Topping of Arundo donax as a Pre-Treatment to Biological Control

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ABSTRACT

Multiple methods have been employed to control invasive giant reed populations in the southwestern U.S., including modes of mechanical and chemical control, with little success of control at a landscape or temporal scale, except in localized areas at great cost. The recent release and successful establishment of biological control agents in the Lower Rio Grande Basin, which includes south Texas, represent a potential large-scale, long term, and inexpensive option, but little is known regarding the synergistic effects of mechanical control on host plant suitability for biological control. We hypothesized that timed cutting treatments can increase host plant suitability for biological control. We documented phenotypic, plastic responses of giant reed to cutting at different heights, to explore whether cutting can serve as an effective control measure and as a pre-treatment to biological control, particularly with the arundo wasp *Tetramesa romana*, which deposits eggs into shoot tips. Ground level cutting (mowing) is unsuccessful at reducing total biomass or height, whereas cutting giant reed at 1 m or more above gro und level (topping) reduces final height. Mowed areas were recolonized by emergent ramets that 9 months later had allocated 60% of total biomass to main shoots and achieved significantly more height gain than did plants cut to 1 m and 2 m. Topping at 1 m or more above ground level induced the production of lateral side shoots at the expense of upward growth. Plants left uncut, topped at 2 m or 1 m had 28-fold, 42-fold, and 8-fold more exit holes, respectively, than did plants cut to ground level, which typically had 0 to 2 holes per plant (F_{3,56} = 21.7, P < 0.001, site effect, $F_{2,56} = 0.5$, P = 0.60, interaction, $F_{6,56} = 2.9$, P = 0.01). Other factors, such as shoot tip tissue quality or wasp density might have also influenced the ability of field shoots to support arundo wasp populations. We discuss the implications of plant growth responses to different cutting treatments in the context of a large-scale biological control program.

Additional Index Words: giant reed, South Texas, integrated control, weed management

An aggressive rhizomatous invader, giant reed (Arundo donax L.) is considered one of the greatest threats to the health and value of riparian ecosystems in southwestern US and northern Mexico, with great negative impact to biodiversity and ecological processes (Cushman and Gaffney, 2010; Herrera and Dudley, 2003; Hong and Hu, 2007; Lambert et al., 2010; McGaugh et al., 2006). Giant reed has invaded a significant portion of the Rio Grande River basin, threatening water supply for agriculture and development in south Texas as a result of this weed's high evapo-transpirative capacity (Gowda et al., 2011; Watts and Moore, 2011). Additionally, giant reed presents considerable obstacles for the protection of the international border by law enforcement (Cleere, 2007) and agricultural inspectors, by both significantly

reducing visibility within the enforcement areas, and by providing favorable habitat for agriculturallydamaging cattle ticks (Racelis et al., 2012). Despite its wide range of ecological, economic, and agricultural implications, little success has been achieved in controlling giant reed. Until 2009, a combination of mechanical cutting and chemical control using herbicides was the only available option for decelerating giant reed invasion (Newhouser et al., 1999; Spencer et al., 2008), and this strategy involved high economic costs, due to the need for repeated treatments, and substantial negative collateral impacts due to applicator imprecision (Puertolas et al., 2010). These options are only available for local treatments and are thus not considered a wide-scale option for control of giant reed in the Lower Rio Grande Basin, in which giant reed occupies at least 11,000 ha of largely remote terrain (Yang et al., 2011). The recent release and establishment of biological control agents in Texas (the arundo wasp *Tetramesa romana* Walker), and the arundo armored scale (*Rhizaspidiotus donacis* Leonardi)) (Goolsby et al., 2011; Racelis et al., 2009) provide a long term, broad-scale control option for all areas of North America containing invasive giant reed populations.

