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Impact of the biological control agent Tetramesa romana (Hymenoptera: Eurytomidae) on Arundo donax (Poaceae: Arundinoideae) along the Rio Grande River in Texas

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6   **Impact of the biological control agent *Tetramesa romana* (Hymenoptera: Eurytomidae) on**
7   ***Arundo donax* (Poaceae: Arundinoideae) along the Rio Grande River in Texas.**

8

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26   Five years post release of the arundo gall wasp, *Tetramesa romana*, into the riparian habitats of the lower
27   Rio Grande River, changes in the health of the invasive weed, *Arundo donax*, or giant reed, have been
28   documented. These changes in plant attributes are fairly consistent along the study area of 558 river miles
29   between Del Rio and Brownsville, TX, and support the hypothesis that the arundo wasp has had a
30   significant impact as a biological control agent. Plant attributes were measured prior to release in ten
31   quadrats at each of ten field sites in 2007, and measured again at the same undisturbed sites, 5 years after
32   the release of *T. romana*, in 2014. Above ground biomass of *A. donax* decreased on average by 22%
33   across the ten sites. This decline in biomass was negatively correlated to increased total numbers of *T.*
34   *romana* exit holes in main and lateral shoots per site in 2014 compared to 2007. Changes in biomass, live
35   shoot density and shoot lengths, especially the positive effect of galling on main and lateral shoot
36   mortality, appear to be leading to a consistent decline of *A. donax*. Economically, this reduction in *A.*
37   *donax* biomass is estimated to be saving 4.4 million dollars per year in agricultural water. Additional
38   impacts are expected as populations of the wasp increase and as other biological control agents such as
39   the arundo scale, *Rhizaspidiotus donacis*, become more widespread.

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42   **Key words**: biological control, giant reed, carrizo cane, invasive weeds, gall-forming wasp, pathogenic
43   landscape

