-D, the dimethylamine salt of 2,4-D and dicamba, the monoethanolamine salt of clopyralid, the monoammonium salt of glufosinate, the isopropylamine salt of glyphosate, the potassium salt of picloram, and the butoxyethyl ester of triclopyr were applied logarithmically from 8.9 to 0.28 kg/ha. Additionally, metsulfuron was applied logarithmically from 0.063 to 0.002 kg/ha, and glufosinate, glyphosate, and 2, 4-D amine were applied at a constant rate of 2.2 kg ai/ha in combination with a logarithmic rate (v/v) of pelargonic acid, from 20 to 0.625%. Herbicide treatments were applied to mugwort that averaged 37.5 cm in height in 1998, and 40 cm in 1999, which was in the vegetative stage of growth during both years. At each location, individual plots were 1.8 m wide by 24.4 m long, and all herbicide treatments were arranged in a randomized complete block design with three replications. In both locations, visual ratings of mugwort control were taken throughout the growing season at 2-wk intervals. These ratings were based on a scale of 0 to 100, with 0 equal to the mugwort vigor and ground cover observed in the untreated control plots, and 100 equal to complete mugwort control. Data were subjected to analysis of variance, and means were separated using Duncan's new multiple range test at the 5% level. Due to significant treatment-by-year interactions, the results from each year are presented separately.

Sequential Herbicide Trial. In 1998, a sequential herbicide trial was established in Scott County, VA on a Hagerstown silty clay loam (fine, mixed, mesic, Typic, Hapludalfs), and this trial was repeated in Nelson County, VA on a Lew clay loam

loamy-skeletal, siliceous, mesic Glossic Fragiudults) during 1999. At the time of the first herbicide applications, mugwort covered approximately 80% of the experimental area at the Scott County site, and approximately 85% of the experimental area at the Nelson County site. In both trials, 2, 4-D amine, 2, 4-D ester, clopyralid, dicamba, glufosinate, glyphosate, picloram, and triclopyr were each applied once, twice, or three times sequentially at 7-wk intervals. The application rates for these herbicides are shown in Tables 1 and 2. All herbicide treatments were applied at a constant speed of 5 km/h with a hand-held CO_2-powered backpack sprayer set to deliver 210 L/ha. All treatments were arranged in a randomized complete block design with four replications, and the individual plots were 1.8 m wide by 3.7 m long in 1998 and 1.8 m wide by 4.6 m long in 1999. In 1998, the first herbicide applications were made on June 8 to mugwort that averaged 6.4 cm in height, while in 1999, the first herbicide applications were made on June 15 to mugwort that averaged 7.6 cm in height. Visual estimates of mugwort control with one, two, or three applications of the same herbicide were made throughout the growing season, and these ratings were based on a scale of 0 to 100, as discussed previously. Data were subjected to a factorial analysis of variance and means were separated with an LSD at the 5% level. Due to significant treatment-by-year interactions, the results from each year are presented separately.

Sequential Mowing Trial. A sequential mowing trial was established on a Ross silt loam (fine-loamy, mixed, mesic cumulic Hapludolls) in Craig County, VA in 1998 and repeated in Nelson County, VA during 1999 on a Lew clay loam (loamy-skeletal, siliceous, mesic Glossic Fragiudults). Mugwort covered approximately 95 to 99% of the experimental area at the Craig County site, and 90 to 95% of the experimental area at the

Nelson County site. The experimental design was a split plot with herbicide treatments as whole plots and the number of mowings prior to herbicide application as subplots. Whole plots were completely randomized with four replications. Individual plots were 1.8 m wide by 1.8 m long in the 1998 field trials, and 1.8 m wide by 3 m long in the 1999 field trials. In both years, half of each plot was mowed with a Troy-Bilt® sickle bar mower[3] either once or twice sequentially at 5-wk intervals and the other half of each plot was left to grow undisturbed. Each herbicide treatment was applied to an entire plot, consisting of both mowed and unmowed mugwort, and these applications occurred approximately 5 wk after each mowing. From this experimental design, two separate strategies for controlling mugwort were evaluated. First, in the mowed portion of each plot, the effect of applying herbicides to either unmowed mugwort, or to mugwort regrowth following one or two sequential mowings was evaluated. Second, in the unmowed portion of each plot, the effect of applying herbicides to mugwort in the vegetative stage of growth was compared to the effect of applying herbicides to mugwort in the flowering stage of growth. The herbicides and rates applied in both trials were identical to those evaluated in the sequential herbicide trials, and all herbicide treatments were applied at a constant speed of 5 km/h with a CO_2-powered backpack sprayer set to deliver 210 L/ha. At the time of the first herbicide application timing in 1998, mugwort regrowth from the initial mowing averaged 31 cm in height, while the unmowed mugwort averaged 71 cm in height and was in the vegetative stage of growth. In 1999, mugwort regrowth from the initial mowing averaged 8 cm in height, while the unmowed mugwort averaged 74 cm in height and was also in the vegetative stage of growth. At the time of the second herbicide application timing, the average height of mugwort regrowth from

[3] Troy-Bilt Manufacturing Co., 102nd St. and 9th Ave., Troy, NY 12180.

the sequential mowing was 15.2 cm in 1998, and 6.4 cm in 1999. At this same timing, the unmowed mugwort was in the flowering stage of growth during both years, and averaged 94 cm in 1998 and 122 cm in 1999. Visual ratings of mugwort control were made throughout the growing season, and these ratings were based on the scale discussed previously. All data were subjected to analysis of variance to test for individual factor effects and interactions, and means were separated with an LSD at the 5% level. There was not a significant treatment-by-year interaction with the sequential mowing trials, therefore the results are presented as an average of both years.

RESULTS AND DISCUSSION

Logarithmic Sprayer Trial. During both years, all rates of picloram provided 100% control of mugwort at four months after treatment (Figures 1 and 2). Although picloram is not currently registered in Virginia on grass pastures or hayfields, these results indicate that complete eradication of mugwort plants and rhizomes from heavily infested areas may be achieved with relatively low picloram application rates. Clopyralid also provided 100% control of mugwort at all rates in 1998, and at the 1.1, 2.2, 4.4, and 8.9 kg/ha rate in 1999 (Figures 1 and 2). Additionally, in 1999 the 0.28 kg and 0.56 kg clopyralid rates provided 87 and 95% mugwort control, respectively. These results reveal that complete eradication of mugwort plants and rhizomes can also be achieved with clopyralid, which is currently registered for use on grass pastures and hayfields in Virginia.

Of the remaining growth regulator herbicides evaluated in these experiments, dicamba provided the next highest level of mugwort control (Figures 1 and 2). In 1998, only the

8.9 kg dicamba rate provided 90% mugwort control while in 1999 both the 8.9 and 4.4 kg dicamba rates provided 90% mugwort control. The more commonly used dicamba rates of 0.28, 0.56, and 1.1 kg/ha provided 62, 68, and 71% mugwort control in 1998, respectively, and 53, 58, and 62% mugwort control in 1999. These results indicate that relatively good mugwort suppression may be achieved with the 0.28, 0.56, or 1.1 kg dicamba rate, but regrowth from mugwort rhizomes should be expected during the subsequent growing season. Additionally, the 4.4 and 8.9 kg dicamba rates will provide relatively good control of mugwort at 4 mo after treatment, but complete mugwort eradication will more than likely not be achieved with a single application of any dicamba rates evaluated.

In both the Westmoreland County and Nelson County field trials, a gradual increase in mugwort control was observed with each corresponding increase in the rate of triclopyr (Figures 1 and 2). In 1998, mugwort control ranged from 20% at the 0.28 kg/ha rate, to 50% at the 8.9 kg/ha rate. Similarly, mugwort control ranged from 28% at the 0.28 kg/ha rate to 62% at the 8.9 kg/ha rate in 1999. Therefore, the results from both years indicate that that the higher triclopyr rates may provide a moderate level of mugwort suppression, but the lower rates of 0.28, 0.56, 1.1, and 2.2 kg/ha provide very little control of mugwort at 4 mo after treatment.

