City of Austin
WATERSHED PROTECTION

Arundo donax Herbicide Survival Assessment
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Abstract

Arundo donax *(giant cane) is a tall, perennial, reed-like grass which has been designated a prohibited exotic species by the Texas Parks and Wildlife Department. It is considered to be invasive in Central Texas especially in riparian areas. Patches of* A. donax *have been identified around Lady Bird Lake in Austin, TX and mapped using GIS. Four treatment methods, based on physical cutting of stalks and herbicide type, were evaluated based on their ability to control the patches around the lake in October 2011. Two herbicide solutions were used: imazamox alone on patches with proximal non-target vegetation and glyphosate/imazamox mix in monoculture patches, since glyphosate can impact non-target plants. Patches were grouped according to canopy cover, slope steepness, and distance from the water, and then revisited in 2012 when the number of live stalks and dead stalks were counted. The fraction of dead stalks was compared between treatment types and environmental conditions. Results showed that the environmental conditions did not significantly affect the fraction of dead* A. donax*, while the treatment methods did have a significant effect. The uncut* A. donax *patches sprayed with imazamox and the cut patches sprayed with a glyphosate/imazamox mix had significantly higher fractions of dead stalks. This suggests that the most effective herbicide treatments would be to either spray patches of uncut, full height* A. donax *with imazamox where there is proximal non-target vegetation, or to spray monoculture patches with the glyphosate/imazamox mix.*

Introduction

Giant cane (*Arundo donax*) is tall, perennial, reed-like grass which can grow to over 20 ft in height (Stuhlman 1947). It is believed that this plant is native to Asia (Mariani et al. 2010) but has been planted throughout the subtropical and warm-temperate regions of the world (Perdue 1958). *Arundo donax* (hereafter called *Arundo*) can grow in many adverse conditions but it is a hydrophyte and grows best along lakes, rivers, and other sites where abundant moisture is available (Mackenzie 2004). *Arundo* can use up to 2,000 L of water per meter of plant growth and grows at a rate of up to four inches a day (Perdue 1958; Iverson 1994; Khudamrongsawat et al. 2004). The plant spreads mainly by rhizomes, but can readily invade disturbed riparian zones through stems washed ashore during flooding or the spreading of cut stems left by mowing. Once established, this plant creates poor habitat for local wildlife. The stems and leaves contain materials such as silica, triterpenes, sterols, cardiac glycosides, and hydroxamic acid that make it unpalatable and possibly harmful to many organisms (Jackson and Nunez 1964; Chandhuri and Ghosal 1970; Ghosal et al. 1972; Zuñiga et al. 1983). *Arundo* can also affect watershed hydrology through its potentially high rates of evapotranspiration as well as channel morphology by reducing velocities and altering sediment transport (California Invasive Plant Council 2011). Unlike most riparian plants, *Arundo* is highly flammable throughout most of the year and can increase the risk of fire in a riparian zone (Scott 1994). *Arundo* has also been designated by the Texas Parks and Wildlife Department as a prohibited exotic species. For all these reasons, it is important to properly manage *Arundo* so that is does not dominate a riparian zone plant community.

A total of 3.5 acres of *Arundo* have been mapped along both sides of Lady Bird Lake's 5-mile shoreline. The City of Austin plans to eliminate this non-native species locally around the lake so that native species can re-establish in those patches. Elimination of *Arundo* may take multiple herbicide applications. As this is a one year study, the assumption is made that the treatment method which kills the highest fraction of *Arundo* within the study period will eliminate the *Arundo* with the least amount of applications over time and thus be the most efficient and cost effective method.