44

1 **Introduction**
2 *Arundo donax* L. (Poaceae; Arundinoideae), also known as giant reed or carrizo cane, is a tall (2-10 m)
3 perennial grass native to the Mediterranean and Caspian Basins, Arabian peninsula and east to Indian
4 subcontinent (Hardion et al. 2014). It was likely introduced to North America from Spain during
5 colonization (Tarin et al. 2013) and is now a widespread invasive weed in the bi-national Rio Grande
6 Basin of the southwestern U.S. and northern Mexico (IMTA 2008). Globally, giant reed is also invasive
7 in South Africa, Australia, New Zealand, and the Cook Islands; biological control programs have been
8 initiated in these places (Space and Flynn 2002; Nel 2004;; Howell 2008; Sands et al. 2014). In the
9 riparian habitat along the Rio Grande, large areas of giant reed cause serious ecological impacts by
10 displacing native vegetation (Everitt et al. 2005; Yang et al. 2009, 2011, 2012; Racelis et al. 2012a) and
11 creating a pathogenic landscape which facilitates the invasion of cattle fever ticks from Mexico (Racelis
12 et al. 2012b, Esteve-Gassent et al. 2014). Giant reed also interferes with law enforcement activities along
13 the international border and competes for scarce water resources in an arid region already experiencing
14 extended drought and potential changes in rainfall patterns because of climate change (Goolsby and
15 Moran 2009, Yang et al. 2009, IPCC 2013, Texas Water Development Board 2015). Prior to 2009, and
16 still in large part today, *A. donax* has been managed in limited areas by costly mechanical and chemical
17 means (Vartanian 1998). Classical biological control may be the most cost-effective and sustainable
18 option for management of the weed over large areas, such as the Rio Grande and Nueces River Basins in
19 Texas, the Sacramento-San Joaquin Delta in California, and other arid watersheds (Tracy and De Loach
20 1999; Goolsby and Moran 2009, Lambert et al. 2010). A biological control program was initiated in
21 2005, and two insects, the arundo gall wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) and
22 the arundo scale, *Rhizaspidiotus donacis* (Leonardi) (Homoptera: Diaspididae) were released in 2009 and
23 2011 respectively (Racelis et al. 2010, Goolsby et al. 2011). A third agent, the leafsheath mining fly,
24 *Lasioptera donacis* Coutin (Diptera: Cecidomyiidae), is being evaluated for environmental safety and
25 efficacy.
26   *Tetramesa romana* is a shoot-galling wasp host specific to the genus *Arundo* and native to
27 Mediterranean Europe (Goolsby and Moran 2009). Female wasps reproduce via parthenogenesis and
28 deposit eggs into the shoots and lateral shoots of giant reed. Larval development induces gall formation,
29 affecting plant growth and development (Moran and Goolsby 2009, Moran et al. 2013). In even-aged
30 cohort studies in a greenhouse, galling of main shoots decreases main shoot length by up to 90% and, for
31 several weeks, induces increased production of lateral shoots (Goolsby et al. 2010). Aerial releases of the
32 European arundo wasps started in 2009 and by 2012 they were established along the entire length of the
33 Rio Grande between Del Rio and Brownsville, TX (Racelis et al. 2010). However, before release of the
34 European wasps in 2009, adventive populations of *T. romana* from unknown origins were detected with
35 very limited distributions along the Rio Grande within the urban areas of Eagle Pass and Laredo, TX
36 (Goolsby et al. 2009). In 2014, *Tetramesa romana*, primarily one single genotype, which matches
37 populations imported from Mediterranean Spain is now widespread on the Rio Grande and appears to be
38 having an impact on giant reed (Goolsby et al. 2014).
39   In 2007, prior to release of the arundo wasps from Spain, surveys were conducted along the Rio
40 Grande to determine the above ground standing biomass of giant reed. *Tetramesa romana* was not
41 present at any of the sites in this study in 2007. These same sites were revisited in 2014 to measure the
42 difference in biomass. Between 2007 and 2014, the Lower Rio Grande Basin experienced both historic
43 drought and floods. Although the weather conditions were variable during this time period, it should be
44 noted that the Rio Grande below Amistad Dam near Del Rio, TX is highly controlled, with steady