During both years, there was no significant difference in the level of mugwort control achieved with either 2, 4-D amine or 2, 4-D ester when evaluated at equivalent rates. Mugwort control ranged from 20 to 42% with all rates of 2, 4-D amine in both years, and also with all rates of 2, 4-D ester in 1998. Similarly, mugwort control with 2, 4-D ester ranged from 0 to 30% in 1999 (Figures 1 and 2). These results indicate that neither

formulation of 2, 4-D provides an acceptable level of mugwort control at 4 mo after treatment, even at the rates of 4.4 and 8.9 kg/ha.

Some of the lowest levels of mugwort control were observed with metsulfuron during both years (Figures 3 and 4). In 1998, mugwort control with 0.002 to 0.063 kg metsulfuron ranged from 3 to 13%, and in 1999 mugwort control ranged from 0 to 27%. These results clearly indicate that metsulfuron should not be selected as a method of either suppressing or controlling mugwort when applied in a single application at the rates evaluated in these logarithmic sprayer trials.

In 1998, all rates of glyphosate provided good to excellent (75 to 98%) control of mugwort, but in 1999 the 0.28, 0.56, and 1.1 kg glyphosate rates provided much lower levels (30 to 60%) of mugwort control (Figures 5 and 6). This reduction in mugwort control may be partially explained by the drought encountered at the Nelson County experimental site during 1999. However, in both 1998 and 1999, the 4.4 and 8.9 kg glyphosate rates provided essentially complete control of mugwort at 4 mo after treatment. Additionally, the 2.2 kg glyphosate rate afforded 98% mugwort control in 1998, and 88% mugwort control in 1999. Collectively, these results indicate that the 2.2, 4.4, and 8.9 kg glyphosate rates are capable of providing levels of mugwort control that are statistically similar to the control afforded by either picloram or clopyralid.

Variable levels of mugwort control were also observed with glufosinate during 1998 and 1999. For example, in 1998, mugwort control with the 0.28 to 8.9 kg glufosinate rates ranged from 22 to 43%, while in 1999 mugwort control ranged from 0 to 7%. Once again, the reduced level of control observed in 1999 may be partially explained by the drought that occurred at the Nelson County experimental site. Nevertheless, the control

afforded by glufosinate in 1998 alone indicates that this herbicide provides very little control of mugwort at any of the rates evaluated. During both years, glufosinate applications provided essentially complete control of existing mugwort vegetation at one week after treatment (data not shown), but extensive mugwort regrowth from underground rhizomes was observed in both trials by one month after treatment.

The addition of a logarithmic range of pelargonic acid rates to a constant rate of 2.2 kg glyphosate did not significantly enhance the control of mugwort compared to applications of 2.2 kg glyphosate alone (Figures 7 and 8). As mentioned previously, the application of 2.2 kg glyphosate alone provided 98% control of mugwort in 1998 (Figure 5). During this same year, however, the highest level of mugwort control observed with the pelargonic acid plus glyphosate treatment combination was 78%, and this was achieved with the lowest rate of pelargonic acid included in these experiments (Figure 7). Similarly, the 2.2 kg glyphosate rate provided 88% mugwort control in 1999 (Figure 6), and the highest level of mugwort control observed with the pelargonic acid plus glyphosate combination was 58%, and this was achieved with the 1.25% v/v pelargonic acid rate. Therefore, the overall response observed during both years was a gradual increase in mugwort control with decreasing rates of pelargonic acid. This suggests that pelargonic acid is antagonistic to glyphosate and consequently should not be included with glyphosate treatments as a method to enhance mugwort control. Although the precise mechanism of antagonism was not investigated in these experiments, severe wilting and darkening of treated mugwort plants was observed within an hour after treatment in both years, especially with higher application rates. These symptoms are consistent with the primary mechanism of action of pelargonic acid, which is rapid cell

death through a decline in the intracellular pH and loss of membrane integrity (Hatzios 1998). Therefore, these higher pelargonic acid application rates may have disrupted the uptake and/or translocation of glyphosate, which would explain the gradual increase in mugwort control observed with the lower pelargonic acid rates.

As with glyphosate, the addition of a logarithmic range of pelargonic acid rates to a constant rate of 2.2 kg glufosinate did not significantly enhance the control of mugwort compared to applications of glufosinate alone (Figures 7 and 8). Unlike the response observed with glyphosate, however, a slight increase in mugwort control was observed during both years when increasing rates of pelargonic acid were added to the constant rate of glufosinate. Since the level of mugwort control achieved with pelargonic acid plus glufosinate was not significantly different from the mugwort control achieved with glufosinate alone, it seems likely that this response may almost entirely be attributed to a greater degree of leaf tissue desiccation with higher pelargonic acid application rates.

The addition of a logarithmic range of pelargonic acid rates to a constant rate of 2.2 kg 2, 4-D amine did not significantly enhance the control of mugwort at any pelargonic acid rate during 1998, but did significantly enhance mugwort control at the 2.5% v/v pelargonic acid rate during 1999 (Figures 7 and 8). Mugwort control with the pelargonic acid plus 2, 4-D amine combination was extremely variable during 1999, making it difficult to draw any definitive conclusions pertaining to the effects of pelargonic acid on mugwort control with 2, 4-D amine. However, 57% mugwort control was achieved when pelargonic acid was applied at the 2.5% v/v rate in combination with 2.2 kg 2, 4-D amine during 1999, and this level of control was significantly better than the 32% mugwort control afforded by 2.2 kg of 2, 4-D amine alone. In 1998, a gradual increase in mugwort

control was also observed with increasing pelargonic acid rates, from the 0.625% to the 5% rate. However, the levels of control provided by these combinations were not significantly better than the control afforded by 2.2 kg of 2, 4-D amine alone. Collectively, the results from both years suggest that pelargonic acid at the rates of 2.5 to 5% v/v may enhance the uptake and translocation of 2, 4-D amine. This synergistic activity of pelargonic acid has been reported with other systemic postemergence herbicides (Hatzios 1998), but additional research will be required to determine the specific interaction that exists between pelargonic acid and 2, 4-D amine, and the value of applying these herbicides in combination on perennial weeds like mugwort.

Sequential Herbicide Trial. Picloram provided 100% mugwort control during both years when applied in a single application of 1.1 kg/ha, and no significant effects of sequential applications of this herbicide were observed (Tables 1 and 2). Additionally, picloram was the only herbicide that provided complete mugwort control with just one application in either year.

As observed in the logarithmic sprayer trial, the next highest level of mugwort control was achieved with clopyralid. In both years, two sequential applications of 0.28 kg clopyralid provided a significantly higher level of mugwort control than one clopyralid application, but three sequential clopyralid applications did not provide a significantly higher level of mugwort control than two. Two sequential applications of clopyralid provided 99% control of mugwort in 1998, and 98% control of mugwort in 1999, indicating that essentially complete mugwort eradication can also be achieved with two sequential clopyralid applications.

In 1998, only two sequential applications of 2.2 kg dicamba were required to provide 100% mugwort control, while in 1999 each additional dicamba application resulted in a significantly higher level of mugwort control (Tables 1 and 2). Similarly, each sequential application of 2.2 kg triclopyr provided a significantly higher level of mugwort control during both years. However, the highest level of mugwort control achieved with three sequential triclopyr applications was 94% in 1998, and 64% in 1994. These results indicate that relatively good mugwort control can be achieved with triclopyr when applied in three sequential applications, but equivalent or higher levels of mugwort control can be achieved with dicamba when applied in only two sequential applications.