Three herbicides are currently used as the most effective control for *Arundo*: imazapyr, imazamox and glyphosate. Imazapyr and imazamox work by inhibiting the enzyme acetolactate synthase (ALS), a key enzyme in the biosynthesis of branched-chain amino acids such as leucine, isoleucine, and valine. Without these amino acids plant growth will stop within hours. These two herbicides have a low mammalian toxicity as the ALS enzyme is found only in plants (BASF 2008; Tu et al. 2004). Glyphosate is a non-selective herbicide that works by inhibiting the enzyme enolpyruvylshikimate-3-phosphate (EPSP) synthase, a key enzyme in the biosynthesis of aromatic amino acids such as phenylalanine, tryptophan, and tyrosine. Without these amino acids plant growth will stop within hours much like with imazamox. Unlike imazamox, the enzyme inhibition of glyphosate is not limited to strictly plants, but the EPA has declared glyphosate to be non-carcinogenic for humans (Miller et al. 2010).

While herbicide selectivity is often a function of concentration, treatment scale and timing, imazapyr is generally considered a non-selective herbicide. It is rapidly taken up through foliage and roots and is active in soil so it can impact adjacent desirable plants (Netherland 2009). Imazamox is more selective, being taken up rapidly through the foliage but very slowly through the roots, with many tree species showing tolerance (Burns 2009). They are both translocated easily by the plant from the foliage to the roots, allowing for better effectiveness when the plant is actively moving nutrients to the roots during late summer/early fall. Glyphosate is non-selective and is taken up by the foliage and translocated to the roots as well. It binds quickly to the soil so is not taken up by plant roots, but can impact adjacent plants through drift or overspray (Miller et al. 2010).

The potential to impact non-target vegetation was one of the greatest concerns to the City, due to the density of high value woody tree species in the lake's riparian zone, and the presence of these species in or near many patches of *Arundo*. Imazapyr can move between plant species via intertwined root grafts or movement of soil particles, so it may affect adjacent, non-target vegetation. It is recommended that imazapyr not be applied within twice the distance of the dripline or canopy of desirable trees (Tu et al. 2004). Due to this distance restriction, imazapyr was not used for any *Arundo* control along Lady Bird Lake.

There is no root uptake of imazamox by trees, making it safe to use around desirable hardwoods. Most of the *Arundo* patches on Lady Bird Lake are mixed with desirable woody species such as *Taxodium distichum* and *Acer negundo,* so imazamox alone was used in these patches.

Glyphosate has also been shown to be very effective in *Arundo donax* management (Bell 1997), but can impact non-target vegetation if contact occurs through foliar spray or drift. The primary reason for using a glyphosate/imazamox mix for *Arundo* control is the synergistic effect provided, which gives better results than either chemical used alone (J. Crosby personal communication, August 25, 2011). This mixture of glyphosate/imazamox was used on monoculture patches of *Arundo* with no adjacent woody vegetation.

Some treatment recommendations for *Arundo* management include a ‘cut, re-grow, spray’ strategy, where plants are cut to the ground, then allowed 3 to 6 weeks for re-growth to a 4 ft minimum height prior to foliar herbicide application. This practice may limit the potential for public exposure during application (since the plants are only 4 ft, not 30 plus ft tall), but it also reduces the amount of herbicide sprayed and absorbed by the plant on a per treatment basis and may require multiple follow up treatments for complete control. The cost of cutting and transporting the material for the ‘cut, re-grow, spray’ method is also far more expensive than the additional herbicide used to spray full uncut *Arundo* stalks. Thus it is important to the City to identify if these treatment recommendations result in faster, more effective *Arundo* control over simply spraying uncut *Arundo* with either imazamox alone or a glyphosate/imazamox mixture. This report defines which herbicide and treatment recommendations result in the most efficient and cost effective elimination of *Arundo* with the least negative impact on public use and the environment.

Methods

In 2011, volunteers with the LBJ Wildflower Center’s Citizen Scientist program mapped approximately 3.5 acres of *Arundo* along 5 miles of shoreline on Lady Bird Lake. The *Arundo* clusters were designated as 56 numbered patches. In August 2011, most of the patches were cut near ground level and allowed to re-grow prior to herbicide treatment. Due to the lack of precipitation following the cutting, growth of certain *Arundo* patches was very slow and thus the patches varied in height when the herbicide treatment occurred in October 2011. All but two patches were treated with 5% Imazamox by volume of product (product used is 12.1% active ingredient.) while the remaining two patches were treated with an imazamox/glyphosate mixture (2% to 1% by volume of product, respectively, with glyphosate product 53.8 % active ingredient). Both treatments included a 1% by volume methylated seed oil as a surfactant, and used a high pressure spray rig to deliver the herbicide. Patches of *Arundo* were thus placed in 4 treatment categories based on the height of stalks at the time of herbicide treatment, whether or not the patch was cut, and type of herbicide used in the treatment (Table 1).

