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Evaluation of Glyphosate, Flumioxazin and Imazamox against Japanese Knotweed s. l.

by LeeAnn M. Glomski

PURPOSE: The purpose of this work was to evaluate flumioxazin and imazamox alone and in combination with glyphosate as potential alternatives for controlling Japanese knotweed at Times Beach, a 56-acre nature preserve located in Buffalo, New York.

BACKGROUND: Times Beach is a 56-acre nature preserve located in Buffalo, New York. The site was initially constructed by the USACE Buffalo District as a confined disposal facility (CDF) in 1971. The CDF was partially filled and closed in 1976 at the request of the Ornithological Society of Buffalo (Simmers and Lee 1997). The site consists of three distinct ecological zones (aquatic, wetland and upland) and is utilized by more than 200 species of resident and migratory birds (Andrle 1986, Simmers and Lee 1997). Approximately 46 acres of Times Beach has been designated as a state wetland by the New York State Department of Environmental Conservation. Times Beach is located adjacent to the Buffalo River Area of Concern (AOC) and within the Niagara River Bi-national AOC. The site is dominated by invasive species such as phragmites (*Phragmites australis* (Cav.) Trin. Ex Steud.), Japanese knotweed (*Polygonum cuspidatum* Siebold & Zucc.), common buckthorn (*Rhamnus cathartica* L.) and mugwort (*Artemisia vulgaris* L.).

The U.S. Army Engineer Research and Development Center in collaboration with the U.S. Army Corps of Engineers (USACE) Buffalo District developed an invasive species management and restoration plan for Times Beach. Removal of invasive species combined with enhancement of native plant communities will ultimately result in repaired ecosystem function. The Times Beach management and restoration plan was initiated in October 2012 with funding from the U.S. Environmental Protection Agency's (EPA) Great Lakes Restoration Initiative (GLRI). Because the majority of the site is classified as a state wetland, only aquatic herbicide formulations registered by the U.S. EPA and the state of New York can be applied. There is little information in the peer-reviewed literature regarding chemical control of Japanese knotweed. Fact sheets published by numerous state and federal agencies recommend using the aquatic formulations of glyphosate, imazapyr or triclopyr to control Japanese knotweed. Reports from the field indicate glyphosate provides poor control on established stands (Soll 2004) and imazapyr is not registered for aquatic use in New York State (NYSDEC 2012). Triclopyr was thus the only aquatic herbicide option for controlling Japanese knotweed at Times Beach. To effectively address the Japanese knotweed infestation at Times Beach, a closer look into its biology and ecology – as well as alternative control options – is needed.

There is debate surrounding the nomenclature and classification of Japanese knotweed. In general, Japanese knotweed is referred to as *Polygonum cuspidatum* in North America and *Fallopia japonica* (Houtt.) Ronse Decr. in Europe. Due to the varieties and hybrids that are possible, herein the taxon will be referred to as Japanese knotweed sensu lato (s. l.) which "encompasses *Fallopia japonica* var. *japonica*, *Fallopia sachalinensis* (F. Schmidt ex Maxim) Ronse de Craene (giant knotweed), *Fallopia* x *bohemica* (Chrtek et Chrtkova) J. P. Bailey (the hybrid between *F. japonica* var *japonica* and *F. sachalinensis*) and backcrossed plants" (Grimsby and Kesseli 2010).

Japanese knotweed s.l. is an invasive rhizomatous perennial native to Japan, Korea, Taiwan and northern China (Barney et al. 2006). It was introduced to the U.S. in the 1880s as an ornamental plant (Child and Wade 2000) and is currently found in all states except Alabama, Arizona, Florida, North Dakota, New Mexico, Nevada, Texas and Wyoming (USDA 2012). In the Northeastern U.S., Japanese knotweed s.l. was most likely introduced by seed dispersal and populations, then expanded vegetatively (Grimsby et al. 2007). Japanese knotweed s.l. is one of the world's worst invasive species (Lowe et al. 2000). Outside of its native range, it causes large changes to the ecosystems it invades by reducing biodiversity and increasing biomass production by two to six times that of native vegetation (Aguilera et al. 2010). Japanese knotweed s.l. has hollow, bamboo-like stems that form dense thickets (Child and Wade 2000). Not only can few native plants survive under the dense summer canopy of Japanese knotweed s.l. but persistent stem litter also prevents other species from establishing (Beerling et al. 1994). To overwinter, buds form at rhizome nodes and at stem bases in the fall. New shoots form rapidly from these buds in the spring giving Japanese knotweed s.l. a competitive advantage over native species. It can grow up to 3 m in one season (Child and Wade 2000). Research has shown that Japanese knotweed s.l. is allelopathic, which also hinders the growth of native plants (Vrchotova and Sera 2008; Fan et al. 2009; Murrell et al. 2011). Besides its impact on native plants, Japanese knotweed s.l. also impacts the foraging success of green frogs (Maerz et al. 2005) and stream detrital food webs (Lecerf et al. 2007).