During 1998, two sequential applications of 4.5 kg 2, 4-D amine provided 90% mugwort control, and this level of mugwort control was not significantly different from that provided by three sequential 2, 4-D amine applications during the same year (Tables 1 and 2). In 1999, however, mugwort control decreased with the second sequential 2, 4-D amine application, and then significantly increased with the third sequential 2, 4-D amine application. This phenomenon was also observed with 2, 4-D ester and glyphosate in the 1999 trial, and most likely is a result of the extremely dry conditions encountered at the time of the second herbicide application at the Nelson County site. Mugwort control with two sequential applications of 4.5 kg 2, 4-D ester was statistically similar to that provided by two sequential applications of 4.5 kg 2, 4-D amine in 1998, but unlike 2, 4-D amine, three sequential applications of 2, 4-D ester provided a significantly higher level of mugwort control than two sequential 2, 4-D ester applications. Nevertheless, mugwort control was statistically similar with 2, 4-D amine and 2, 4-D ester when applied in three sequential applications during both years, and the minimum level of

control achieved in either year with three sequential applications of these herbicides was 90%. Although the results from the second herbicide applications in 1999 make it difficult to draw any definitive conclusions pertaining to either 2, 4-D amine or 2, 4-D ester, it seems clear that both herbicides are capable of providing excellent mugwort control when applied in three sequential applications of 4.5 kg/ha

During 1998, an application error was made at the time of the first metsulfuron application that prevented further observations pertaining to this treatment in this year. In 1999, however, each sequential metsulfuron application provided a significantly higher level of mugwort control, but the highest level of mugwort control achieved with three sequential metsulfuron applications during this year was 84% (Table 2). This level of mugwort control was relatively low compared to that observed with three sequential applications of the other herbicides evaluated in this experiment. These results indicate that metsulfuron will provide unsatisfactory mugwort control, regardless of the number of sequential metsulfuron applications.

The level of mugwort control achieved with one application of 4.5 kg glyphosate was 83% in 1998 and 85% in 1999. During both years, a single application of 4.5 kg glyphosate provided one of the highest levels of mugwort control when compared to a single application of all of the other herbicides evaluated in the experiment. For example, in 1998 the level of mugwort control achieved with one glyphosate application was statistically similar to that provided by a single clopyralid application, and in 1999 a single glyphosate application provided a significantly higher level of mugwort control than a single clopyralid application. However, one application of glyphosate still did not provide the level of control afforded by a single application of picloram in either year. In

1998, a second sequential glyphosate application did not significantly enhance mugwort control compared to one glyphosate application alone, but as mentioned previously, a decline in mugwort control was observed with the second glyphosate application in 1999. Once again, this makes it difficult to draw any definitive conclusions pertaining to the second sequential application of glyphosate. However, the results from 1998 alone seem to indicate that the level of mugwort control achieved with two sequential applications of glyphosate will be slightly higher, although not significantly different from, the level of mugwort control achieved with a single glyphosate application. Alternatively, three sequential glyphosate applications provided a significantly higher level of mugwort control than one glyphosate application in both years. This suggests that each sequential glyphosate application should provide minor increases in mugwort control until essentially complete mugwort control is achieved.

The effect of each sequential herbicide application was perhaps most obvious with glufosinate. In both years, each sequential application of 1.7 kg glufosinate provided a significantly higher level of mugwort control (Tables 1 and 2). In 1998, mugwort control was 31, 74, and 100% with one, two, and three glufosinate applications, respectively. Similarly, mugwort control was 40, 54, and 95% with one, two, and three glufosinate applications during 1999. These results indicate that mugwort regrowth will likely occur with either one or two sequential glufosinate applications, but also that excellent mugwort control can be achieved when glufosinate is applied in three sequential applications. Therefore, the sequential herbicide treatment strategy was probably most successfully demonstrated with glufosinate, which provided little to no control of mugwort when applied in a single application at any rate in the logarithmic sprayer trial.

One of the most significant conclusions that can be drawn from both sequential herbicide trials is that excellent mugwort control may be achieved with three sequential applications of almost any of the herbicides included in these experiments. In 1998, all of the herbicides evaluated provided a statistically similar level of mugwort control when applied in three sequential applications, and these levels of mugwort control ranged from 94 to 100%. In 1999, mugwort control was also statistically similar with three sequential applications of 2, 4-D amine, 2, 4-D ester, dicamba, clopyralid, picloram, glufosinate, and glyphosate. This suggests that a similar level of control will be achieved with three sequential applications of any of the herbicides evaluated in these experiments, except for metsulfuron and possibly triclopyr. Therefore, the question of which herbicides eliminate mugwort most effectively and/or economically should be answered with the herbicides that provide the highest level of mugwort control when applied in either one or two applications. Obviously, the most effective control of mugwort with only one herbicide application was achieved with picloram. However, as mentioned previously, picloram is not currently registered for use in Virginia. Relatively good, although significantly lower, levels of mugwort control were also achieved with a single application of either clopyralid or glyphosate, but complete mugwort control is more likely to occur with two sequential applications of these herbicides. Additionally, the results from both years suggest that two sequential applications of dicamba will also provide essentially complete mugwort control. Lastly, with the exception of metsulfuron and possibly triclopyr, the remainder of the herbicides evaluated in these experiments are capable of providing excellent mugwort control, but three sequential applications of these herbicides will be required.

Sequential Mowing Trial. Table 3 provides an average of the results from both years pertaining to the effect of the timing of herbicide application on mugwort control at six weeks after treatment (WAT). As in the logarithmic sprayer and sequential herbicide trials, 100% mugwort control was achieved when picloram was applied in the vegetative stage of growth, but this level of control decreased slightly, although not significantly, when picloram was applied to mugwort in the flowering stage of growth. The average level of mugwort control afforded by an application of 0.28 kg clopyralid was much lower than that observed in either the logarithmic sprayer or sequential herbicide trials, but again there was no significant difference between applications of clopyralid to vegetative vs. flowering stage mugwort. Similarly, there was no significant difference in the level of mugwort control achieved at 6 WAT when 2, 4-D amine, 2, 4-D ester, or dicamba was applied to mugwort in the vegetative vs. the flowering stage of growth. However, there was a significant difference in the level of mugwort control achieved at these application timings with triclopyr, metsulfuron, and glyphosate, but no consistent trend in mugwort control with any one particular application timing was observed. For example, both triclopyr and glyphosate provided a significantly higher level of mugwort control when applied in the vegetative stage of growth, but metsulfuron provided a significantly higher level of mugwort control when applied in the flowering stage of growth.

In an effort to determine the overall effect of applying herbicides to mugwort in the vegetative vs. the flowering stage of growth, the average level of mugwort control with all of the herbicides evaluated in these experiments is presented in Table 4 for each application stage and each year. In 1998, an average of 60% mugwort control was

achieved when herbicides were applied to mugwort in the vegetative stage of growth, while an average of 59% mugwort control was achieved when these herbicides were applied to mugwort in the flowering stage of growth. Similarly, an average of 54% mugwort control was achieved in 1999 when these herbicides were applied to mugwort in both the vegetative and the flowering stage of growth. These results suggest that, in general, there will not be a significant difference in the level of control achieved when herbicides are applied to mugwort in the vegetative or the flowering stage of growth.

As explained previously, another component of the sequential mowing trials was an evaluation of the effect of mowing through the application of herbicides to unmowed mugwort, or to mugwort regrowth following either one or two sequential mowings. These results are presented in Table 5 as an average of the mugwort control observed at 2 months after treatment (MAT) during both years. Once again, 1.1 kg picloram provided 100% mugwort control at each application, and in this case there was no significant effect of mowing. Similarly, relatively good mugwort control was achieved with all glyphosate applications, but there was no significant difference in the level of control observed with applications of 4.5 kg glyphosate to unmowed mugwort, mugwort that had been mowed once, or mugwort that had been mowed twice.

With clopyralid, dicamba, metsulfuron, and glufosinate, however, there was a significant effect of two sequential mowings (Table 5). For example, applications of 0.28 kg clopyralid to unmowed mugwort, mugwort that had been mowed once, and mugwort that had been mowed twice provided 43, 30, and 76% mugwort control, respectively. Therefore, two sequential mowings significantly enhanced the control of mugwort with clopyralid compared to either one mowing or to unmowed mugwort. Similarly,

applications of 2.2 kg dicamba and 1.7 kg glufosinate to mugwort that had been mowed twice provided 87 and 74% mugwort control, respectively, and these levels of control were significantly higher than the control achieved when these herbicides were applied to either unmowed mugwort or mugwort that had been mowed once. Two sequential mowings also significantly enhanced the control of mugwort with 0.01 kg metsulfuron, but as in the logarithmic sprayer and sequential herbicide trials, the overall level of mugwort control was relatively poor with any metsulfuron treatment.