Table 1: *Arundo donax* patch characteristic and herbicide for each treatment.

	Treatment 1	**Treatment 2**	**Treatment 3**	**Treatment 4**
Cut/Uncut	Cut	Cut	Uncut	Cut
Height of Stalk	< 4ft.	4 – 10ft.	> 10ft.	> 10 ft.
Herbicide	imazamox	imazamox	imazamox	imazamox/ glyphosate

Each patch was then classified according to canopy cover, steepness of slope, and distance from water. Patches were classified as having ‘closed’ canopy if there was greater than 50% canopy cover above the *Arundo* patch, while ‘open’ canopy patches had less than 50% canopy cover. ‘Steep’ slope patches had a slope greater than 60º and ‘gentle’ slope patches had a slope less than 60º. The degree of the slope was measured by clinometer. ‘Far’ patches were patches that were greater than 5ft from the edge of the water and ‘near’ patches were less than 5ft from the edge of the water. Thus patches were stratified into eight classifications (Table 2). Large patches of *Arundo* were sometimes designated as having two classifications. For instance, a patch could be both near and far if the patch of *Arundo* began at the bank and stopped 15ft away from the lakeshore.

Table 2: Environmental criteria for each classification.

Classification	Canopy Cover	Slope	Distance from Water
CGF	Closed (> 50%)	Gentle (< 60º)	Far (> 5ft.)
CGN	Closed (> 50%)	Gentle (< 60º)	Near (< 5ft.)
CSF	Closed (> 50%)	Steep (> 60º)	Far (> 5ft.)
CSN	Closed (> 50%)	Steep (> 60º)	Near (< 5ft.)
OGF	Open (< 50%)	Gentle (< 60º)	Far (> 5ft.)
OGN	Open (< 50%)	Gentle (< 60º)	Near (< 5ft.)
OSF	Open (< 50%)	Steep (> 60º)	Far (> 5ft.)
OSN	Open (< 50%)	Steep (> 60º)	Near (< 5ft.)

A design matrix was created using herbicide treatment for each patch and classification. When possible, two patches were inserted into this matrix for each combination of classification and herbicide treatment. Some herbicide treatments were limited to a small number of *Arundo* patches. For example, treatment 4 was limited to patch numbers 11 and 12. Thus these two patches were inserted into every classification for treatment 4. Also, larger patches were sometimes inserted more than once in the design matrix because they contained more than one classification. To avoid confusion, the chosen patches were designated as sampling units for each classification and herbicide treatment. Initially there were two sampling units for each classification within treatments; however, some classifications within treatments were sampled more than twice or only once due to complications in the field. The sampling units collected are displayed in Table 3. Figure 1 shows the location of each *Arundo* patch examined in the design matrix.

Table 3: *Arundo donax* patch numbers for each treatment and canopy/slope/distance from water classification.

Classification	Treatment 1	Treatment 2	Treatment 3	Treatment 4
CGF	52	20 and 42	22, 25, and 35	12
CGN	27 and 52	42 and 50	22 and 25	11
CSF	40 and 41	3 and 14	26	--
CSN	17	3 and 14	26	--
OGF	6, 21, and 27	29 and 48	22 and 25	11 and 12
OGN	6, 21, and 27	1 and 48	22 and 25	12*
OSF	33	4 and 7	31	--
OSN	16, 18, and 19	4 and 7	31	11 and 12

*Two samples were collected for classification OGN in patch 12.