The spread of Japanese knotweed s.l. can be attributed to rhizomes, seeds, and dispersal of stem or rhizome material. The rhizome system is extensive, reaching 2 m deep and extending 7 m away from the parent plant (Child and Wade 2000). The spread from rhizomes is estimated at several meters per year (Child and Wade 2000). Plants produce seed in late summer and it has been reported that outside of its native range, the spread of Japanese knotweed s.l. via seeds is minimal (Child and Wade 2000). However, in the Northeastern U.S., Japanese knotweed s.l. produces a viable seed with high germination rates (Forman and Kesseli 2003, Bram and McNair 2004). Forman and Kesseli (2003) reported Japanese knotweed s.l. seeds are viable under a variety of environmental conditions and can survive through winter. Dormancy or cold treatment is not required for germination and germinated seeds can grow into healthy adult plants. Germinated seedlings cannot survive under a well-established Japanese knotweed s.l. canopy but can survive when dispersed into open areas (Forman and Kesseli 2003). At one study site in Pennsylvania, a single stem was estimated to produce over 127,000 seeds annually (Bram and McNair 2004). Given the number of seeds produced and the high germination rate, seeds do play an important role in the rapid spread of Japanese knotweed s.l. Dispersal of stem and rhizome fragments also contributes to the spread of this weed. These fragments can be transported by water and eventually establish new colonies downstream (Beerling et al. 1994, Barney et al. 2006). New plants can form from stem segments as small as one node (De Waal 2001) and rhizome segments as small as 8 g (Beerling 1990).

Japanese knotweed s.l. grows best in full sunshine (Grime et al. 1988) and is commonly found along river banks, forest edges, rights-of-way, gardens, and disturbed sites (Barney et al. 2006). Japanese knotweed s.l. can tolerate soils with high salt or heavy metal content as well as soils with a pH of 3.0 to 8.5 (Child and Wade 2000). Although it prefers nutrient-rich soils, Japanese knotweed s.l. can thrive on soils poor in nutrients (Barney et al. 2006). It has also been found growing in coastal salt marshes along Long Island (Richards et al. 2008). Regardless of habitat, once Japanese knotweed s.l. becomes established it is difficult to control.

The two most common control options for Japanese knotweed s.l. are cutting and foliar herbicide applications. Regardless of control method, however, depletion of the rhizome system is necessary to control Japanese knotweed s.l. Seiger and Merchant (1997) reported that cut plants accumulated less underground biomass than uncut plants but the timing of cutting didn't influence the amount of underground biomass at the end of the season. The authors also noted that Japanese knotweed s.l. reaches maximum height within three to four weeks of breaking dormancy; therefore, to prevent replenishment of rhizome reserves, plants should be cut every four weeks throughout the growing season (Seiger and Merchant 1997). It may take more than one year of cutting to achieve control (Seiger and Merchant 1997) and care must be taken when disposing of cut material to prevent the spread of Japanese knotweed s.l.