1   conveyance flows of water for downstream agricultural and municipal users (U.S. Dept. of Interior 2013).
2   Giant reed grows in the riparian zone where its roots can reach the shallow water table (Moore et al. 2010,
3   Watts and Moore 2011). Since hydrological conditions on the Rio Grande are fairly stable for giant reed,
4   the most obvious change in the environment during the study period has been the establishment of the
5   arundo wasp. This study is designed to measure the presumed impact of the arundo wasp on above
6   ground biomass and other plant attributes of giant reed.
7
8   **Materials and Methods**
9   **Field Sampling.** Above ground biomass of *A. donax* was measured at 10 sites on the Rio Grande in May
10 of 2007 and November 2014. These sites were located approximately every 50 miles between Del Rio
11 and Brownsville, TX and were representative of the 558 river miles between the mouth of the Rio Grande
12 and Amistad Dam. Stands of *A. donax* were mature with several years of growth, located in the riparian
13 zone, and in sites which had not been disturbed by mowing, burning, herbicide use, or a major flood in
14 July 2010. At each location, ten quadrats ($0.25m^2$) were haphazardly selected and the GPS coordinates
15 recorded (Garmin Etrex 20). A PVC frame was used to delineate the boundaries of the quadrat. In each
16 quadrat, all *A. donax* shoots (stems/canes) were cut at ground level, removed and measured. In 2007,
17 shoot lengths were measured and categorized as live or dead. Based on shoot dissections, as well as non-
18 destructive surveys of wasp exit holes on 50 lateral shoots per site, repeated several times in 2007, wasps
19 were not present at these sites in 2007. In 2014, we returned to the same sites, within a few meters given
20 the accuracy of the GPS unit, and made the same measurements, but also included shoot diameter and
21 number at the base of the main stem, numbers and lengths of side shoots at each node, number of arundo
22 wasp exit holes per side shoot, and numbers of exit holes on the main shoot. To calculate above ground
23 biomass, we used the formula developed by Spencer et al. (2006). Due to the bulk of the material
24 harvested, we used wet weights to assess biomass in both years.
25
26 **Data Analysis.** Biomass estimates were averaged across 10 quadrats with each site. Shoot height (both
27 live and dead shoots), live, dead and total shoot density, and the proportion live shoots per quadrat were
28 also averaged across quadrats, while exit holes were summed. Average plant biomass and density values
29 for each site were compared between 2007 and 2014 using generalized linear ANOVA models using SAS
30 9.4 (PROC GLIMMIX) (SAS Institute, 2012), with Gaussian distribution assumptions for all variables
31 except dead shoot density (exponential) and proportion live shoots. Binomial distribution models were
32 used for all analyses involving proportions). Otherwise, the distributions that were selected minimized
33 Akaike's Information Critical Criterion (AICC). A random site factor was included in these models.
34 Pearson correlations and, if significant, linear regression analyses were used to examine associations
35 between the total number of exit holes per site per m total shoot length (main and lateral shoots
36 combined) and the 2007 to 2014 change in biomass, as well as between exit holes and average live shoot
37 density per m^2. To examine site-to-site variation in wasp galling and the influence of galling on main and
38 lateral shoot size and mortality in 2014 data, similar models were used, but with a random factor of
39 quadrat nested within site. Due to non-normality of the data, models with log-normal distribution
40 assumptions were used for analyses of site effects on density of main shoot exit holes per m main shoot
41 length and lateral shoot exit holes per m lateral shoot length. These models were also used to examine
42 effects of main shoot galling on main shoot length and width, and on density of lateral shoots. Spearman
43 correlations were used to examine associations between the proportion of main shoots with galls and the
44 proportion of main shoots (galled or ungalled) with at least one galled lateral shoot; and also proportion

1 main shoots and proportion lateral shoots galled. A similar approach was used to test for correlations
2 between galled shoot proportion per quadrat and dead shoot proportion per quadrat, separately for main
3 and lateral shoots. Chi-square tests (maximum likelihood, SAS PROC FREQ) examined differences in
4 the proportion of galled vs. ungalled main or lateral shoots that were dead, considering the response of
5 each main shoot, (n = 821) or lateral shoot (n = 11,403), to galling individually.

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8 **Results**
9 **Pre and post-release comparisons of giant reed stands along the Rio Grande.** Average live biomass
10 per m^2, estimated from average live shoot height and live shoot density, declined by 22% between 2007
11 and 2014 across the 10 Rio Grande sites:, a significant decrease (Table 1). Eight of ten sites showed a
12 decrease over this time period (Fig. 1A). Quadrat averages for main shoot length (live and dead shoots
13 combined) did not vary pre-and post-wasp release, and differences in height (shoot length) pre vs post-
14 release did not show a clear trend across sites (Table 1, Fig. 1B). Similar results were found for lengths of
15 live shoots only (data not shown). Total and live shoot density did not vary between 2007 and 2014
16 (Table 1). The density of dead shoots increased 6.5-fold between 2007 and 2014 (Table 1), and the
17 proportion of live shoots per plot declined significantly (by 18%, Table 1). Eight of 10 sites showed a
18 decline in proportion live shoots (Figure 1C). Across the 10 sites, the decline in biomass from 2007 to
19 2014 was negatively correlated to the total exit hole density per site (per m summed live and dead main
20 and lateral shoot length) (r = -0.74, P = 0.014). Live shoot density per m^2 in 2014 was also negatively
21 correlated to summed exit holes per m length (r = -0.77, P = 0.009). Regressions of these two impact
22 measures on total exit hole density per site per m of total shoot length (galled and ungalled, live and dead
23 main and lateral shoots indicated negative relationships (Fig. 2).