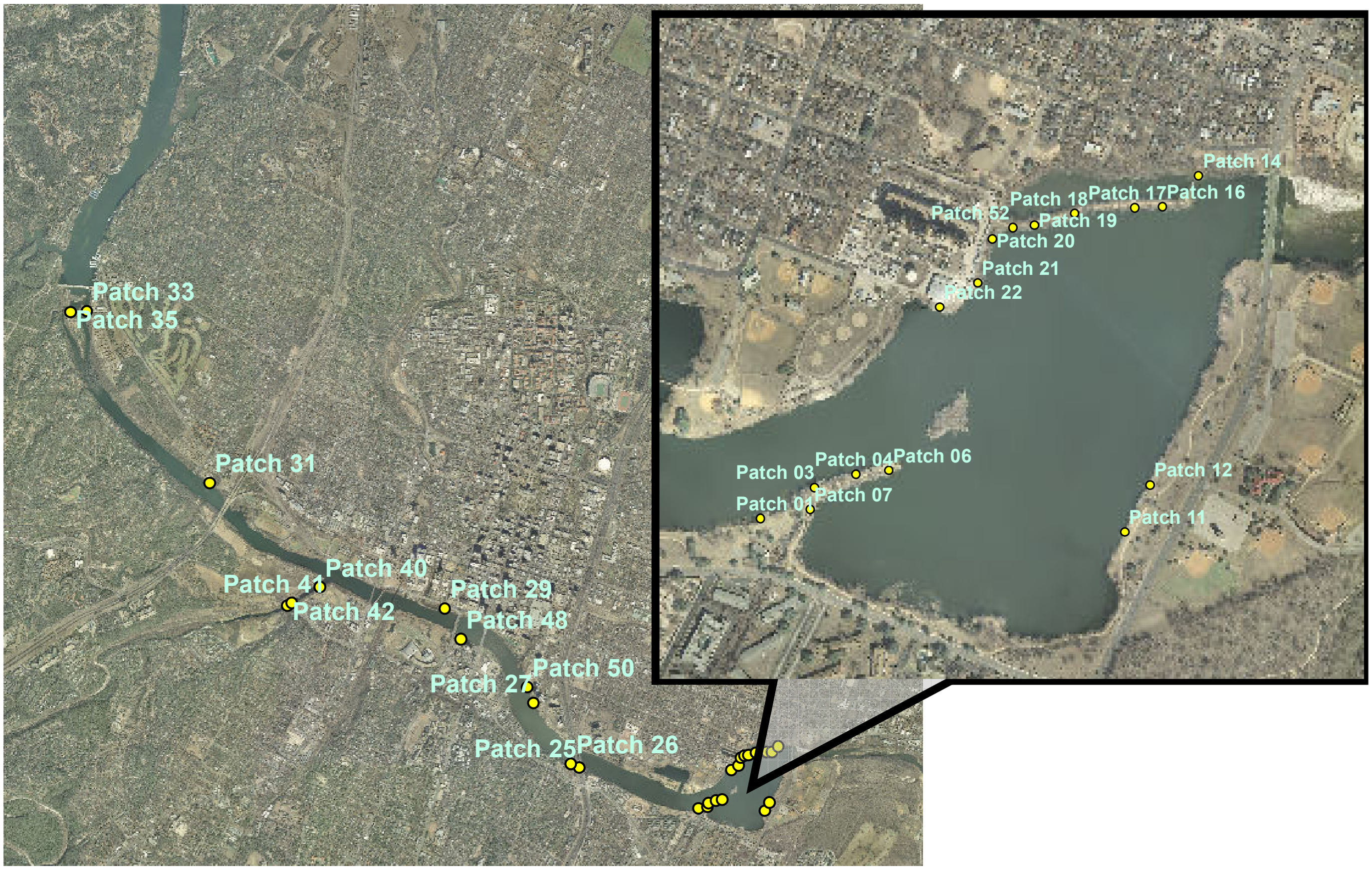

Figure 1: Map of Lady Bird Lake in Austin, TX, and the *Arundo donax* patches that were analyzed in this report.