A number of herbicides and herbicide combinations have been evaluated for control of Japanese knotweed s.l. Both picloram (Scott and Marrs 1984) and imazapyr (Figueroa 1989) are reported to be effective but picloram is not approved for use in aquatic sites and will not be discussed further. The Michigan Department of Natural Resources (MI DNR 2012) reports imazapyr is the most effective herbicide for Japanese knotweed s.l. to date but due to soil activity, treated sites shouldn't be re-planted for at least a year following treatment. There is conflicting information about the effectiveness of glyphosate and triclopyr on Japanese knotweed s.l. Numerous researchers have reported glyphosate to be effective at controlling Japanese knotweed s.l. (Ahrens 1975; Figueroa 1989; Gover et al. 2005); however, Soll (2004) provides two examples of a 7-8 percent glyphosate application with different outcomes. One application was made on first year plants and provided good control, whereas the second application was made on well-established plants and provided poor control. Although not addressed in the paper, this difference in control may be attributed to differences in age of treated plants and the extent of their rhizome systems. In most cases, Japanese knotweed s.l. treated with glyphosate is known to re-grow the year after herbicide application and therefore requires follow-up treatments (MI DNR 2012). Triclopyr has been reported to provide over 90 percent control on small patches of Japanese knotweed s.l. (age of plants not reported) the first year of treatment (Soll 2004) but the MI DNR (2012) considers triclopyr to provide "some control" and to be less effective than imazapyr or imazamox. The MI DNR (2012) has conducted some field trials with imazamox and considered it to be equal to imazapyr in its effectiveness to control Japanese knotweed s.l. Effective herbicide combinations that can be used in aquatic sites include glyphosate + imazapyr (Gover et al. 2005) and carfentrazone-ethyl combined with triclopyr or glyphosate (Skibo and Isaacs 2006). Regardless of which herbicide is used, it may take two or more years of treatment to remove established patches of Japanese knotweed s.l. and even after treatment has begun, new shoots can continue to sprout up to 20 feet from the treated stand (Soll 2004).

The cost to control invasive knotweeds is high. Germany estimated the annual cost to control knotweed along its rivers to be $8 million (Reinhardt et al. 2003); whereas Britain estimates it would cost them $2.6 billion (DEFRA 2003). In the U.S., the Washington Department of Agriculture and state Salmon Recovery Funding Board contributed roughly $6 million to control knotweed infestations in 2004 (PNWER 2012).

At Times Beach, Japanese knotweed s.l. is found in patches along the edges of both the forest and wetland (Figure 1). Although imazapyr can effectively control Japanese knotweed s.l., it is not registered for aquatic use in New York State (NYSDEC 2012) and is not recommended for use on vegetation that is in close proximity to trees as it is highly mobile in the soil and roots and can be transferred among intertwined root systems (Tu et al. 2001). Imazamox, which has the same mode of action as imazapyr (both are ALS inhibitors), is safe to use near trees (Burns 2009) and would therefore be a possible alternative to imazapyr at Times Beach; however, little research has been done to document the effectiveness of imazamox on Japanese knotweed s.l. Another alternative herbicide is flumioxazin which is pending registration in New York State[1]. Flumioxazin is a newly registered herbicide for use in aquatic sites and has the same mode of action as carfentrazone-ethyl (both are protox inhibitors). Carfentrazone-ethyl has activity on Japanese knotweed s.l. but is more effective when combined with glyphosate or triclopyr (Skibo and Isaacs 2006). No work to date has been done to evaluate the effectiveness of flumioxazin on Japanese knotweed s.l. Therefore, the objective of these studies was to determine a dose response for imazamox and flumioxazin with small greenhouse plants and evaluate the potential for enhanced activity when adding these herbicides with glyphosate.

Figure 1. Young Japanese knotweed s.l. plants growing along the wetland edge at Times Beach.

MATERIALS AND METHODS: Japanese knotweed s.l. was collected from Times Beach, Buffalo, NY and cut into 8-cm stem sections. Leaves from the lower half of each stem section were removed and the cut ends of each stem were dipped into Schultz TakeRoot® (Spectrum Brands, Madison, Wisconsin) rooting hormone$_2$. Fifty stem sections were planted in 10-cm diameter pots of a 50/50 mixture of peat and vermiculite and 50 in Oasis Rootcubes® (Figure 2). The stems were then placed under a misting system in the greenhouse. The misting system ran on a 2 hr on: 2 hr off cycle over a 24-hr period. Plants remained under the misting system for 30 days at which time roots had developed. Plants in rootcubes were then planted into 10-cm diameter pots of Miracle-Gro (Scott's Miracle-Gro, Marysville, Ohio) potting mix. All plants were allowed to grow for an additional 30 days outside of the misting system before being treated with herbicide. There was no visual difference between plants started in rootcubes versus peat/vermiculite; therefore, both sets of plants were used for herbicide evaluations. Plants were 20 to 25 cm in height at the time of treatment. Since small greenhouse grown plants can be

Figure 2. Japanese knotweed s.l. planted in a rootcube.