24

25 **Effects of main and lateral shoot galling by *T. romana* in 2014.**
26 *Wasp damage levels*. Across 10 sites (100 quadrats), 11.4% of main shoots on average per quadrat had
27 exit holes indicative of main shoot galling by *T. romana* (lower and upper confidence limits, 8.3–14.4%).
28 From 2-27% of main shoots were galled at each site (Fig. 3A), and main shoot galling did not vary
29 between the 10 Rio Grande sites (F = 0.64, df = 9, 90; P = 0.7597). Exit hole density on galled main
30 shoots averaged 5.44 ± 0.67 holes per m main shoot length and did not vary among sites (Fig. 3C) (F =
31 1.01, df = 9, 39, P = 0.4517). The density of lateral shoots produced did not vary significantly between
32 main shoots that had been galled and those which had not (across all 821 shoots, total lateral shoot density
33 was 5.3 ± 0.2 per m main shoot length; live lateral shoot density was 2.2 ± 0.1 per m; galling effect, $P \geq$
34 0.49). An average of 47.3% of main shoots (galled or ungalled) in each quadrat had at least one galled
35 lateral shoot (lower and upper 95% confidence limits, 40.8%–53.8%). Site averages ranged from 1.4%–
36 73.0% (Fig. 3A), but despite this variation, lateral shoot galling did not vary significantly among sites (F
37 = 1.34, df = 9, 90, P = 0.2262). The proportion of main shoots that were galled per quadrat was positively
38 correlated to the proportion of main shoots per quadrat (galled or ungalled) that had at least one galled
39 lateral shoot (r = 0.26, P = 0.0086, n = 100), as was proportion of main shoots galled and proportion of
40 lateral shoots that were galled (summed across all main shoots per quadrat) (r = 0.37, P = 0.0002, n = 99).
41 The proportion of lateral shoots galled per quadrat averaged 13.9% (lower and upper 95% confidence
42 limits, 11.3–16.5%), ranging from 0.2–31.2% (Fig. 3B), but not varying across sites (F = 0.57, df = 9, 89,
43 P = 0.8191). Exit hole density on galled lateral shoots averaged 3.8 ± 0.7 holes per m lateral shoot length,
44 ranging from 0.1 to 14.3 holes per m, and varying significantly across sites (F=4.55, df = 9, 74, P <