Strategies to increase the speed and efficacy of biological control in the field may be needed. It is important in a biological control program to maximize settling, establishment, and proliferation of biological control agents by releasing agents when preferred tissues are most readily available on the plant (Briese, 2004; van Klinken and Raghu, 2006), either by timing field releases to match plant phenology (Spencer and Ksander, 2006), or by increasing the availability of feeding and reproduction sites through modification of plant architecture or chemistry, for example by topping or pruning weed ramets (see for example Bottrell et al., 1998; Corteseroa et al., 2000). Greenhouse, adventive range and field research site observations suggest that 'topping' of giant reed stems - cutting to heights $\geq 1m$ - can prematurely induce lateral (side)shoot initiation and growth (A.R. and P.M., pers. obs.). These lateral shoots are readily used both as sites of oviposition by the arundo wasp (Moran and Goolsby, 2009), and as settling sites for the arundo armored scale (Moran and Goolsby, 2010). The goal of the research described here was to determine whether the observed lateral shoot growth reaction of giant reed occurs under subtropical arid summer field conditions, and whether this reaction promotes increased host plant suitability for field applications of biological control of giant reed by the arundo wasp. By cutting field populations of giant reed at three different heights, we examined how giant reed responds to different modes of repeated cutting and the implications of these plant responses in the context of a large-scale biological control program.

MATERIALS AND METHODS

Site selection and treatment. Field populations of giant reed used in this study are located north of Laredo, Texas (N 27°36.201', W99°34.875'), where giant reed has infested large swaths (ca. 500 meters wide) of riparian habitat adjacent to the Rio Grande River (Yang et al., 2011). Soil types in the study area are dominated by deep, non-saline loamy soils (Sanders and Gabriel, 1985). The climate is considered to be hot semi-arid (Köppen climate classification BSh) (Peel et al., 2007), where monthly temperatures and rainfall respectively average 12.9 C and 2.3 cm in January, and 30.2 C and 3.3 cm in July (Sanders and Gabriel, 1985). These areas were historically dominated by vegetation characteristic of the Tamaulipan biotic province (Everitt et al., 2002), though few plant species other than giant reed were found in study area. The arundo wasp *Tetramesa romana* has been present at the site since at least 2007 (Racelis et al., 2009).

In February 2010, standing, mostly-dormant ramets of giant reed within the study area were mowed to the ground using a boom mower. This initial treatment was administered before the active growth period of giant reed, as measured by the accumulation of degree days described by Spencer and Ksander (2006). By May 2010, the plots were repopulated with freshly emerged ramets of giant reed at a density of 10.8 ± 3.2 stems/ m² with an average height of 327 ± 23 cm. At this time, three 20 m² plots were subdivided into four- 5 m^2 subplots for a total of 12 subplots. Subplots were subjected to one of four cutting treatments applied in a randomized block design, hereafter referred to as the treatment cut. Standing ramets within each subplot were cut with hand loppers (Fiskars[™], Madison WI) at 2 m, 1 m, or 0 m (ground level), and the remaining subplot left was uncut as a control. All cut material was left on the ground.

Data collection and analysis. Subplots were revisited in the fall (early November 2010) near the end of the active growth period (Spencer and Ksander, 2006). An average of the selected six ramets per subplot was used to compare differences among treatments in average stem height, lateral shoot length, and biomass allocation. These six randomly selected ramets were cut at ground level, taking care to avoid ramets located within 0.5 m of the plot edge, recording final main stem height (base to top-most node) and number of lateral shoots. Main stem height gain after treatment was determined for the cutting treatments by subtracting height at cut (e.g., 0, 1, 2 m) from final main stem height. Counts of lateral shoots were expressed per m final main shoot height to correct for variable main shoot height. Fifty lateral shoots of varying lengths were selected, their lengths measured, and then dried to allometrically estimate total lateral shoot length for each ramet (length of lateral shoots in $cm = (dry weight in grams +4.167)/0.0857, R^2 =$ 0.823).

In order to estimate differences in biomass allocation among treatments, each ramet was partitioned into four components--main stem, main stem leaves, lateral shoots, and lateral shoot leavesand dried to a constant dry weight for 48 h at 60°C in a drying oven (Salvislab©; Schweiz, Switzerland). To determine if cutting treatments promotes increased host plant suitability to the arundo wasp, stem components (including main and lateral stems) were inspected for evidence of wasp reproduction by counting adult exit holes. Counts of exit holes were divided by total shoot length (final main shoot length plus allometric regression-estimated lateral shoot length, based on lateral shoot weight). The distributions of all these variables did not meet normality requirements, so all data were converted to ranks and analyzed with Kruskal-Wallis analysis of variance with treatment and plot as main factors plus interaction term (6 plants sampled in each subplot, 3 subplots per treatment; n = 18 total plants sampled per treatment). A random factor consisting of individual plants nested within treatment was included in the model. Type 3 sums of squares and Tukey separation of least-square means of ranks were used to determine significance.