[1] Personal communication, Jim Petta, Aquatics Territory Manager, Valent Corporation, Corpus Christi, Texas.

more sensitive to herbicide treatment than plants in the field, low rates of both herbicides were evaluated in these studies to determine a dose response.

Flumioxazin Evaluation: Plants started in peat/vermiculite were used in this evaluation. Flumioxazin and flumioxazin + glyphosate were applied on August 22, 2012 as a foliar spray. Flumioxazin was applied as Clipper® (Valent U.S.A. Corporation, Walnut Creek, California) at rates of 0.054, 0.107 and 0.214 kg ai ha^{-1} with and without 2 kg ai ha^{-1} glyphosate applied as AquaPro® (SePRO Corporation, Carmel, Indiana). Glyphosate at 2 kg ai ha^{-1} alone and an untreated control were also included. The non-ionic surfactant Thoroughbred® (Winfield Solutions LLC, St. Paul, Minnesota) was added to all herbicide treatments at a rate of 0.5% v:v. Treatments were applied using a CO_2-pressurized sprayer (Bellspray, Opelousas, Louisiana) equipped with a hand-held, single-nozzle (TeeJet® solid cone spray tip) spray header calibrated to deliver a spray volume of 935 L ha^{-1} (100 gal A^{-1}).

Treatments were randomly assigned and replicated three times. All living aboveground plant material was harvested four weeks after treatment (WAT) and dried at 65 C to a constant weight. Biomass data were subjected to one-way analysis of variance (ANOVA) and means separated via the Student-Newman-Keuls method (SNK, α=0.05).

Imazamox Evaluation: Plants started in rootcubes were used in this evaluation. Imazamox and imazamox + glyphosate were applied on August 22, 2012 as a foliar spray using the same equipment and spray volume as described above. Imazamox was applied as Clearcast® (SePRO Corporation, Carmel, Indiana) at rates of 0.14, 0.28 and 0. 56 kg ai ha^{-1} with and without 2 kg ai ha^{-1} glyphosate applied as AquaPro® (SePRO Corporation, Carmel, Indiana). Glyphosate at 2 kg ai ha^{-1} alone and an untreated control were also included. The methylated seed oil Inergy® (Winfield Solutions LLC, St. Paul, Minnesota) was added to all herbicide treatments at a rate of 1.0% v:v.

Treatments were randomly assigned and replicated four times. All living aboveground plant material was harvested six WAT and dried at 65 C to a constant weight. Due to the slow activity of imazamox, this study was allowed to run an additional two weeks. Biomass data were subjected to one-way analysis of variance (ANOVA) and means separated via the Student-Newman-Keuls method (SNK, α=0.05).

Herbicide interaction for both the flumioxazin and imazamox evaluations was calculated using the following equation (Colby 1967):

$$E = (X + Y) - (XY/100)$$

Prior to estimating the herbicide response, biomass data were converted to percent control. In the above equation, E is the expected control with herbicides A + B; X is the observed control with herbicide A; and Y is the observed control with herbicide B. The herbicide combination is antagonistic when the observed response is less than the expected; when greater than the expected, the combination is synergistic. When the observed and expected responses are equal the combination is additive. Expected and observed values were calculated for each replication and subjected to a Wilcoxin Rank Sum Test.

RESULTS AND DISCUSSION:

Flumioxazin Evaluation: Within the first WAT flumioxazin and flumioxazin + glyphosate-treated plants showed symptoms of necrosis on leaves, stems and flowers. For most treated plants, symptoms progressed through three WAT. Symptoms did not progress with the two lower rates of flumioxazin alone and there was healthy green tissue present at the time of harvest. No symptoms were observed on the plants treated with glyphosate alone throughout the four week evaluation period. The highest rate of flumioxazin alone and all combinations reduced biomass of Japanese knotweed s.l. when compared to the untreated reference and those treated with glyphosate alone. Percent control for these treatments ranged from 76 to 93 percent. Combining glyphosate with flumioxazin did not enhance treatment performance (Figure 3).