One of the unexpected responses observed in this trial was the significantly higher level of control achieved with all of these herbicides, except for picloram and glyphosate, when applied to unmowed mugwort compared to mugwort that had been mowed only once (Table 5). This response could be a result of increased herbicide uptake due to the greater size and leaf area of the unmowed mugwort at the time of the herbicide applications compared to the size and leaf area of the mugwort that had received one mowing. For example, the average height of the unmowed mugwort was 72.5 cm at the time of herbicide application, while the average height of the mugwort that had been mowed once was 19 cm. Another possible explanation for this response is a reduction in the translocation of these herbicides to the rhizomes of mugwort that had been mowed once compared to the translocation of herbicides in unmowed mugwort. This reduced translocation could be a result of the upward flow of herbicides into mugwort shoots at the time of these herbicide applications. This upward flow of carbohydrates could be due to the mobilization of carbohydrate reserves in mugwort rhizomes either in response to the first mowing, or more simply due to a seasonal carbohydrate fluctuation at the time of these herbicide applications. As discussed previously, few studies have sufficiently

investigated the translocation of herbicides in mugwort, and currently no study has determined the seasonal carbohydrate fluctuations in mugwort rhizomes. Therefore, future research should be conducted to determine the potential relationship between optimum herbicide application timing and seasonal carbohydrate fluctuations in mugwort.

There was no significant difference in the level of mugwort control afforded by either 2, 4-D amine or 2, 4-D ester at any application timing, and two sequential mowings provided a significantly higher level of mugwort control than one mowing alone with both of these herbicides (Table 5). Unlike dicamba, clopyralid, metsulfuron, and glufosinate, however, the level of mugwort control achieved with either 2, 4-D amine or 2, 4-D ester when these herbicides were applied to mugwort that had been mowed twice was not significantly different from that observed when these herbicides were applied to unmowed mugwort. A similar response was observed with 2.2 kg triclopyr, which provided 46, 38, and 49% control when applied to unmowed mugwort, mugwort that had been mowed once, and mugwort that had been mowed twice, respectively.

These results with 2, 4-D amine, 2, 4-D ester, and triclopyr make it difficult to draw any absolute conclusions pertaining to the effects of mowing on mugwort control. However, an average of 59, 48, and 66% mugwort control was achieved with the herbicides evaluated in these experiments when applied to unmowed mugwort, mugwort that had been mowed once, and mugwort that had been mowed twice, respectively (Table 6). These results reveal a general trend toward increased mugwort control with sequential mowings. As with other perennial weeds like tropical soda apple (Mislevy et al. 1999), this response is more than likely due to the depletion of carbohydrates in the

perennating rootstock with each sequential mowing. However, additional research involving the quantification of carbohydrate levels in mowed and unmowed mugwort rhizomes is needed to confirm this conclusion. The collective results presented in Table 6 also reveal a slight decline in the level of mugwort control observed with these herbicides when applied to mugwort mowed once compared to unmowed mugwort. As explained previously, this response may be due to the greater size and leaf area of the unmowed mugwort compared to the mugwort that had been mowed once, or may also be explained by a greater degree of herbicide translocation towards the mugwort rhizomes in unmowed compared to mowed plants. In either case, the generally higher level of mugwort control observed with two mowings compared to one mowing alone could be explained by a gradual depletion of the carbohydrates in mugwort rhizomes with each sequential mowing. This response has been observed with tropical soda apple, where each sequential mowing provided a significantly lower percentage of total nonstructural carbohydrates in the crowns of these plants, and at the same time a significantly higher level of control of this weed (Mislevy et al. 1999). These results indicate that mowing severe mugwort infestations at least two times sequentially prior to the application of a herbicide can be an effective method of enhancing mugwort control. Additionally, these results suggest that mowing mugwort infestations more than two times sequentially prior to herbicide application may provide even higher levels of mugwort control with the herbicides evaluated in these experiments.

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Table 1. Mugwort control following three sequential herbicide treatments applied at 7-wk intervals in Scott County, VA during 1998.

Herbicide	Rate	Mugwort control[a]		
		One application	Two applications	Three applications
	kg/ha		%	
2, 4-D amine	4.5	49	90	100
2, 4-D ester	4.5	28	88	100
Dicamba	2.2	39	100	100
Triclopyr	2.2	36	82	94
Clopyralid	0.28	85	99	100
Picloram	1.1	100	100	100
Glufosinate	1.7	31	74	100
Glyphosate	4.5	83	92	100
Untreated	----	0	0	0
LSD (0.05) between herbicide treatments within an application (columns):				14
LSD (0.05) between applications within an herbicide treatment (rows):				10

[a] Represents visual control ratings taken 4.5 mo after the first herbicide treatment.

Table 2. Mugwort control following three sequential herbicide treatments applied at 7-wk intervals in Nelson County, VA during 1999.

Herbicide	Rate	Mugwort control [a]		
		One application	Two applications	Three applications
	kg/ha		%	
2, 4-D amine	4.5	51	44	90
2, 4-D ester	4.5	70	25	95
Dicamba	2.2	60	80	95
Triclopyr	2.2	16	53	64
Clopyralid	0.28	75	98	96
Picloram	1.1	100	100	100
Metsulfuron[b]	0.01	24	48	84
Glufosinate	1.7	40	54	95
Glyphosate	4.5	85	76	96
Untreated	----	0	0	0
LSD (0.05) between herbicide treatments within an application (columns):				7
LSD (0.05) between applications within an herbicide treatment (rows):				4

[a] Represents visual control ratings taken 4.5 mo after the first herbicide treatment.

[b] Applied with X-77 Spreader ® (non-ionic surfactan) at 0.25% v/v.

Table 3. Influence of the timing of herbicide application on mugwort control 6 wk after treatment (6 WAT) during 1998 and 1999.

Herbicide	Rate	Herbicide application timing	
		Vegetative[a]	Flowering[b]
	kg/ha	Visual control ratings (%)	
2, 4-D amine	4.5	71	75
2, 4-D ester	4.5	82	78
Dicamba	2.2	54	57
Triclopyr	2.2	33	41
Clopyralid	0.28	41	46
Picloram	1.1	100	98
Metsulfuron[c]	0.01	26	5
Glufosinate	1.7	73	79
Glyphosate	4.5	92	85
Untreated	----	0	0
LSD (0.05) between herbicides within an application timing (columns):		8	
LSD (0.05) between application timings within an herbicide (rows):		6	

[a] Indicates herbicide applications to mugwort 53 cm in height and in the vegetative stage of growth.
[b] Indicates herbicide applications to mugwort 94 cm in height and in the early bloom stage of growth.
[c] Applied with X-77 Spreader ® (non-ionic surfactant) at 0.25% v/v.

Table 4. Influence of the timing of herbicide application on mugwort control 6 wk after treatment (6 WAT) when averaged over all herbicides during 1998 and 1999.

	Herbicide application timing	
Year	Vegetative[a]	Flowering[b]
	Visual control ratings (%)	
1998	60	59
1999	54	54
LSD (0.05) between years (columns):	6	
LSD (0.05) between application timings within a year (rows):	6	

[a] Indicates herbicide applications to vegetative-stage mugwort 53 cm in height.
[b] Indicates herbicide applications to early-bloom-stage mugwort 94 cm in height.

Table 5. Influence of mowing regime and herbicides on mugwort control 2 mo after treatment (2 MAT) during 1998 and 1999.

Herbicide	Rate	Mowing regime		
		0 Mowings	1 Mowing[a]	2 Mowings[b]
	kg/ha	Visual control ratings (%)		
2, 4-D amine	4.5	75	55	73
2, 4-D ester	4.5	79	52	77
Dicamba	2.2	73	64	87
Triclopyr	2.2	46	38	49
Clopyralid	0.28	43	30	76
Picloram	1.1	100	100	100
Metsulfuron[c]	0.01	22	11	29
Glufosinate	1.7	65	48	74
Glyphosate	4.5	87	87	90
Untreated	----	0	0	0
LSD (0.05) between herbicides within a mowing treatment			6	
LSD (0.05) between mowing treatments within an herbicide (rows):			3	

[a] Indicates one preherbicide mowing conducted five weeks before herbicide application.

[b] Indicates two sequential preherbicide mowings conducted five weeks before herbicide application.

