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Evaluating the Potential for Differential Susceptibility of Common Reed (*Phragmites australis*) Haplotypes I and M to Aquatic Herbicides

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Notes and Commentary

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Common reed (*Phragmites australis*) is an invasive perennial grass in aquatic and riparian environments across the United States, forming monotypic stands that displace native vegetation that provides food and cover for wildlife. Genetic variation in global populations of common reed has given rise to two invasive haplotypes, I and M, in the United States. Our objectives were to (1) determine if any differences in herbicide efficacy exist with respect to common reed haplotypes I and M and (2) screen for other labeled aquatic herbicides that may have activity on common reed haplotypes I and M, most notably imazamox and diquat. A replicated outdoor mesocosm study was conducted in 1,136-L (300-gal) tanks using haplotypes I and M of common reed. Restriction fragment length polymorphism methodologies were used to verify the identification of I and M haplotypes used in this study. Diquat at 2.2 (1.9) and 4.5 (4.0) kg ai ha⁻¹ (lb ai ac⁻¹), glyphosate at 2.1 (1.8) and 4.2 (3.7) kg ae ha⁻¹ (lb ae ac⁻¹), imazamox at 0.6 (0.5) and 1.1 (0.9) kg ai ha⁻¹ (lb ai ac⁻¹), imazapyr at 0.8 (0.7) and 1.7 (1.5) kg ai ha⁻¹ (lb ai ac⁻¹), and triclopyr at 3.4 (3.0) and 6.7 (5.9) kg ae ha⁻¹ (lb ae ac⁻¹) were applied to the foliage of common reed. After 12 wk, no difference ($P = 0.28$) in herbicide tolerance was seen between the two haplotypes with respect to biomass. The 4.2-kg ae ha⁻¹ rate of glyphosate and the 0.8- and 1.7 kg ai ha⁻¹ rates of imazapyr reduced common reed by > 90% at 12 wk after treatment (WAT). Imazamox at 0.6 and 1.1 kg ai ha⁻¹, and triclopyr at 3.4 and 6.7 kg ae ha⁻¹ reduced common reed biomass (62–86%) at 12 WAT, though regrowth occurred. Diquat did not significantly reduce biomass by 12 wk. Glyphosate and imazapyr were the only herbicides that resulted in > 90% biomass reduction and corroborate control from previous studies.

Nomenclature: Diquat; glyphosate; imazamox; imazapyr; triclopyr; common reed, *Phragmites australis* (Cav.) Trin. ex Steud PHRCO.

Key words: *Phragmites australis*, invasive plant management, wetland, chemical control, riparian.

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Common reed is a perennial aquatic and riparian grass that is found on every continent except Antarctica (Holm et al. 1977) and has the widest distribution of any flowering plant (Tucker 1990). In the United States, common reed is found in every state except Alaska and Hawaii. Today, rapid expansion of common reed is attributed to multiple introductions of nonnative strains, likely from Europe and Asia in the late 1700s (Saltonstall 2002). Anthropogenic effects such as hydrologic alterations and disturbances (Roman et al. 1984), and increased eutrophication of the environment through nutrient runoff (Chambers et al. 1999) have all been factors in common reed expansion. The primary source of propagation for common reed is vegetative growth through the formation

Management Implications

Common reed (*Phragmites australis*) is an invasive perennial grass in aquatic and riparian environments across the United States. Common reed has high genetic variability, with two unique haplotypes, I and M, having the greatest distribution. Our common garden mesocosm study indicated that there was no difference in the susceptibility of the two most common haplotypes (I and M) to a selection of aquatic herbicides. Glyphosate and imazapyr at all rates evaluated provided excellent control of common reed and would be recommended for large-scale applications. Triclopyr provided good control only at the maximum application rate, and regrowth tended to be quicker (as observed new shoot growth) than in plants treated with either glyphosate or imazapyr, but may be a more selective option depending upon associated species. Identifying effective herbicides and use patterns for controlling common reed will provide land managers with necessary information on effective selective and nonselective control of this invasive plant in aquatic systems.

of rhizomes and stolons (Haslam 1973). Rhizomes not only function as the primary means of reproduction (Kilmeš et al. 1999) but they store the majority of the plant's carbohydrates (Fiala 1976; Kilmeš et al. 1999).

Species in the genus *Phragmites* display high phenotypic plasticity, making identification difficult (Clayton 1967; Haslam 1972; Koppitz 1999). Multiple morphological traits have been suggested for differentiation between common reed of various origins, however the sole use of these characteristics as a definitive identification tool is difficult and highly subjective (Saltonstall et al. 2004). A key tool in the identification of invasive lineages of common reed has been the use of polymerase chain reaction, restriction fragment-length polymorphism analyses (PCR-RFLPs) (Saltonstall 2001, 2003). The differences in DNA fragment size can be used to identify unique genetic variants, also commonly referred to as haplotypes.