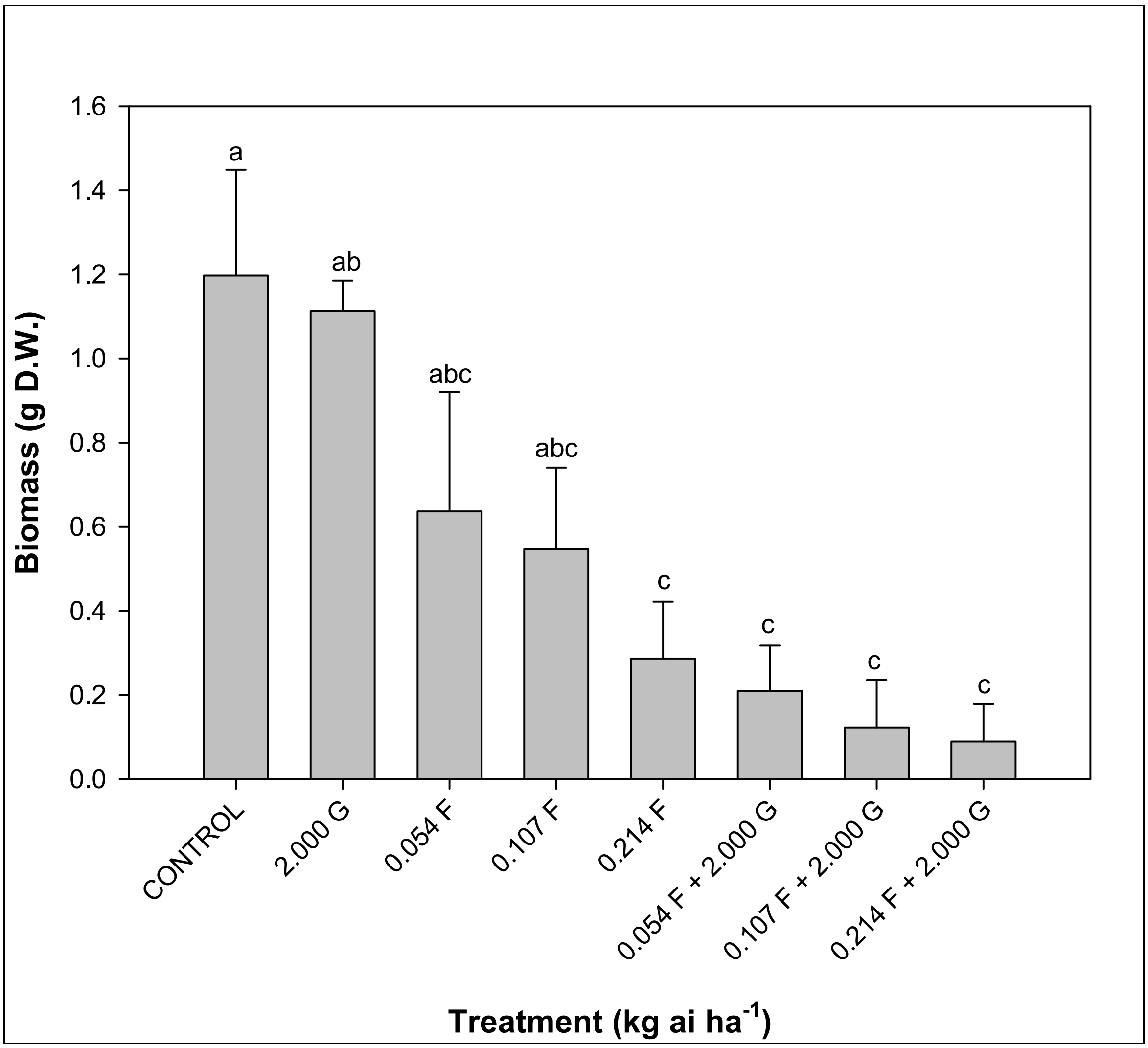

Figure 3. Mean (± SE) Japanese knotweed s.l. biomass 4 weeks after treatment (WAT) with flumioxazin (F) and flumioxazin (F) + glyphosate (G). Each mean represents the average of three replicate treatments. Means sharing the same letter do not significantly differ from each other. Data were subjected to a one-way analysis of variance and means were separated using the Student-Newman-Keuls Method (SNK; α=0.05).