[c] Applied with X-77 Spreader ® (non-ionic surfactant) at 0.25% v/v.

Table 6. Influence of mowing regime and herbicides on mugwort control 2 mo after treatment (2 MAT) when averaged over all herbicides and years.

	Mowing regime		
Yearly average	No mowings	One mowing [a]	Two mowings [b]
	Visual control ratings (%)		
All herbicides	59	48	66
LSD (0.05) between mowing treatments:		3	

[a] Indicates one preherbicide mowing conducted 5 wk before herbicide application.

[b] Indicates two sequential preherbicide mowings conducted 5 wk before herbicide application.

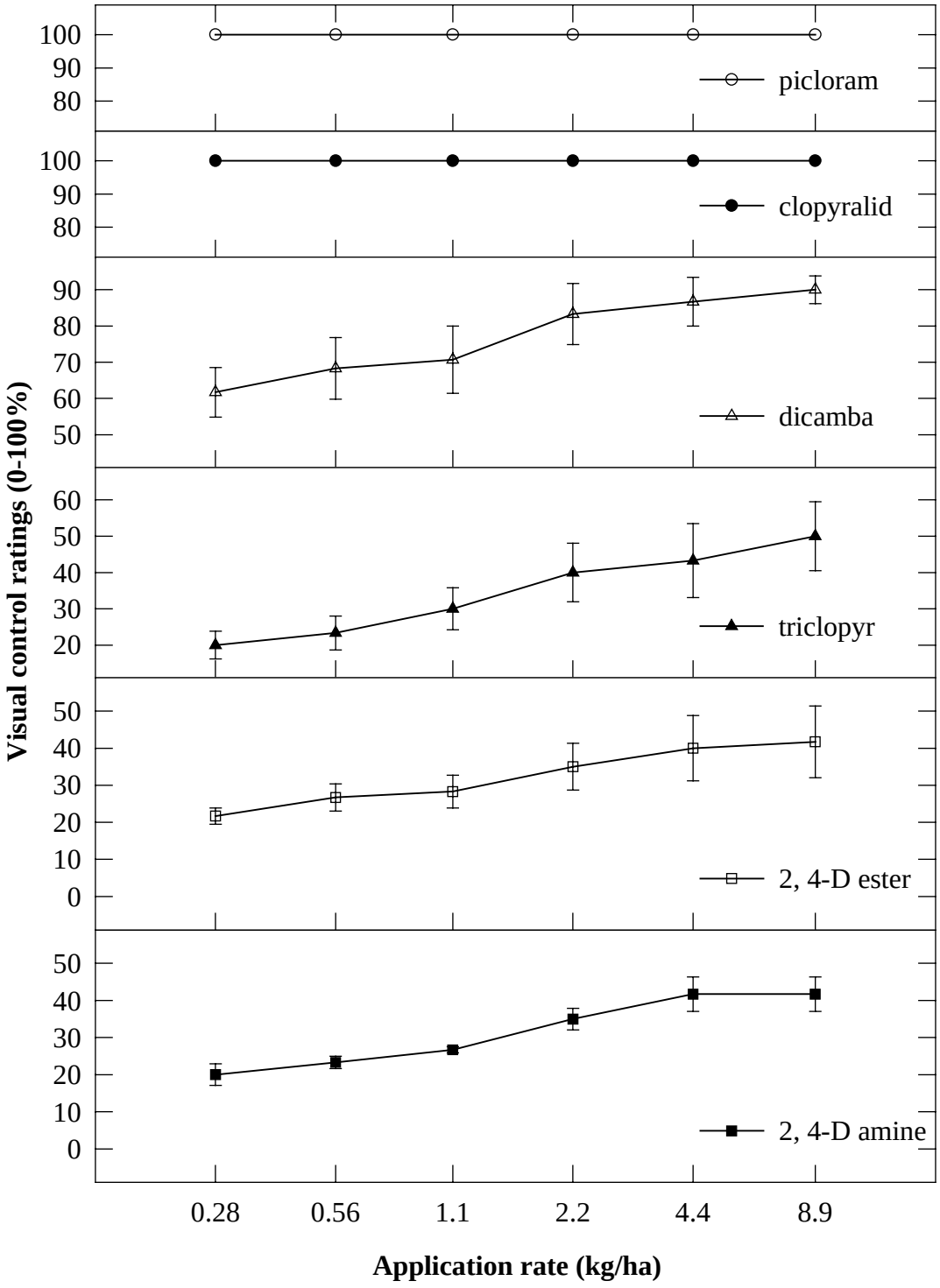

Figure 1. Mugwort control 4 mo after treatment with a logarithmic range of application rates of picloram, clopyralid, dicamba, triclopyr, and the isooctyl ester and the dimethylamine salt of 2, 4-D during 1998. Bars represent the standard error of the mean, P = 0.05.

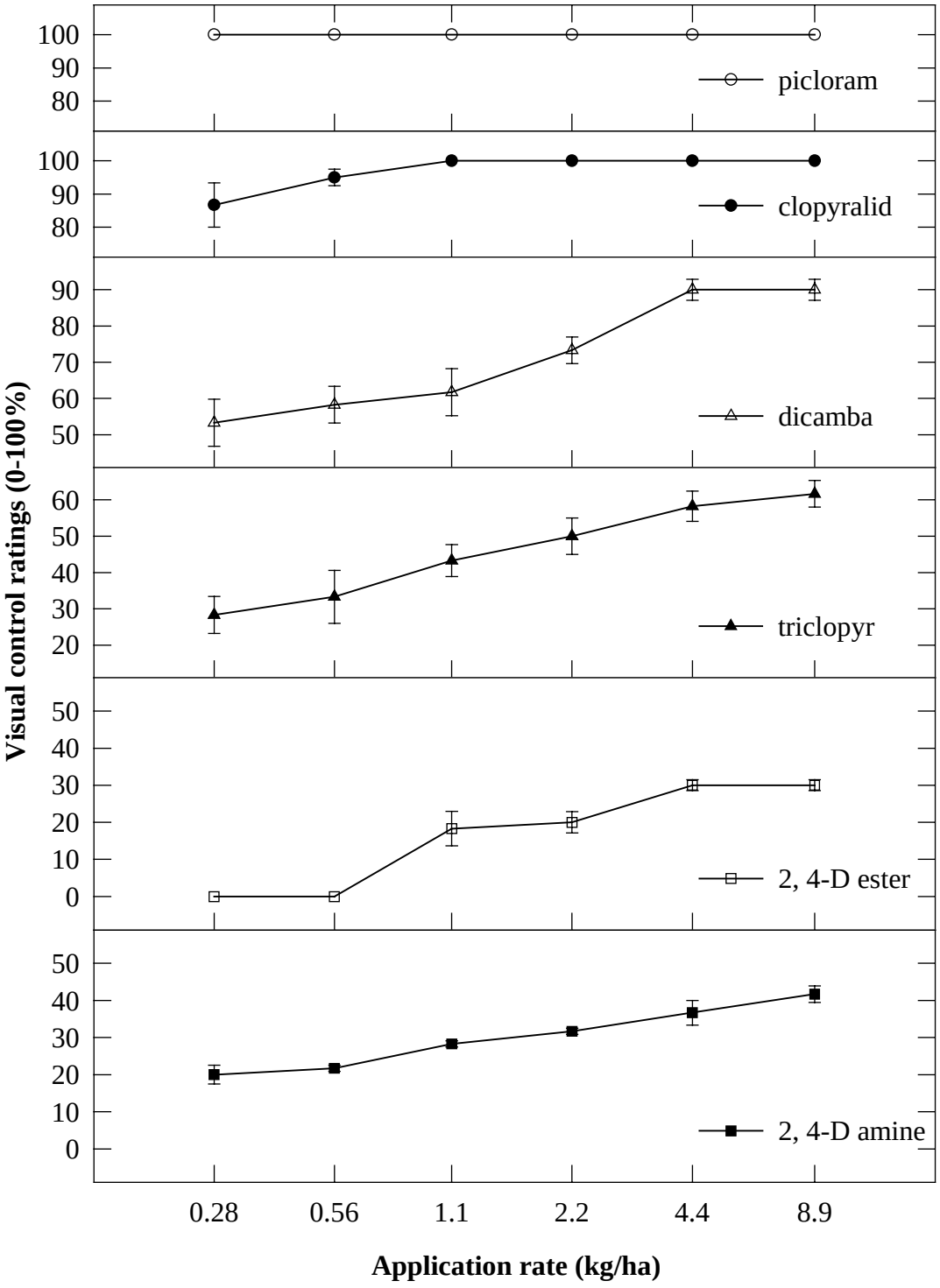

Figure 2. Mugwort control 4 mo after treatment with a logarithmic range of application rates of picloram, clopyralid, dicamba, triclopyr, and the isooctyl ester and the dimethylamine salt of 2, 4-D during 1999. Bars represent the standard error of the mean, P = 0.05.