Haplotype differences have been linked with invasions of nonnative common reed (Saltonstall 2002) and are important for properly identifying an unknown population as well as providing a vital tool in management decision support. These cryptic invasions of common reed over the last 200 yr have resulted in a loss of native common reed and an increase in nonnative haplotypes (Saltonstall 2002). Currently, there are 29 haplotypes of common reed that have been identified worldwide, 13 of which are native to North America; five of these are native to the northeastern portion of North America (K. Saltonstall, personal communication). Of the 29 haplotypes discovered worldwide, haplotypes I and M have the most widespread distribution on multiple continents, with haplotype M being the most common type in North America, Europe, and Asia today (Saltonstall 2002). Haplotypes I and M are the most problematic haplotypes in the United States, though haplotype M is expanding its range at a faster rate than that of haplotype I (Saltonstall 2002).

Haplotype I is thought to have originated in South America and parts of Asia and is the most prevalent haplotype along the Gulf Coast of the United States (Hauber et al. 1991; Saltonstall 2002). Haplotype I has been present along the Gulf Coast since the late 1800s (Saltonstall 2002). Genetic analysis of pre-1910 herbarium samples as well as current samples indicate genetic autonomy and geographic isolation of this haplotype along the Gulf Coast of the United States (Pellegrin and Hauber 1999; Saltonstall 2002) from all other populations of common reed in North America. Haplotype I's closest relative is only found in Asia (Saltonstall 2002). Haplotype M is found throughout Eurasia and Africa and is considered native to those ranges. In North America, haplotype M is replacing native haplotypes in the New England states and has become prevalent in the Midwestern states (Saltonstall 2002). Haplotype M displays invasive characteristics in that it overtakes wetlands and shorelines, produces monotypic stands, and outcompetes native vegetation and wildlife (Able and Hagan 2000; Chambers et al. 1999; Marks et al. 1994; Saltonstall 2002; Windham and Lathrop 1999).

To mitigate the spread of invasive haplotypes, management has utilized herbicides to control this species. Glyphosate and imazapyr provided 82 and 93% control, respectively, under field conditions (Derr 2008a). Triclopyr, which is typically selective for monocot species, reduced common reed shoot, regrowth shoot weight, and stem number by 40 to 92% when applied at 1.12 (0.9) and 6.72 (5.9) kg ae ha⁻¹ (lb ae ac⁻¹), respectively under greenhouse conditions (Derr 2008a). Wipe-on applications of both glyphosate and imazapyr were not considered efficacious (Kay 1995). Imazapyr provided 57 and 75% control of common reed respectively, when 25 and 50% dilutions were applied to plants (Kay 1995). Glyphosate applied using a wiper applicator provided 38 and 33% control when applied at similar rates as imazapyr; however, when glyphosate was applied as a foliar spray it provided 100% control (Kay 1995). Monteiro et al. (1999) reported that cutting common reed prior to herbicide applications had a positive effect. Pursuant to this, increasing the spray volume increased the control of common reed; a higher volume provided > 90% control, resulting in less plant biomass and less plant density than the lower spray volume.

With the ongoing spreading of nonnative common reed haplotypes across North America, an assessment of herbicides is necessary to identify effective chemistries and screen for potential differential susceptibility between haplotypes. Therefore, the objectives of this study were to (1) determine if any differences in herbicide efficacy exist with respect to common reed haplotypes I and M, and (2) screen for other labeled aquatic herbicides that may have activity on common reed haplotypes I and M, most notably imazamox and diquat.

Materials and Methods

Haplotype Identification. Rhizomes of haplotype I were collected from Polecat Bay, east of Mobile, AL (Lat. 30°42'8.18"N, 88°0'25.70"W). Rhizomes of haplotype M were collected from the St. John's Marsh, located on the northern shore of Lake St. Clair, near Harsens Island, MI (Lat. 42°35'97"N, 88°37'30.50"W). Rhizomes were transported to an outdoor mesocosm facility at the R. R. Foil Plant Science Research Facility, Mississippi State University, Starkville, MS, and separate culture populations were established. Leaf tissue samples of each haplotype culture were assayed using PCR-RFLP to identify the variation in chloroplast DNA and verify the haplotype identities used in this study (Saltonstall 2003).