RESULTS

Treatments cut to ground level, 1 m, 2 m, or left uncut, showed differences in recovery of lost shoot height and biomass allocation to main and lateral stems after five months of regrowth. Shoots subjected to no cutting treatment averaged 4 m in height (Table 1). Final shoot height decreased predictably and significantly in shoots cut at 2 and 1 m. In both cases, 0.5 to 1.0 m height gain occurred, due to emergence of an axillary bud that grew straight upward. Shoots cut to ground level were 1.4-fold taller (in terms of total height) than those cut at 1 m and were equal to those cut at 2 m in final height (Table 1). Height gain was 3 -fold more in subplots cut to the ground than in those cut at 1 or 2 m, (Table 1).

Dry biomass of stems and leaves (pooled across main and lateral stems) decreased with decreasing summer cut height (= increasing severity of cut), with shoots in plots cut to ground level or 1 m in the summer respectively 60% to 70% lighter than those left uncut and 40 to 70% lighter than those cut at 2 m, respectively (Table 1). The proportional allocation of total biomass to leaf and stem tissue did not differ among the four cut treatments (Fig. 1; for both stems and leaves, treatment effect, $F_{3,56}$ = 2.4, P = 0.079; site effect, $F_{2,56}$ = 2.9, P = 0.03; interaction $F_{6,56}$ = 1.9, P = 0.09). Shoots from all treatments consisted of 63 to 70% stem tissue and 30 to 37% leaf tissue (Fig. 1).

However, both actual final biomass of main shoots and lateral shoots (stem and leaf tissues combined) and proportional biomass allocation to main and lateral shoot varied significantly among treatments. Main shoots in uncut plots had 2.4-fold more biomass than did main shoots on plants in any other treatment (Table 1). Plants from plots cut to ground level attained similar or greater main shoot biomass as did plants cut to 1 or 2 m (Table 1). Main shoot growth occurred at the expense of lateral shoot growth, since the biomass, number and length of lateral shoots was at least 93%, 54%, and 83% lower in plants cut to ground level than in plants cut to 1 m, 2 m, or uncut, respectively (Table 1), with no differences among these latter three treatments. Plants cut to ground level allocated 93% of their biomass to main shoots, significantly more than all other treatments (treatment effect, $F_{3.56} = 46.4$, P < 0.001, site effect, $F_{2.56} = 3.7$, P = 0.01, interaction $F_{6,56}$ = 2.2, P = 0.05). Plants left uncut allocated 60% to main shoots, significantly more than plants cut to 1 m (38%) and to 2 m (31%) (Fig. Percent biomass allocation to lateral shoots 1). showed the opposite, significant trend from main shoots. Stems cut at 1 or 2 m allocated 62% and 69%, respectively, of their biomass to lateral shoots (Fig. 1).

Exit holes made by emerging T. romana arundo wasps were found on ramets from all four cut treatments. However, the abundance and density of arundo wasp exit holes varied significantly. Plants cut to 2 m or left uncut in the summer had 42-fold and 28fold more exit holes, respectively, than did plants cut to ground level (Fig. 2), which typically had 0 to 2 holes per plant (F_{3,56} = 21.7, P < 0.001, site effect, F_{2,56} = 0.5, P = 0.60, interaction, $F_{6,56} = 2.9$, P = 0.01). Plants cut to 1 m in the summer had 8-fold more exit holes than those cut to ground level, but this difference was not significant. The four cutting treatments differed in the same way when exit holes were expressed as densities per m total shoot length (= final measured main shoot length + regression-estimated total lateral shoot length) ($F_{3,56} = 15.6$, P < 0.001, site effect, $F_{2,56} = 0.55$, P = 0.58, interaction $F_{6,56} = 2.3$, P = 0.05) (Fig. 2).