For each sampling unit, data was collected within a 1 m^2 randomly placed quadrat. Data collection included the number of brown *Arundo* stems, number of green *Arundo* stems, and the height of each green *Arundo* stem. The brown stems were designated as dead while green stems were designated as alive. The fraction of dead stems was then calculated by dividing the number of brown stems by the total number of stems. While the height of each live stem was collected it was not used in this analysis. The height data was collected in response to a desire to measure growth rates for *Arundo* in these patches at some point in the future. Thus height of live *Arundo* stems will no longer be discussed in this report.

The primary analysis objective of this report is to determine whether there was a significant difference in the fraction of dead *Arundo* stems between treatment methods. A secondary analysis objective of this report is to determine whether the canopy cover, slope steepness, or distance from water measures have any effect on the fraction of *Arundo* killed by an herbicide treatment. Initial sample design was set up as a multi-factor randomized complete block design with replications within blocks. The primary factor considered in this block design was the treatment method followed by secondary factors of canopy cover, slope steepness, and distance from the water. The levels of the secondary factors were designated as the blocks in this design. Initial analysis chosen for this study was the Mack-Skillings test, which is a distribution-free test (Hollander and Wolfe 1999; Mack and Skillings 1980). Unfortunately, the complications encountered in the field led to an incomplete block design with some blocks having multiple replications and some blocks not having any data. Mack (1981) developed a technique to handle this more generalized design which involved calculating the Kruskal-Wallis statistic for each block between treatments and combining the Kruskal-Wallis statistic into one overall statistic (SKW). If the SKW is greater than some critical value based on the significance level in question, then there is a significant difference in the fraction of dead *Arundo* stalks between primary factor levels. In order to find this critical value, the user must either calculate their own null distribution based on the data, approximate the critical value using a chi-square distribution, or approximate the critical value using an F-distribution. Calculating a null distribution is cumbersome and only useful for that particular data set. The chi-square distribution is used for large sample approximations. When sample sizes within blocks reach 10, then the chi-square approximation begins to give an accurate critical value. Below this sample size the chi-square approximation tends to be far too conservative (Mack 1981), which means that the actual significance level achieved by the results is less than intended. Put another way, even if the test fails to designate a difference between primary factors there may still actually be a difference between the primary factors. With small sample sizes in each block, the F-distribution approximation has been shown to give the most accurate results (Mack 1981). For this technique to be mathematically sound, each block must contain the same number of observations. This now becomes a problem as there is not the same number of observations in each block in this study.

A different way to combine these blocks would be to combine the significance level produced by each Kruskal-Wallis test instead of the actual test statistic. Three well known procedures for combining significance levels include the Fisher's procedure, inverse normal procedure, and Tippett's procedure. It has been shown that the Fisher's procedure is the best to use when the number of treatments is greater than or equal to 3 for each block (Koziol and Perlman 1978). Thus the Kruskal-Wallis procedure was run on each block in the design and the p-value from each test was combined using the Fisher's procedure in order to decide if there was a significant difference in the fraction of *Arundo* killed between herbicide treatments. Fisher's procedure will conclude that there is a significant difference in fraction of *Arundo* killed between herbicide treatments if:

$$-2\sum_{i=1}^{n} \log P_i \geq X^2_{2n,\alpha}$$

where n is the number of blocks, P_i is the p-value from the i^{th} block's Kruskal-Wallis test, and $X^2_{2n,\alpha}$ is the upper α percentage point of the X^2_{2n} (chi-square) distribution. Similarly, Wilcoxon rank sum tests were performed on the data to test for differences in the fraction of *Arundo* killed between different canopy cover, different slope steepness, or different distance from water using Fisher's procedure to combine blocks. Finally, multiple comparison tests were performed on the data to determine which herbicide treatments were different from one another using the Dwass, Steel, Critchlow-Fligner procedure (Hollander and Wolfe 1999).