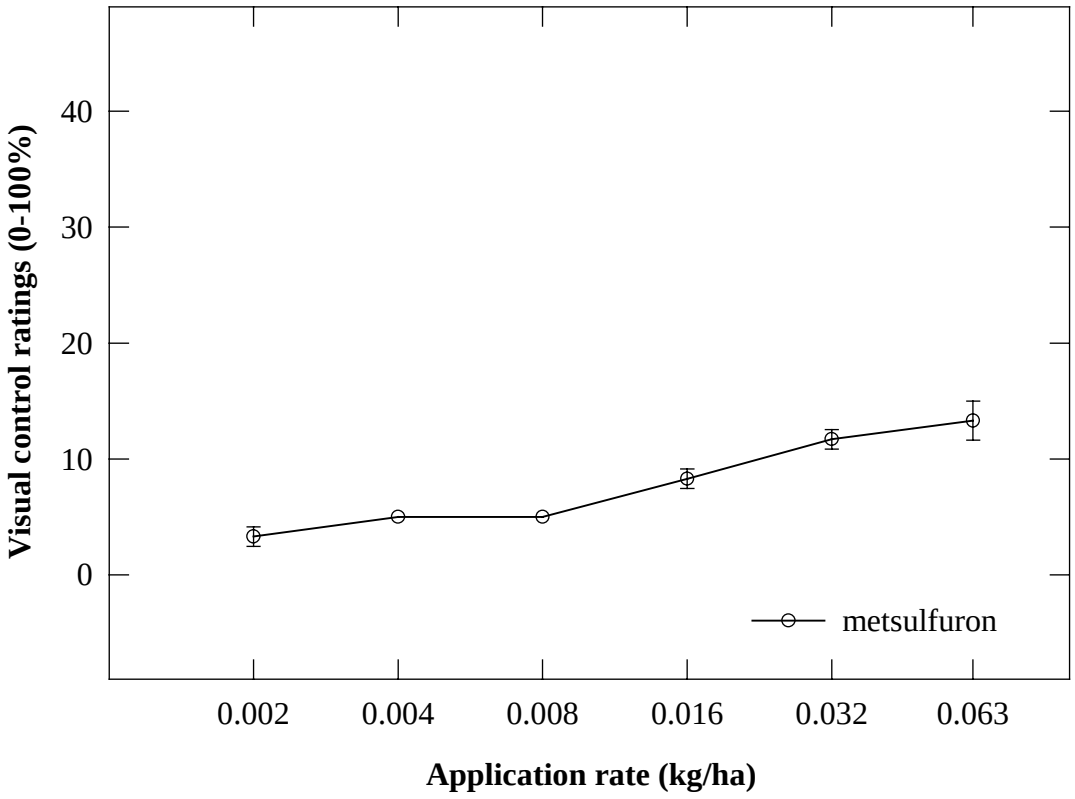

Figure 3. Mugwort control 4 mo after treatment with a logarithmic range of metsulfuron application rates during 1998. Bars represent the standard error of the mean, P = 0.05.

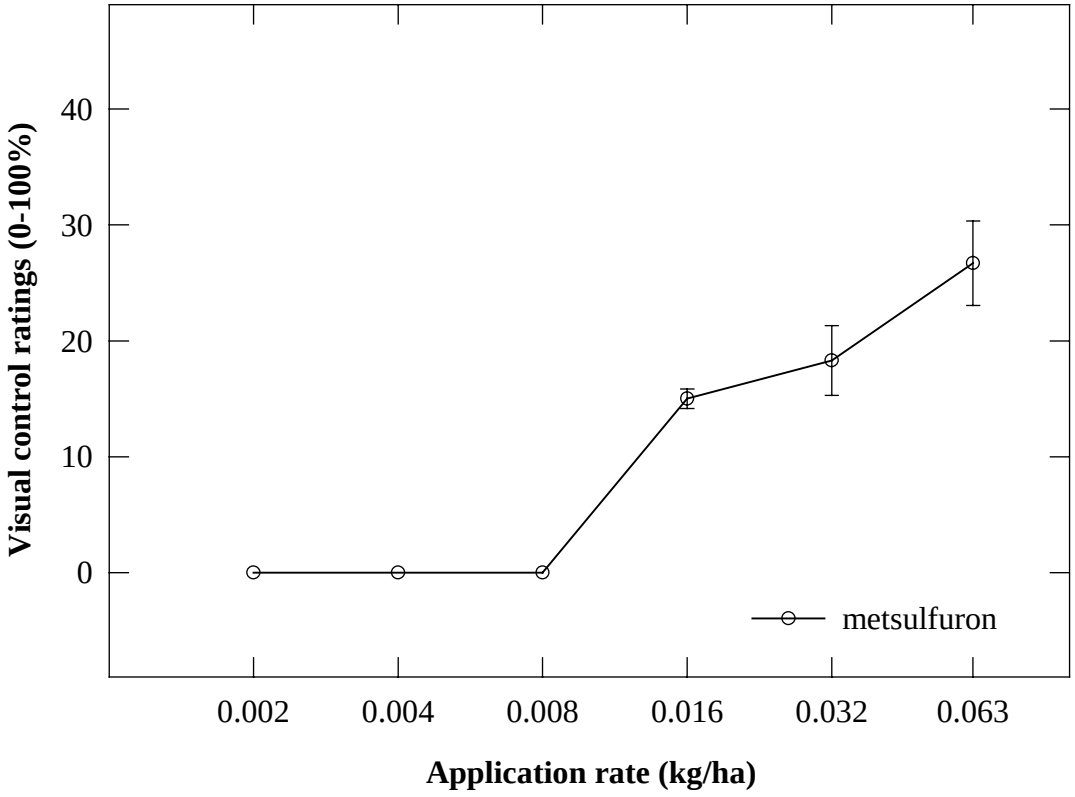

Figure 4. Mugwort control 4 mo after treatment with a logarithmic range of metsulfuron application rates during 1999. Bars represent the standard error of the mean, P = 0.05.