1   0.0001) (Fig 3C). Two sites in Del Rio, one in Brownsville, and one in Eagle Pass comprised the group of
2   sites with the highest exit hole density on lateral shoots. Exit hole densities at each site on main and
3   lateral shoots are higher in Figure 3 than in the regressions in Figure 2, because density calculations in
4   Figure 3 considered only the total length of galled main or galled lateral shoots per quadrat, while
5   densities in Figure 2 reflect total shoot length (galled + ungalled).
6
7   *Effect of wasp galling on shoot size and death.* Main shoots that had been galled by *T. romana* were
8   significantly shorter (by 0.7 m or 23%) compared to ungalled main shoots (Table 2). Galled shoots were
9   approximately 3 mm thinner (21% thinner) in diameter than ungalled shoots (Table 2). The proportion of
10 galled main shoots per quadrat was positively correlated to the proportion dead main shoots per quadrat (r
11 = 0.34, *P* =0.0005, n = 100). On a quadrat basis, the proportion of galled shoots that were dead (0.28,
12 0.17–0.39 lower-upper 95% C.I.) did not differ from the proportion of ungalled shoots that were dead
13 (0.31, 0.23–0.39 95% C.I.) (*F* = 0.11, df 1, 46, *P* = 0.741). However, on the basis of 821 individual main
14 shoots, galled shoots were 10% more likely to be dead (33 of 96 total) than were ungalled shoots (178 of
15 725 total) (Table 3).
16   Galled lateral shoots were approximately 6 cm (1.4-fold) longer on average per quadrat than
17 ungalled lateral shoots: a significant difference (Table 2). When analyzed on the basis of individual lateral
18 shoots, galled lateral shoots were again longer (20.4 ± 0.49 cm, n = 2,012) than ungalled laterals (16.5 ±
19 0.21 cm, n = 9,391) (*F* = 73.7, df 1, 11401, *P* < 0.0001). However, when the analysis was restricted to
20 dead and live lateral shoots 10 cm or longer (to avoid inclusion of commonly-encountered broken-off
21 lateral shoots), galled (31.7 ± 0.68, n = 1,161) and ungalled (31.7 ± 0.35, n = 4,239) lateral shoots did not
22 differ in length (*F* = 0, df 1, 5398, *P* = 0.97). The proportion of galled lateral shoots per quadrat was
23 positively correlated to the proportion dead lateral shoots (r = 0.26, P =0.009, n = 99). On a quadrat basis,
24 the proportion of summed galled lateral shoots that were dead per quadrat (0.60, 0.53–0.66 95% C.I.) was
25 near-significantly greater than the proportion of summed ungalled lateral shoots that were dead per
26 quadrat (0.46, 0.40–0.51 95% C.I.) (*F* = 3.68, df 1, 79, *P* = 0.059). Across 11,403 individual lateral
27 shoots, galled lateral shoots were 17% more likely to be dead (1,336 of 2,010 total) than were ungalled
28 lateral shoots (4,610 of 9,393 total) (Table 3).
29
30
31 **Discussion**
32 Five years post-release of 1.2 million arundo wasps into the infested riparian habitats of the lower Rio
33 Grande River; we clearly see differences in the health of *A. donax*. These changes in plant attributes are
34 fairly consistent along the 558 river miles between Del Rio and Brownsville, TX comprising the study
35 area, and support the hypothesis that the arundo wasp is having a significant impact as a biological control
36 agent. These results are consistent with pre-release efficacy studies in greenhouses showing up to 90% of
37 shoot stunting and increased mortality of galled (Goolsby et al. 2010; Moran et al. 2013). Galling by
38 other *Tetramesa* wasps species attacking economic grass crops causes similar damage, including stunting
39 of shoots, and shoot 'lodging' (collapse) through breakage of galled regions (Holmes and Blakely 1971;
40 Dubbert et al. 1998; Shanower and Waters 2006). Dicot-galling insects have similar negative effects on
41 shoot height/length (Fay et al. 1996; Goolsby et al. 2004; Balciunas & Smith 2006; Djamankulova et al.
42 2008). Insect galls are metabolic sinks for plants that limit growth (Dennill 1988). Harris and Shorthouse
43 (1996) describe the attributes of a successful gall inducer in weed biological control. *Tetramesa romana*
44 fits all of the attributes that are applicable to a gall inducer of a grass, which include: 1) the gall is a