Due to rapid foliar desiccation following application, flumioxazin has limited phloem movement (Senseman 2007) and is therefore not likely to impact the rhizomes of well-established plant stands. Flumioxazin alone may be more appropriate for rapidly controlling new infestations of Japanese knotweed s.l. before the rhizome system has had a chance to develop. Because glyphosate is translocated and accumulates in underground tissue (Senseman 2007), the flumioxazin + glyphosate combination treatment may be more effective for control of plants with established rhizomes. However, results from the present study did not show a difference between observed and expected responses; therefore, the flumioxazin + glyphosate combination is not synergistic but is additive (Table 1). An additive interaction means the two herbicides did not hurt or enhance each other and that the herbicides can be tank mixed. Tank mixing herbicides is cheaper and less time consuming then applying each herbicide separately.

Table 1. Calculating the herbicide antagonistic, synergistic or additive response of Japanese knotweed s.l. to combinations of flumioxazin (F) plus glyphosate (G) 4 weeks after treatment (WAT) and imazamox (I) plus glyphosate (G) 6 WAT. There were no significant differences between any of the observed and expected responses according to a Wilcoxin Rank Sum Test.

Herbicide (g ai ha^{-1})	Observed Response	Expected Response	Difference in Response	Interaction
0.054 F + 2.0 G	92.5 ± 7.5	77.8 ± 10.4	+14.7	Additive
0.107 F + 2.0 G	89.7 ± 9.5	57.6 ± 15.1	+32.1	Additive
0.214 F + 2.0 G	82.5 ± 9.0	50.7 ± 22.0	+31.8	Additive
0.125 I + 2.0 G	30.3 ± 14.4	42.9 ± 13.3	-12.6	Additive
0.250 I + 2.0 G	29.5 ± 10.4	46.3 ± 6.9	-16.8	Additive
0.500 I + 2.0 G	91.3 ± 8.7	94.2 ± 3.8	-2.9	Additive

<u>Imazamox Evaluation</u>: Some minor leaf discoloration was observed on imazamox and imazamox + glyphosate-treated plants two WAT. By four WAT, necrotic leaves were observed on plants treated with the highest rate of imazamox alone or with glyphosate and no new growth was observed. The two lower rates of imazamox alone or with glyphosate had new growth present at four WAT but the leaves were small when compared to the untreated control. At six WAT, the highest rate of imazamox and imazamox + glyphosate had brown stems, leaves and flowers. All other treated plants still had healthy green tissue present at the time of harvest. No symptoms were observed on the plants treated with glyphosate through six WAT. Compared to untreated plants, only the highest rate of imazamox applied alone and in combination with glyphosate significantly reduced Japanese knotweed s.l. biomass. The treatment effects were statistically similar, reducing biomass by an averaged 92 percent. All other treatments were similar to the untreated control (Figure 4).

Unlike flumioxazin, imazamox can translocate in both the phloem and xylem (Senseman 2007), impacting underground plant tissues. It is also safe to use near hardwoods (Burns 2009) and therefore may be a good option at Times Beach where Japanese knotweed s.l. occurs along the forest edge. Based on field trials, MI DNR (2012) recommends applying imazamox alone as a five percent solution. No information could be found on the effectiveness of imazamox + glyphosate against Japanese knotweed s.l. as an operational field application and the herbicide interaction in this study was additive

(Table 1). Because the addition of glyphosate to imazamox did not increase efficacy, it is likely imazamox alone would be effective at controlling Japanese knotweed s.l.