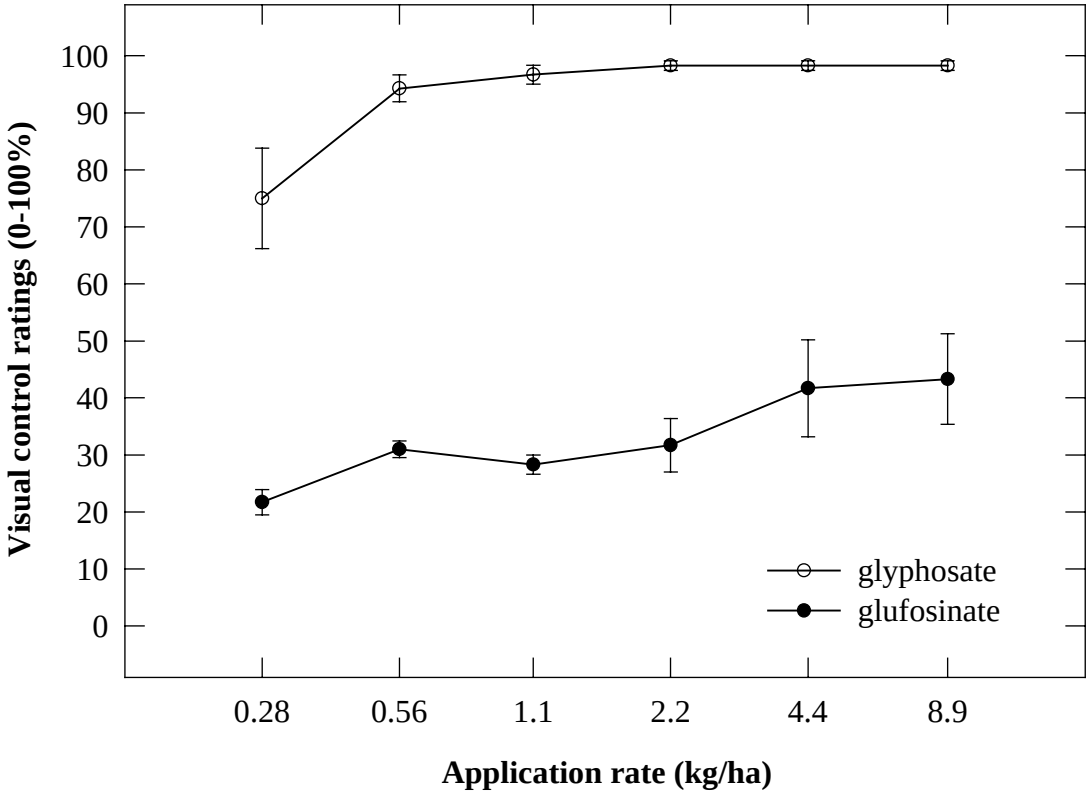

Figure 5. Mugwort control 4 mo after treatment with a logarithmic range of glyphosate and glufosinate application rates during 1998. Bars represent the standard error of the mean, P = 0.05.

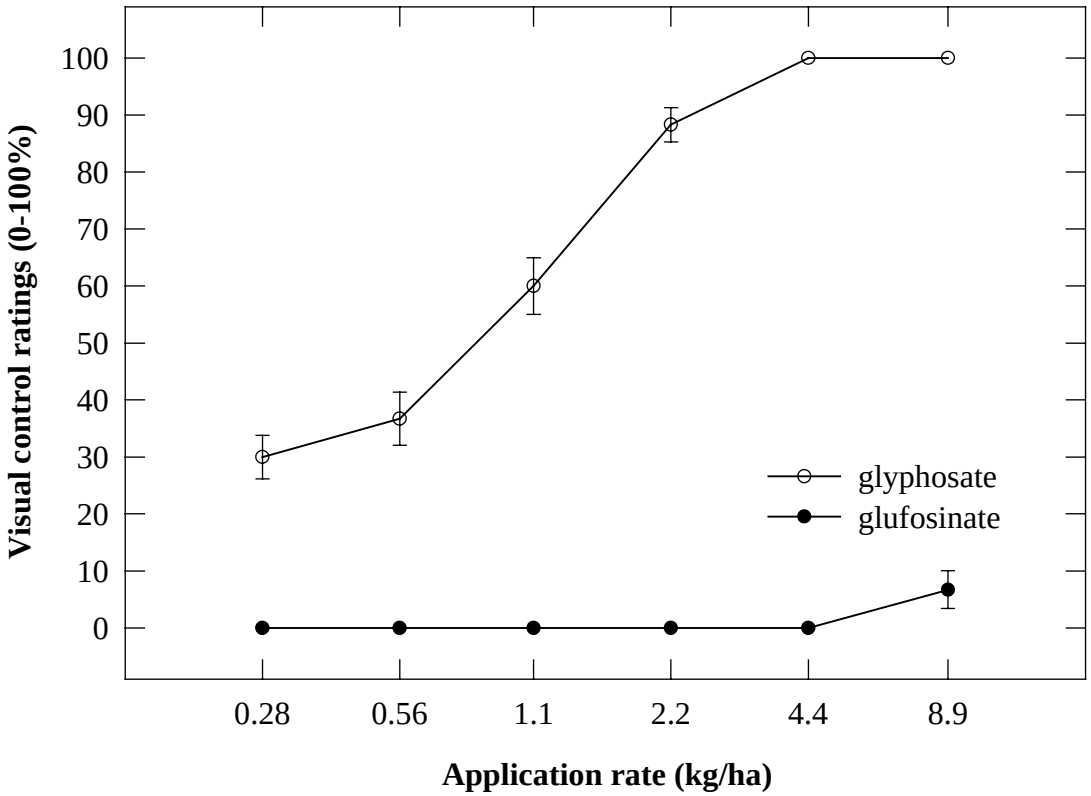

Figure 6. Mugwort control 4 mo after treatment with a logarithmic range of glyphosate and glufosinate application rates during 1999. Bars represent the standard error of the mean, P = 0.05.

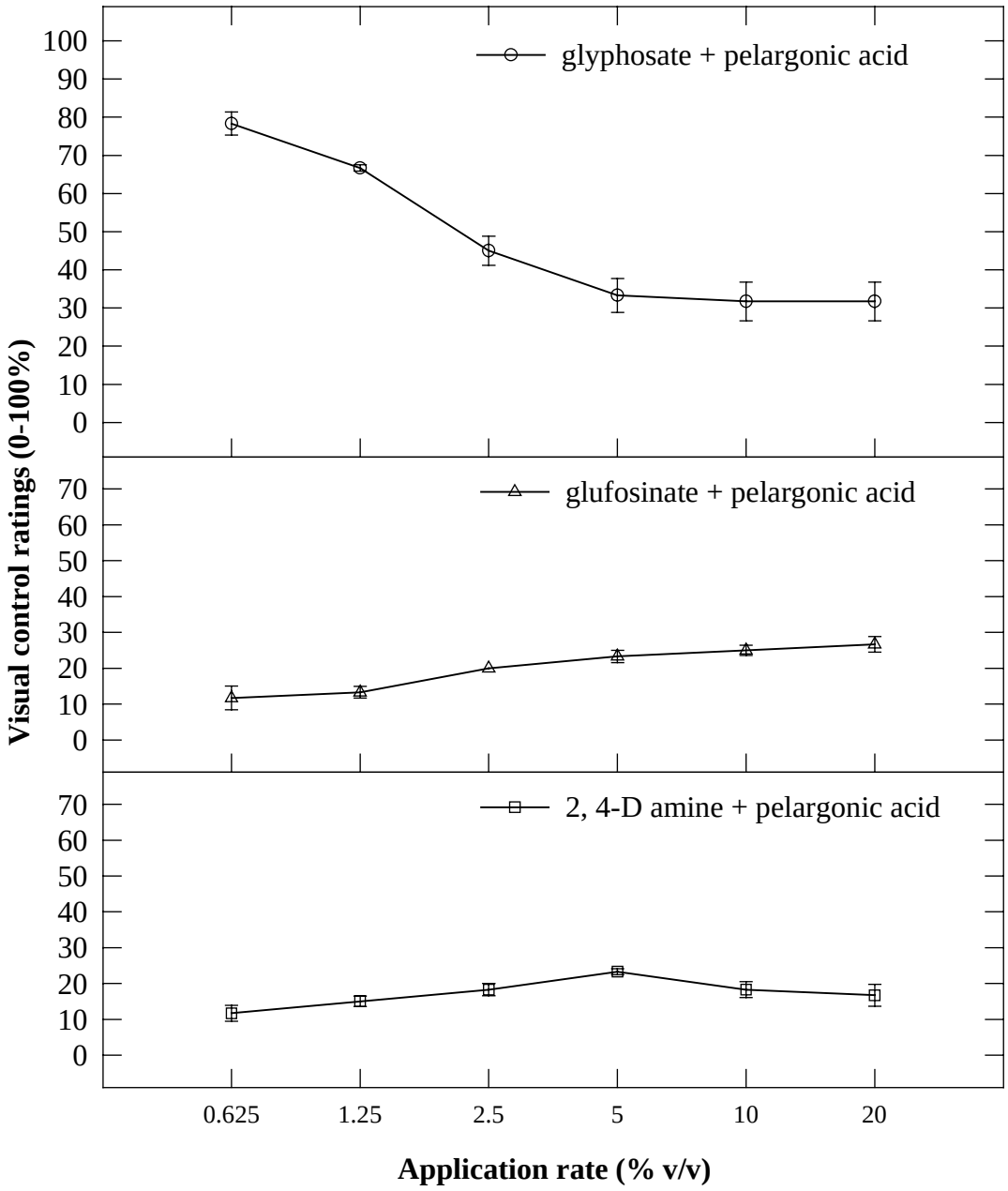

Figure 7. Mugwort control 4 mo after treatment with a logarithmic range of pelargonic acid application rates in combination with a constant rate (2.2 kg/ha) of glyphosate, glufosinate, or the dimethylamine salt of 2, 4-D during 1998. Bars represent the standard error of the mean, P = 0.05.