Planting. Common reed rhizomes were taken from the haplotype cultures and two 20 cm (8 in) rhizome segments of haplotypes I or M were planted in separate 18.9-L (5 gal) plastic pots that were filled with soil. All pots were amended with 2 g L⁻¹ (0.32 oz gal⁻¹) of 19-6-12 fertilizer (Osmocote®, Scotts-Sierra Horticultural Products Company, Marysville, OH). A total of 104 pots were planted for each haplotype. Two pots of each haplotype were then placed into each of 52 1,136-L tanks with water depth of 15 cm that was maintained throughout the trials. Plants were allowed to grow for approximately 6 wk or until plants were 100 cm tall, an accepted treatment height in previous trials (Derr 2008a).

Treatment. Following the growth period, foliar applications of diquat (Reward®, 4.5 and 2.2 kg ai ha⁻¹, Syngenta Crop Protection Inc., Greensboro, NC), glyphosate (isopropylamine salt, Rodeo®, 4.2 and 2.1 kg ae ha⁻¹, Dow AgroSciences LLC, Indianapolis, IN) and (monopotassium salt, Touchdown Hi-Tech®, 4.2 and 2.1 kg ae ha⁻¹, Syngenta Crop Protection Inc., Greensboro, NC), imazamox (Clearcast®, 1.1 and 0.6 kg ai ha⁻¹, SePRO Corporation, Carmel, IN), imazapyr (Habitat®, 1.7 and 0.8 kg ai ha⁻¹, BASF Corporation, Research Triangle Park, NC), and triclopyr (Renovate3®, 6.7 and 3.4 kg ae ha⁻¹, SePRO Corporation, Carmel, IN) were made to common reed. Treatments were applied using a CO₂-pressurized backpack sprayer (R&D Sprayers, Opelousas, LA) calibrated to deliver a spray volume of 187 L ha⁻¹. A nonionic surfactant (Dyne-Amic®, Helena Chemical, Collierville, TN) was added to the spray solution at a rate of 0.25% v/v. Barriers were placed around each tank during application to prevent herbicide drift. At 12 WAT, the aboveground biomass of common reed was harvested and dried in a forced-air oven at 70 C (158 F) for 72 h, then weighed to determine dry mass of plants.

Experimental and Statistical Design. The study was conducted for 12 wk, from July to October 2008, and was repeated when growing conditions were favorable. The

study was conducted as a split plot design with the whole plot factor being two rates of diquat, glyphosate (isopropylamine and mono-potassium), imazamox, imazapyr, triclopyr, and an untreated reference. The subplot factor was haplotype, I and M, of common reed. Each treatment was replicated four times in the 1,136-L tanks. Herbicide treatment data were analyzed by fitting mixed models using the Mixed Procedure (SAS software, SAS Institute Inc., Cary, NC). Common reed shoot biomass was included in the model as the dependent variable. Treatment, haplotype, and the treatment by haplotype interaction term were included as independent variables. Trial, the interaction terms tank by trial, and the treatment by tank within trial were included as a random effects to account for their influence on the results. Haplotype ($P = 0.28$) and treatment by haplotype interaction ($P = 0.31$) were not significant, therefore data were pooled by trial and haplotypes to test for treatment effects. Treatment was significant ($P < 0.01$) and means were separated using least squares means and grouped using Dunnett's test. Only the comparisons to the untreated references are reported, and all analyses were conducted at the $P < 0.05$ significance level.

Results and Discussion

The application of imazapyr resulted in > 90% biomass reduction for both rates against common reed (Figure 1). Previous studies have shown similar results with imazapyr where applications successfully controlled common reed under greenhouse and field conditions (Derr 2008a; Mozdzer et al. 2008). Pursuant to this, Mozdzer et al. (2008) reported imazapyr applications to be more effective than glyphosate applications, reducing common reed biomass by 95 and 79%, respectively. In the current study, glyphosate applied at the higher rate resulted in > 90% control regardless of the salt formulation (Figure 1). Control of common reed when using glyphosate has been reported to increase with increasing application rates (Ailstock et al. 2001; Derr 2008a, Kay 1995; Riemer 1976). Although glyphosate effectively controls common reed at label rates (4.2 to 8.8 L ha⁻¹), repeat applications have been reported as necessary to maintain control of existing common reed populations (Derr 2008a; Riemer 1976).

Unlike the nonselective systemic herbicides imazapyr and glyphosate, triclopyr is typically selective for grass species, though it suppresses growth of common reed (Derr 2008a). Triclopyr at 3.4 and 6.7 kg ae ha⁻¹ reduced common reed biomass by 68 and 86%, respectively, compared to untreated reference plants, though regrowth occurred by 12 wk as new shoots were observed growing from the sediment in treated containers. Control of common reed with triclopyr as observed in this study corroborates results reported by Derr (2008a), where