1 moderately powerful metabolic sink and galls persist over the entire growing season; 2) galls can be
2 produced on many parts of the *A. donax* plant and change plant architecture; and 3) galls do not have
3 stomata; 4) parasitism of the galls is extremely low in the introduced range. *Tetramesa romana*
4 (Hymenoptera: Eurytomidae) is the first *Tetramesa* species released in a classical biological control of
5 weeds program. However, other species of Eurytomidae have been evaluated for biological weed control
6 programs including a *Eurytoma* sp. stem borer mother-of-millions weed *Bryophyllum delagoense*
7 (Ecklon and Zeyher) Schinz in Australia (Witt et al. 2004), a *Eurytoma* sp. seed-feeder *for Asparagus*
8 *asparagoides* (L.) Druce (Liliaceae) in Australia (Kleinjan & Edwards 2006), and *Eurytoma attiva* Burks,
9 a seed feeder against *Cordia curassavica* (Jacq.) R. & S. in Malaysia, (Simmonds 1980). Based on their
10 narrow host specificity and demonstrated impacts, *Tetramesa* spp., especially gall-makers of grasses,
11 should be considered as biological control agents based.
12   The impacts of *T. romana* are subtle and have taken time to manifest on *A. donax*, a clonal grass
13 with large carbohydrate reserves accessible through its extensive and interconnected rhizome system (Kui
14 et al. 2013), which grows under optimum conditions along a river used for continuous conveyance flows.
15 Our studies focused on comparison before and after release of *T. romana* on above- ground plant
16 attributes and biomass. The increase in dead shoot density and concomitant decline in proportion live
17 shoots per plot by 2014 suggest that *T. romana* is reducing recruitment of live main shoots. Actual wasp
18 damage levels were 11% of main shoots and 14% of lateral shoots on average across the ten Rio Grande
19 sites, suggesting that wasp galling damage and its negative effects on arundo will continue to increase and
20 develop over time. Site-to-site variation was substantial, although not significant except in the case of
21 density of lateral shoot exit holes per m lateral shoot length. Climatic factors (Racelis et al. 2009) and
22 possibly edaphic factors related to soil nitrogen (Moran and Goolsby 2014) or plant water deficit (Moran
23 2015) from underlying hydrology of the riverbank could have led to variable shoot tip suitability for the
24 wasp. The variability across the field sites on the Rio Grande did not show a geographic pattern.
25 Significant impacts were documented in Brownsville in the subtropics near the Gulf of Mexico and 350
26 miles inland in the arid, warm temperate climate near Del Rio, TX. A previous study showed that *T.*
27 *romana* reaches highest densities during prolonged periods of sunny weather without rain, and weather
28 conditions in Texas since 2010 have been favorable for the wasp (Goolsby et al. 2014). If weather
29 conditions change, i.e. the occurrence of an El Niño event, which favors increased rainfall, wasp damage
30 levels could decrease due to the negative direct effects of high rainfall on wasp populations (Racelis et al
31 2009). However, there is great potential for resurgence of native riparian vegetation, especially with
32 increased rainfall, even in dense *A. donax* stands (Racelis et al. 2012a). Additional studies are needed to
33 document potential changes in the riparian vegetation related to *T. romana* impacts on *A. donax*; and to
34 determine if the variability in impact in some locations is temporary, or due to other underlying biotic or
35 abiotic factors. This information may be useful in other areas where *T. romana* is being released or is
36 proposed, including Arizona and California in the USA, the Cook Islands, New Zealand and South
37 Africa.
38   The fact that 47% of main shoots (galled or ungalled) on average at each site had at least one
39 galled lateral shoot suggests that the wasp is well- established at most or all of the ten Rio Grande sites.
40 The positive correlations between main shoot and lateral shoot galling suggest that the presence of lateral
41 shoots enhances *T. romana* establishment, and agrees with observations (J.A.G., P.J.M., and A.E.R.,
42 unpublished) that emerging wasps tend to begin ovipositing on or close to their parental plant, if shoot
43 tips are available. The incidence of lateral shoot galling exceeded that of main shoot galling in this study
44 and in other observations at Rio Grande field sites (Goolsby et al. 2014). In this field study, main shoot