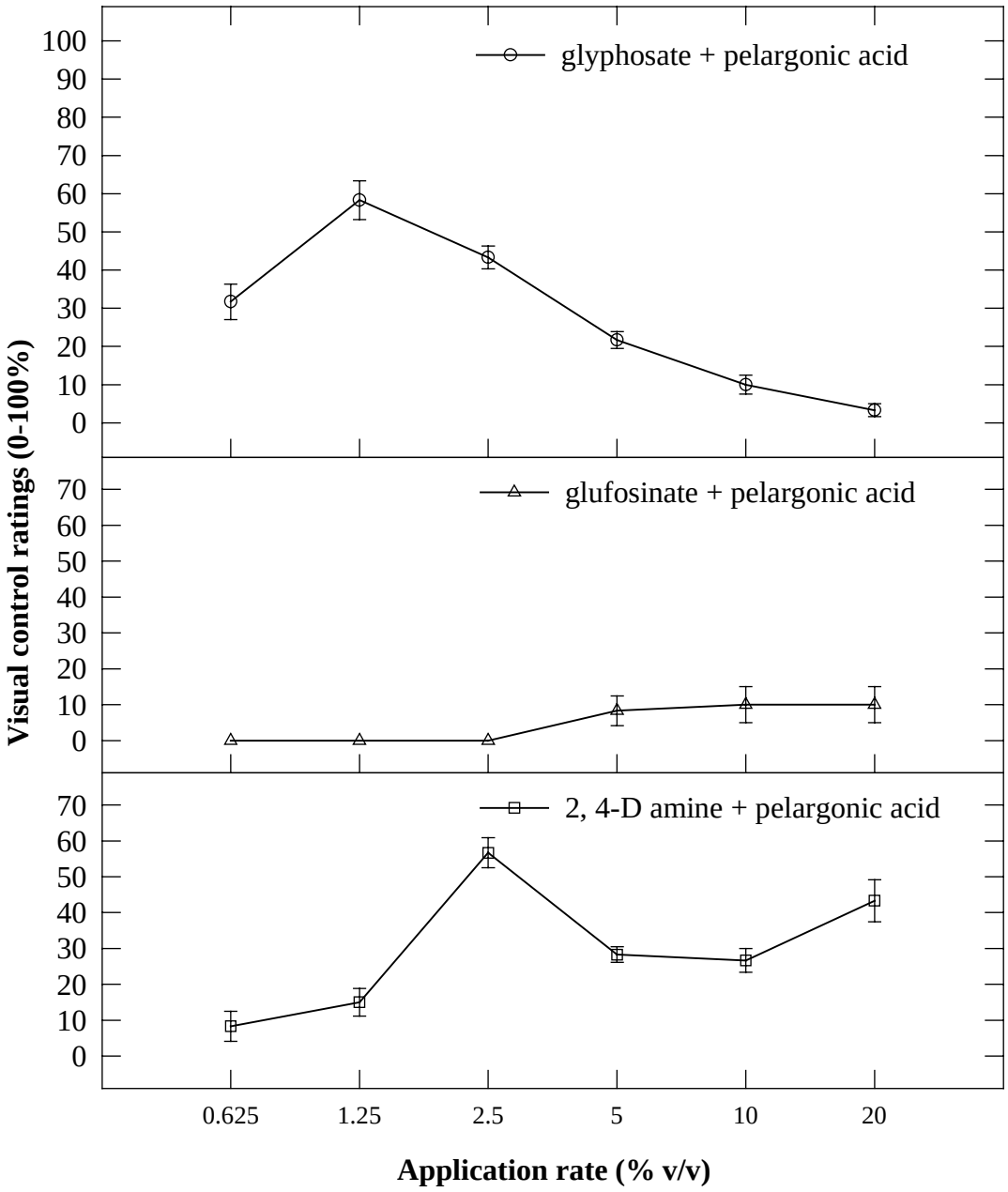

Figure 8. Mugwort control 4 mo after treatment with a logarithmic range of pelargonic acid application rates in combination with a constant rate (2.2 kg/ha) of glyphosate, glufosinate, or the dimethylamine salt of 2, 4-D during 1999. Bars represent the standard error of the mean, P = 0.05.

Chapter V

Summary and Conclusions

Herbicide resistance is defined by the Weed Science Society of America (1998) as the "inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type." Based on this definition, we conclude that the johnsongrass biotype investigated in this research is resistant to the aryloxyphenoxypropionate (APP) herbicides fluazifop-P and quizalofop-P and the cyclohexanedione (CHD) sethoxydim. Additionally, these experiments have revealed that resistance in the johnsongrass biotype is not due to reduced absorption or translocation, or to increased metabolism of the APP and CHD herbicides in the resistant compared to the susceptible biotype. Similarly, resistance to the APP and CHD herbicides is not a result of an insensitive acetyl-coenzyme A carboxylase (ACCase) enzyme in the resistant compared to the susceptible biotype, as is the case with the majority of APP- and CHD-resistant weed biotypes identified thus far (Devine 1997). However, these experiments have revealed that the specific activity of ACCase in the resistant johnsongrass biotype is two- to three-times greater than that detected in the susceptible johnsongrass biotype. Based on this observation, we are able to conclude that an overproduction of the ACCase enzyme is the biochemical mechanism responsible for resistance in the johnsongrass biotype identified in these experiments. This mechanism of resistance has not been previously reported in any naturally-occurring weed biotypes that are resistant to the APP or CHD herbicides. Therefore, these investigations have

contributed significantly to our understanding of the potential mechanisms of resistance that may develop in a resistant weed biotype.

Previous researchers have identified various cultural control strategies that may be used alone or in combination with herbicides to achieve optimum control of troublesome perennial weeds like tropical soda apple (*Solanum viarum* Dunal.), cogongrass [*Imperata cylindrica* (L.) Beauv.], horsenettle (*Solanum carolinense* L.), honeyvine milkweed [*Ampelamus albidus* (Nutt.) Britt], and many more (Gorrell et al. 1981; Mislevy et al. 1999; Moshier et al. 1986; Willard et al. 1996). Our experiments with mugwort have identified several strategies that are capable of enhancing mugwort control compared to the application of certain herbicides alone. For example, a significantly higher level of mugwort control was achieved with the majority of herbicides included in these experiments when they were applied to mugwort that had received two sequential pre-herbicide mowings compared to mugwort that had not been mowed. Additionally, a significantly higher level of mugwort control was observed when the majority of these herbicides were applied in sequential applications conducted at 7-wk intervals. These experiments have also revealed a strategy that does not enhance mugwort control, such as the application of a herbicide at different growth stages. For example, when averaged over all of the herbicides included in these experiments, there was no significant difference in the level of mugwort control achieved when these herbicides were applied to mugwort in the vegetative stage of growth compared to the flowering stage of growth. Lastly, these experiments have identified several herbicides that are capable of providing relatively good to excellent mugwort control without the use of any additional cultural control strategies. For example, a single application of picloram (4-amino-3, 5, 6-

trichloro-2-pyridinecarboxylic acid) provided essentially 100% mugwort control in every evaluation, regardless of the timing of herbicide application, the number of sequential herbicide treatments, or the number of pre-herbicide mowings. Clopyralid (3, 6-dichloro-2 pyridinecarboxylic acid) provided the next highest level of mugwort control at standard use rates, followed by glyphosate [*N*-(phosphonomethyl) glycine] and dicamba (3, 6-dichloro-2-methoxybenzoic acid). All other herbicides evaluated in these experiments provided relatively poor mugwort control, even at exceptionally high use rates. Collectively, the results from these experiments should prove useful in reducing any mugwort infestations that currently exist in VA crops and forages, as well as preventing the spread of mugwort in the future.

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VITA

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