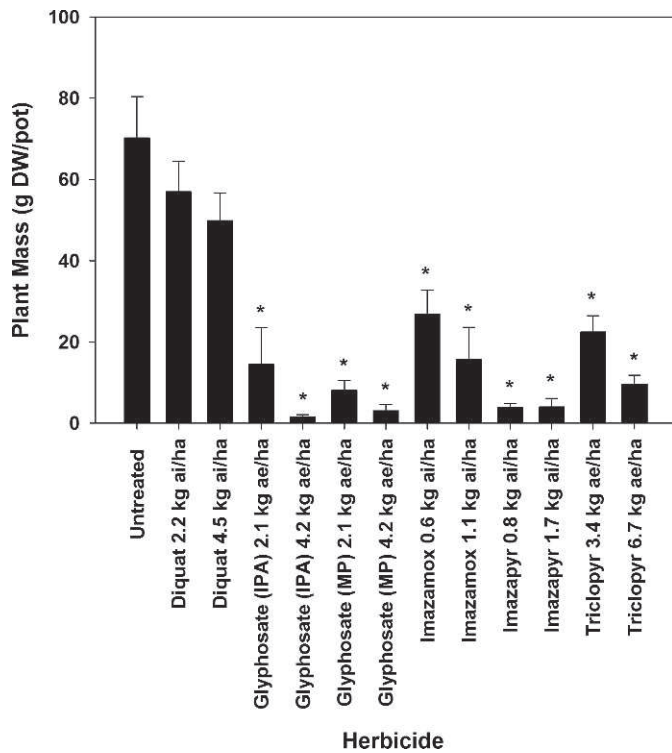


Figure 1. Mean (\pm 1SE) biomass of common reed 12 wk after treatment with select herbicides. There were no significant differences between trial and haplotypes, therefore data were combined. Mean comparisons between herbicide treatments and the untreated reference were made using the Dunnett's test at a 0.05 level of significance. Significant differences are denoted by an asterisk. The isopropylamine salt formulation of glyphosate is denoted as IPA and mono-potassium salt formulation is denoted as MP.

reductions in regrowth shoot weight of 40 to 92% were observed following triclopyr applications. The use of triclopyr may offer a more selective option depending on rate and associated species than using glyphosate or imazapyr; however, further research is needed to understand triclopyr's mechanism of action in monocotyledon species and to ascertain the level of selectivity that could be achieved if using this herbicide with respect to the nontarget plant associations.

Applications of imazamox resulted in a growth reduction of 62 and 78%, respectively, for 0.6 and 1.1 kg ai ha⁻¹, though imazamox had more of a growth regulating effect on common reed than actually causing plant mortality. Biomass 12 WAT was lower with respect to the untreated reference plants (Figure 1). After the initial plant injury following imazamox applications, several new stems began to grow from the nodes of the original culm, an observation frequently referred to as witch's broom. Witch's broom is characterized by the release of apical

dominance and subsequent outgrowth of lateral buds (Murai et al. 1980). Plant hormones are typically produced in the meristematic regions of the plant and enforce apical dominance; treating a plant with an imidazolinone herbicide results in the death of the apical tip of a plant shoot bringing about a decrease in the rate of auxin production and the subsequent release of apical dominance (Shaner 1991). The loss of apical dominance could be an explanation or a secondary sublethal effect of imazamox causing the observed growth of common reed.

In field situations, management of common reed will be most often influenced by habitat type, accessibility, budget constraints, and public perception, all of which will impact herbicide choice. Glyphosate and imazapyr provided the greatest level of control as indicated by percentage of biomass reductions, although imazamox and triclopyr did significantly reduce common reed biomass. Diquat showed early signs of efficacy on common reed, but did not result in control by 12 WAT. It is important to note that no single application of any herbicide or rate in this study completely controlled common reed, an observation seen in similar studies (Derr 2008a; Kay 1995; Monteiro et al. 1999); this reinforces the need for research on other herbicides and management strategies.

Management strategies for common reed could include the newly registered herbicides for aquatic habitats including penoxsulam, flumioxazin, and bispyribac-sodium. However, common reed is not currently listed on the label for any of those herbicides; therefore, the efficacy of these herbicides on common reed is unknown. Mowing has been effective at controlling common reed within season (Cross and Fleming 1989; Derr 2008b; Güsewell 2003). When integrating mowing with herbicide applications it was reported that applying glyphosate 1 mo after a mowing or 2 wk prior to mowing reduced common reed regrowth the following season by 90% (Derr 2008b). However, applying glyphosate alone provided similar control the following season compared to glyphosate combined with a single mowing (Derr 2008b). The success or failure of common reed management may be dictated more by the timing of management practices than the selection of management techniques. For example, it is likely more efficacious to target seedlings instead of more mature plants or to target weak points in the life history of common reed such as times of low carbohydrate storage. However, species specific life history data are often lacking when making decisions with respect to management timing and selection of techniques.

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