DISCUSSION

The results of this double-cut study (initial ground level cut followed by treatment cut) support prior evidence that giant reed displays high tolerance to mechanical control (Newhouser et al., 1999), and indicates differential allocation of resources to main and lateral shoot growth as one possible mechanism of tolerance, with concomitant effects on the suitability of giant reed plants for biological control by the arundo wasp. The results also provide insight for strategies to control giant reed mechanically using ground-mowing or above-ground cutting/topping equipment.

Subplots of giant reed responded to a treatment cut to ground level (mowing) by elongating main shoots faster than ramets in uncut subplots, and much faster than ramets topped to 1 m or 2. The relatively low weights of stem biomass in ground cut plots

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Table 1. Final height, height gain, dry weight (DW) of stem and leaf tissues (main and lateral shoots combined), main and lateral shoot DW (leaf and stem tissue combined)), and the weight, number and length (in cm per cm main shoot length) of lateral shoots (all mean \pm SE) in 5m² plots of giant reed (*Arundo donax*) in Laredo, Texas. All shoots were cut to ground level (mowed) in early spring, and regrowth shoots cut three months later under summer conditions to ground level, topped to 1 m, or 2 m height, or left uncut. Shoots were measured after five additional months.

Variable	Height of treatment cut ¹		Analysis of variance on ranked data (F P)				
	Ground	1 m	2 m	No cut	Cut treatment	Plot	Treatment x Plot
Final height (cm)	273.6 ± 19.7 b	192.8 ± 7.9 c	256.9 ± 5.9 b	401.1 ± 16.1 a	24.1, P < 0.001	2.6, P = 0.09	3.2, P = 0.009
Height gain (cm)	273.6 ± 19.7 a	92.3 ± 7.9 b	56.9 ± 5.9 b	ND3	26.8, P < 0.001	0.5, P = 0.61	2.2, P = 0.08
Total stem DW (g)	55.4 ± 8.8 c	$90.9 \pm 9.7 \text{ bc}$	166.0 ± 15.8 ab	227.1 ± 22.7 a	11.0, P < 0.001	0.03, P = 0.97	2.2, P = 0.055
Total leaf DW (g)	$24.0 \pm 3.7 \text{ b}$	$38.8\pm4.3~b$	93.4 ± 8.1 a	114.6 ± 17.1 a	17.8, P < 0.001	3.9, P = 0.03	5.6, P < 0.001
Main shoot DW (g)	$73.8 \pm 11.0 \text{ b}$	45.4 ± 3.4 c	$79.4 \pm 7.1 \text{ b}$	192.4 ± 16.9 a	32.6, P < 0.001	0.4, P = 0.69	4.2, P = 0.002
Lateral shoot DW (g)	$5.6 \pm 1.1 \text{ b}$	84.3 ± 11.2 a	180.1 ± 17.4 a	149.3 ± 22.8 a	15.9, P < 0.001	4.9, P = 0.01	4.2, P = 0.001
Number of lateral shoots / cm main shoot height	$0.033 \pm 0.008 \ b$	0.071 ± 0.008 a	0.094 ± 0.007 a	0.077 ± 0.009 a	6.9, P < 0.001	7.8, P = 0.001	0.9, P = 0.51
Length of lat- eral shoots/ cm main shoot height	$0.35 \pm 0.04 \text{ b}$	2.92 ± 0.44 a	4.10 ± 0.45 a	2.02 ± 0.27 a	11.0, P < 0.001	2.0, P = 0.15	3.3, P = 0.008

¹Letters indicate Tukey-corrected least-square mean separation of ranked data between summer cutting treatments ($\alpha = 0.05$) (6 shoots measured per subplot, 3 subplots per treatment, n = 18 shoots total). Means with the same letter are not significantly different.

²Degrees of freedom (df) for cutting treatment = 3, 56 except for height gain (df = 2, 42); df for plot = 2, 56 (for height gain, 2, 42); df for treatment × plot interaction = 6, 56 (for height gain, 4, 42). Significant *P*-values and corresponding mean comparisons in bold.

³ND, not determined; shoot height at time of second cutting treatment unknown, height gain not calculated.





Fig. 1. Percent allocation of giant reed plants to stem and leaf tissues (left axis, main and lateral shoots combined) and percent allocation to main and lateral shoots (right axis, stem and leaf tissues combined) in giant reed plants allowed to regrow for five months after being cut under summer conditions to ground level, 1 m, or 2 m, or left uncut.