1   galling did not enhance observed density of total or live lateral shoots per m main shoot length, in contrast
2   to Goolsby et al. (2010) for *A. donax*. An increase in lateral shoot formation was observed in some galled
3   dicots (Kurzfeld-Zexer et al. 2010). However, arundo greenhouse studies involved even-aged cohorts of
4   young (approximately one month-old) main shoots, which showed increased lateral shoot production for
5   six weeks after main shoot galling, after which lateral shoot production was similar to that on ungalled
6   main shoots. Topping of *A. donax* main shoots at 1 to 2m height produces an abundance of lateral shoots
7   (Racelis et al. 2012c), which are favored by the wasp. Therefore, mechanical topping and arundo wasp
8   releases could be integrated in areas along the Rio Grande where immediate control is needed, while
9   favoring wasp establishment for long-term control.
10   The negative correlations between exit holes and 2014 biomass, and between exit holes and live
11 main shoot density, and the subsequent increase in standing dead main shoots, can be attributed to
12 increased mortality of galled compared to ungalled main shoots, although the effect is subtle (10%
13 increase in main shoot death due to galling). Galling of lateral shoots similarly increased lateral shoot
14 mortality (by 17%). Death followed by breakage of main and lateral shoots, and subsequent declines in
15 live shoot density and live biomass, is expected to make *A. donax* stands easier to penetrate physically
16 and visually, as is observed in the native range in the presence of multiple insect natural enemies (Kirk et
17 al. 2003; Goolsby and Moran 2009; Goolsby et al. 2013). Visibility within the stand also relates to light
18 penetration of the plant canopy, which is critical to regeneration of native riparian vegetation. Additional
19 studies are needed to evaluate the change in plant canopy, from increases in mortality of main and lateral
20 shoots.
21   The establishment of arundo wasp in the lower Rio Grande River is producing multiple
22 environmental, political and water conservation benefits. The reduction in *A. donax* biomass will likely
23 allow native flora and fauna to return, which has many multi-trophic benefits environmentally (Racelis
24 2012a). Reduction in biomass increases within stand visibility, which allows for safer and more effective
25 law enforcement activities along the international border. Potential water conservation benefits were
26 estimated at the start of the program by Seawright et al. (2009). A current estimate was calculated using
27 the Seawright model for water conservation and value attributable to the 22 percent reduction in biomass.
28 This suggests a water savings of 6,593 acre feet due reduced consumptive use by *A. donax*, even
29 accounting for water used by regrowth of native riparian plants. Since the U.S. receives about 2/9 of this
30 water, availability to the U.S. would be 2,183 acre feet. This water, available annually, will increase over
31 time, as will the effectiveness and expansion of the biological control. The annual value of the water in
32 agriculture for the Bi-National Rio Grande Valley is an estimated $917,808, where the U.S. portion is
33 $303,848 and one acre foot is valued at $139. Given increasing water issues in the region, and a current
34 market price of $2,000 per acre foot, the value of the water savings for the U.S. would be $4.4 million per
35 year. Impacts from the arundo wasp and other biological control agents are expected to increase the
36 environmental, political and economic benefits realized by the biological control program.
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1

2 Table 1. Comparison of estimated biomass, average main shoot length (live and dead shoots), total, live
3 and dead shoot density and proportion live shoots before (2007) and five years after (2014) release of the
4 arundo wasp at 10 sites along the Rio Grande between Brownsville and Del Rio, Texas. Means with
5 differing letters are significantly different.

Variable	2007[1]	2014[1]	F, df[2]	P
Estimated biomass (m[-2])	111.0 ± 14.2 a	86.9 ± 12.6 b	100, 18	< 0.0001
Main shoot length (m)	3.01 ± 0.18	2.76 ± 0.28	0.53, 18	0.4747
Total Shoot density (m[-2])	29.7 ± 2.0	32.8 ± 2.9	0.77, 18	0.3910
Live shoot density (m[-2])	28.2 ± 2.0	24.4 ± 2.0	1.79, 18	0.1976
Dead shoot density (m[-2])	1.3 ± 0.7 b	8.4 ± 1.9 a	11.1, 18	0.0037
Proportion live shoots	0.96 (0.92-1.0) b	0.78 (0.69–0.88) a	10.9, 18	0.0039

6 [1]Values are mean ± SE, except mean and 95% lower -upper confidence intervals for proportion live shoots.
7 [2]Analysis results from generalized linear ANOVA models with normal distribution assumptions except for dead
8 shoot density (exponential) and proportion live shoot density (binomial). Denominator degrees of freedom shown.
9

10 Table 2. Main and lateral shoot lengths, and width of main shoots, in 2014 sampling of galled and non-
11 galled shoots at 10 Rio Grande sites.