Results

Fisher's procedure performed on the Kruskal-Wallis significance levels for each classification resulted in a value of 33.5 to compare against a critical value based on the chi-square distribution. The value of $X^2_{2n,\alpha}$ is 32.0, with $n = 8$ and a value of 0.01 set for α. Thus there was a significant difference in the fraction of dead *Arundo* stalks between the treatments ($p < 0.01$). Multiple comparison tests showed that Treatment 1 and 2 were not significantly different from each other ($p > 0.2$); Treatment 1 and 3 were significantly different ($p < 0.0005$); Treatment 1 and 4 were significantly different ($p < 0.005$); Treatment 2 and 3 were significantly different ($p < 0.0005$); Treatment 2 and 4 were significantly different ($p < 0.005$); and Treatment 3 and 4 were not significantly different ($0.05 < p < 0.1$). Thus herbicide treatments 3 and 4 killed a significantly higher percentage of *Arundo* stalks compared to herbicide treatments 1 and 2 (Table 4). While there was no significant difference in the fraction of *Arundo* stalks killed between treatment 3 and 4, the p-value was between 0.05 and 0.1 and the sample size was small. A larger sample size might indicate a significant difference in the fraction of *Arundo* killed between these treatments.

Fisher's procedure performed on the Wilcoxon rank sum test significance levels resulted in values of 11.19, 6.55, and 8.72 for canopy cover, distance from water, and slope respectively. The critical value from the chi-square distribution with $\alpha = 0.05$ was 15.51 for each test. Thus there was no significant difference in the fraction of *Arundo* killed between differing canopy cover, distance from water, or slope ($p > 0.05$).

Table 4: Average percent dead *Arundo* stalks in each herbicide treatment for each classification. The percentage of dead *Arundo* stalks was significantly higher in treatments 3 and 4 compared to treatments 1 and 2.

	Treatment 1		**Treatment 2**		**Treatment 3**		**Treatment 4**	
Classification	**n**	**% Dead**	**n**	**% Dead**	**n**	**% Dead**	**n**	**% Dead**
CGF	1	48.6	2	30.0	3	66.3	1	35.7
CGN	2	35.7	2	0.0	2	72.5	1	97.4
CSF	2	28.0	2	11.5	1	75.9	0	--
CSN	1	48.6	2	28.2	1	58.8	0	--
OGF	3	24.5	2	37.0	2	57.3	2	86.5
OGN	3	21.4	2	50.0	2	71.8	2	100.0
OSF	1	17.9	2	11.9	1	87.0	0	--
OSN	3	57.2	2	18.2	1	76.0	2	100.0

Conclusions

From the results it was clear that herbicide treatment 3 and herbicide treatment 4 killed a higher percentage of *Arundo* regardless of environmental conditions. In treatment 3, the *Arundo* was uncut and sprayed with 5% imazamox by volume (as product, not active ingredient). The *Arundo* stalks were greater than 10 ft tall in these patches because they were uncut. In treatment 4, the *Arundo* was sprayed with a mix of 2% imazamox/1% glyphosate by volume (as product, not active ingredient). The *Arundo* in these mixed patches was initially cut but had re-grown to at least 10 ft before the herbicide was applied. While there was no significant difference between these two treatments, the average percent of dead stalks was 70% using imazamox for uncut *Arundo* and 88% using the glyphosate mixture. Given a larger sample size this difference may actually be significant showing that the glyphosate mixture kills the *Arundo* patches the most efficiently. Because the kill percentage was high using the imazamox for uncut *Arundo* and the use of glyphosate can be harmful to surrounding vegetation, further investigation should not be required. Glyphosate mixtures should be used in areas that are free from surrounding vegetation and imazamox should be used everywhere else.

Glyphosate and glyphosate/imazamox mixtures have been shown to be effective management tools for *Arundo* if applied correctly (Bell 1997; BASF 2008; Vollmer et al. 2008). Control levels have been as high as 100% using glyphosate alone (Bell 1997). Thus it was not surprising that the imazamox/glyphosate mixture killed a high percentage of *Arundo.* The issue is that glyphosate is a broad-spectrum herbicide, meaning that all plants in the application area will experience some level of die off. This type of treatment is unwanted in *Arundo* patches surrounded by woody vegetation, thus other treatment options were investigated.