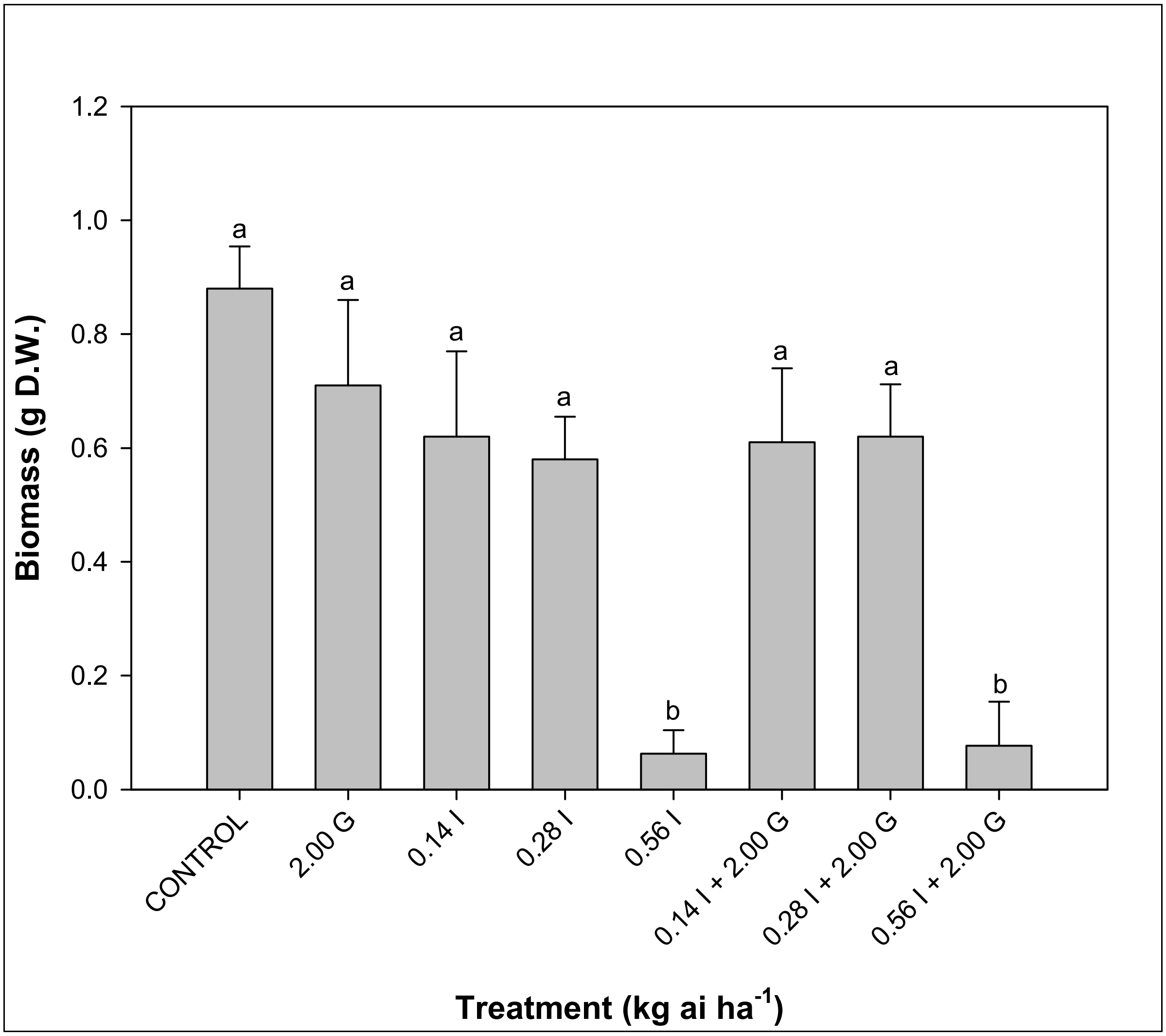

Figure 4. Mean (± SE) Japanese knotweed s.l. biomass 6 weeks after treatment (WAT) with imazamox (I) and imazamox (I) + glyphosate (G). Each mean represents the average of four replicate treatments. Means sharing the same letter do not significantly differ from each other. Data were subjected to a one-way analysis of variance and means were separated using the Student-Newman-Keuls Method (SNK; α=0.05).

As small greenhouse-grown plants can be more sensitive to herbicide treatment than plants in the field, the low rates of both herbicides evaluated in these preliminary studies were used to determine a dose response and are not recommended for use in the field. Given that the high rate (equal to half maximum label rate) of both herbicides with and without glyphosate provided 76 to 93 percent control of small greenhouse grown Japanese knotweed s.l. is a good indication that both products could be effective in the field and be potential alternatives to control Japanese knotweed s.l. at Times Beach.

FUTURE WORK: Field trials evaluating the efficacy of flumioxazin + glyphosate and imazamox with and without glyphosate on established stands of Japanese knotweed s.l. should be conducted. Genetic analysis of the Japanese knotweed s.l. population at Times Beach should also be conducted. There is currently no published work on the effect genotype has on herbicide efficacy. However, as more information becomes available, ascertaining which genotype is present at Times Beach could improve future herbicide recommendations.

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POINTS OF CONTACT: For additional information, contact the author, LeeAnn M. Glomski (972) 436-2215, *LeeAnn.M.Glomski@usace.army.mil*, or the Manager of the Aquatic Plant Control Research Program, Linda S. Nelson (601) 634-2656, *Linda.S.Nelson@usace.army.mil*. This technical note should be cited as follows:

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