Shoot size measure	Galled[1,2]	Ungalled[1,2]	F, df[3]	P
Main shoot length (m)	2.23 ± 0.16 b	2.97 ± 0.11 a	23.3, 47	< 0.0001
Main shoot width (mm)	11.4 ± 0.44 b	14.4 ± 0.45 a	23.5, 47	< 0.0001
Lateral shoot length	22.7 ± 1.5 a	16.6 ± 0.8 b	15.1, 79	0.0002

12 [1]Values are mean ± SE, except 95% lower and upper confidence intervals for proportion dead main and lateral
13 shoots. [2]Refers to main shoot galling for first three rows; lateral shoot galling for last two rows.
14 [3]Analysis results from generalized linear ANOVA models with log-normal distribution assumptions for main shoot
15 length and width, normal distribution for lateral shoot length, and binomial distribution for proportion dead main
16 and lateral shoots. Denominator degrees of freedom shown.
17

18 Table 3. Proportion of dead galled and non-galled individual main and lateral shoots.

Shoot size measure	Galled	Ungalled	χ^2	n	P
Main shoot	0.344	0.246	4.06	821	0.044
Lateral shoot	0.665	0.491	204	11,403	< 0.0001

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21

1   Figure Captions

2

3   Figure 1. Change in (A) live biomass (kg per m^2) estimated from average live shoot height and live shoot

4   density, (B) height (length) of live and dead shoots (m), and (C) proportion of live shoots per plot. Bars

5   represent mean of 10 quadrats per site ± SE (biomass and shoot height) or mean + upper 95% confidence

6   interval (proportion live shoots). Site abbreviations: BR=Brownsville, LI=Los Indios, LAR=Laredo,

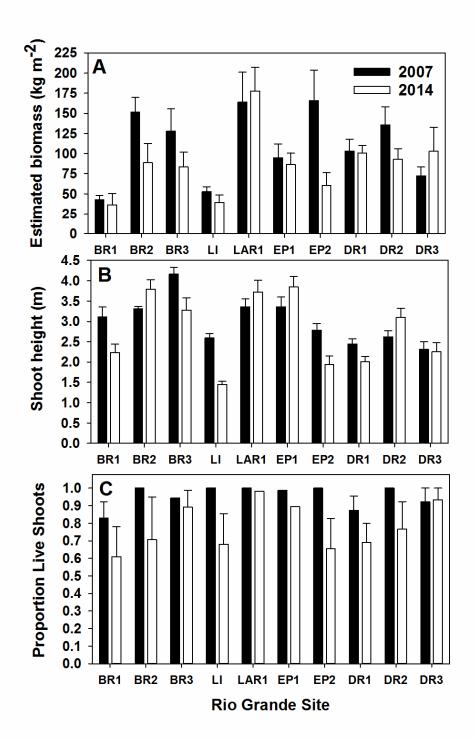7   EP=Eagle Pass, DR=Del Rio.

8

9   Figure 2. Regressions of the change in live biomass between 2007 and 2014 (A), and 2014 live shoot

10   density (B) on wasp exit holes per site per m shoot length, summed across live and dead main shoots and

11   all lateral shoots at each site.

12

13   Figure 3. Wasp damage to arundo shoots in 2014. (A) Average proportion (+ upper 95% confidence

14   interval, C.I.) across 10 quadrats per site of main shoots with main shoot galls (black bars) and of main

15   shoots (either galled or ungalled) with at least one galled lateral shoot (striped bars). (B) Average

16   proportion (+ upper 95% C.I.) of summed lateral shoots in each quadrat that were galled. (C) Average

17   (±SE) density of summed main shoot exit holes per galled main shoot length summed per quadrat (black

18   bars), and of lateral shoot exit holes per m galled lateral shoot length summed per quadrat (white bars).

19   Site abbreviations as in Fig. 1.

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