Fig. 2. Exit holes made by emerging arundo wasp (*Tetramesa romana*) adults as counts per plant (left axis) and as counts per m shoot length (right axis, main and lateral shoots combined) on giant reed plants allowed to regrow for five months after being cut under summer conditions to ground level, 1 m, or 2 m, or left uncut. Means with the same lowercase (exit holes per plant) or uppercase (exit holes per m) letters are not significantly different in Tukey-corrected least-square mean comparisons of ranked data.

suggests that rapid main shoot elongation in these ramets reduced the thickness of stem walls, stem diameters, and possibly the number of leaves. Total resource allocation to stem and leaf tissue (across main and lateral shoots) did not differ among the four cut treatments. However, rapid height gain in main shoots in mowed subplots occurred at the expense of growth of lateral shoots, since proportional allocation to lateral shoots was far lower in ramets from the groundcut subplots than in ramets from 1 m, 2m, and uncut plants. The weight, number and length of lateral shoots were all greatly decreased by mowing. Partial loss of apical dominance in ramets topped to 1 m or 2 m in the summer, as evidenced by total shoot height gain under 1 m, did not lead to significant increases in lateral shoot biomass or length relative to plants left uncut. However, proportional resource allocation to lateral vs. main shoots was elevated by these two topping (1 m and 2 m) treatments.

Exit holes made by emerging arundo wasps were more abundant and dense on plants cut to 2 m or left uncut than on ground-mowed or 1 m-topped plants, indicating that plants and plots subjected to the former two treatments supported larger wasp populations. Wasps require young shoot tips for oviposition (Moran and Goolsby, 2009), and in field surveys in Laredo, Texas, most exit holes were found at the bases of lateral shoots (A.R. and P.M., unpublished data). Ramets subjected to ground mowing, in which where lateral shoots were the least available among the treatments thus supported significantly smaller wasp populations. Shoots topped to 1 m did not follow this pattern; despite producing numbers and biomass of lateral shoot biomass equivalent to those in uncut plants, exit hole abundance was not significantly higher than in shoots in mowed subplots. Other factors, such as shoot tip tissue quality, or microclimatic effects on adult female survival or oviposition site search effort, might have influenced the ability of plants, especially the 1 m topped subplots to support arundo wasp populations.

In our analysis, we found a significant interaction between site and treatment, indicating that mowing/ topping-based differences in the variables tested, including final main shoot height, number and length of lateral shoots, biomass percent allocation and exit hole counts per plant, may have been influenced by site conditions, including shading from neighboring trees, slope, or drainage. Plots were located within 200 m of each other and were thought to be relatively homogenous, but these extraneous factors could have influenced regrowth capabilities of individual ramets.

These results still have significant implications from a control perspective. First, the results demonstrate the ineffectiveness of a single spring ground cut as a treatment for management, as vigorous regrowth occurred in all three large plots within three months. Plants in the uncut subplots attained heights over 4 m by the fall sampling time and produced many lateral shoots, forming dense canopies that were visually similar to those in other plots that were never mowed (P. Moran, pers. obs.). Even combined multiple ground level mowing (ie ground level cut subplots) did not produce immediate benefits in terms of reduction in total biomass or height, as regrowth in these plots approached 3 m in height by early November. However, our findings suggest that multiple ground-level cutting may increase visibility into the stand, due likely to a comparative reduction in lateral shoot production. Visibility is an important metric when considering giant reed management, especially for law and agricultural enforcement along the border (Cleere, 2007; Racelis et al., 2012). Mowing followed by topping of shoots to 1 m is likely most efficacious in terms of improving visibility, as recovery of main shoot height is minimal, though with substantial resource investment in lateral shoots. For integrating mechanical with biological control, mowing followed by topping at 2 m appears to be superior to the other treatments for short-term visibility improvement and long-term establishment of biological control agents. Additional studies are needed to assess effects of different integrated regimes on recruitment and long-term survival of giant reed ramets, and also on other important implications of giant reed management, especially the conservation of water and biodiversity in arid and ecologically sensitive riparian areas.

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