Imazamox has also been shown to be an effective management tool for *Arundo*, and as the more targeted herbicide, it would be ideal for spraying patches of *Arundo* along Lady Bird Lake because of the preponderance of woody vegetation in and around the patches. However, if the imazamox did not kill a high percentage of *Arundo* along Lady Bird Lake and a high number of spray events were required to eliminate the *Arundo*, it might be better to kill the *Arundo* with one or two applications of glyphosate. The results indicated that the imazamox sprayed on the uncut stalks was not less effective than the glyphosate/imazamox mixture, but the imazamox sprayed on the cut stalks regardless of height was less effective.

There are two possible reasons behind the difference in percentage of dead stalks between the treatments. It may be that not enough herbicide was translocated to the roots or not enough herbicide was taken up by the shorter stalks in the first place. In order to eliminate *Arundo* successfully, the root mass must be killed. The imazamox must be translocated from the foliage to the roots after application. Translocation actively occurs in the fall as plants are sending nutrients to the root mass in preparation for winter dormancy. The herbicide was applied in October and should have been within this period of active translocation, but cutting the stalks can induce a growth-phase in the plants, where nutrients are drawn from the roots and less translocation of the herbicide to the roots occurs (Bell 1997). This could create a scenario where not enough herbicide was translocated to the roots in order to kill the plant. It is also possible that the cut stalks did not provide sufficient surface area to absorb enough herbicide, so *Arundo* did not take up enough herbicide to kill the plant. The difference in the amount of herbicide absorbed by the plant could have been the factor affecting the difference in percentage of dead stalks.

Regardless of the biological reason behind the higher percentage killed by the uncut treatment, it seems that a successful management plan for *Arundo* on Lady Bird Lake would be to not cut the stalks prior to spraying with imazamox in areas where there is surrounding woody vegetation. While the amount of herbicide needed is increased in the uncut treatment, the cost to treat the *Arundo* is actually less because the process of cutting is so time and labor intensive and re-growth is so erratic. The glyphosate mix could be used in areas where there is less surrounding vegetation.

While the patches treated with the glyphosate mix were initially cut, *Arundo* had re-grown to over 10 ft before the herbicide treatment. This was considered to be in the same height class as the uncut *Arundo*, thus cutting played little role in the glyphosate mix treatment. The time and resource requirements for cutting large areas of *Arundo* were not worth the small savings in herbicide use, so it is not recommended that plants be cut prior to spraying, regardless of herbicide type. It is also critical to have skilled, licensed professionals conduct very targeted applications when using any type of herbicide, to limit exposure time in public access areas as well as impacts to non-target plants.

Recommendations

Manage *Arundo donax* along Lady Bird Lake or other locations in Austin the using following methods:

- o Where woody vegetation is surrounding or mixed in with an *Arundo donax* patch, imazamox should be sprayed on uncut stalks at label rates.
- o Where woody vegetation is not surrounding or mixed in with an *Arundo donax* patch, a 2% imazamox/ 1% glyphosate (by product volume) solution should be sprayed on uncut stalks.

- All herbicide treatments should be performed between the post-flowering and pre-dormancy stages of the plant, usually between July and October.

- If further resolution is desired in the comparison of imazamox on uncut *Arundo donax* and the glyphosate/imazamox treatment, then future herbicide applications should be designed in such a way to analyze only that question. Since glyphosate can only be used in specific locations, it is not recommended that this resolution is ultimately required.

- Any herbicide treatment in or near any water body on City of Austin property must conform with the TCEQ general pesticide permit, which requires that application records be submitted to Watershed Protection Department IPM staff to allow inspection by TCEQ upon request.

- Any herbicide treatment in or near a public water body requires the submittal of a treatment proposal to Texas Parks and Wildlife Department.

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