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Field impact of the arundo scale, *Rhizaspidiotus donacis* (Homoptera: Diaspididae) on *Arundo donax* on the Rio Grande

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ABSTRACT

The arundo scale, *Rhizaspidiotus donacis* is a biological control agent of the giant reed, *Arundo donax* (Poaceae: Arundinoideae), which is an invasive weed in the riparian habitats of the Rio Grande Basin of Texas, the southwestern U.S.A. and northern Mexico. Nine years after its release in 2010 in Texas, studies were conducted at selected sites with advanced populations to measure its impact on *A. donax*. The above ground biomass of stands of *A. donax* with both the arundo scale, *R. donacis* and the previously released and widely established arundo wasp, *Tetramesa romana* were compared to adjacent stands with only arundo wasp. Stands with arundo scale plus arundo wasp had significantly (55%) reduced biomass compared to stands with the arundo wasp alone. The arundo scale was found to be an effective biological control agent. Its slow dispersal rate from release sites on the Rio Grande River has limited its impact.

Additional index words: biological control of weeds, carrizo cane, post release evaluation

Arundo donax L., also known as giant reed, arundo or carrizo cane, is native to the Old World from the Iberian Peninsula of Europe, across the Mediterranean to south Asia, including North Africa, the Arabian Peninsula and the Caspian Basin. It has been cultivated in the Old World for thousands of years and has been widely introduced around the world as an ornamental, and for its fiber uses. Subsequently, it has become naturalized and invasive in many tropical, subtropical, and warm-temperate regions of the world. *Arundo donax* was introduced into North America from Mediterranean Spain in the early 1500s by colonists for use as roof thatching and quickly became naturalized (Tarin et al. 2013). It is now found throughout the southern half of the United States from Maryland to California but is most invasive in the southwestern U.S. and northern Mexico (Yang et al. 2011, Gowda et al. 2011). Carrizo cane is an invader of riparian habitats, including drainage ditches and irrigation canals throughout the Rio Grande River Basin of Texas and Mexico. *Arundo donax* has historically dominated these habitats where it competes for scarce water resources and reduces riparian biodiversity (Rubio et al. 2014). Arundo also facilitates the invasion of cattle fever ticks from Mexico into Texas and impedes law enforcement activities along the international border (Seawright et al. 2010, Racelis et al. 2012, Esteve-Gassent et al. 2014).

A binational biological control program between the U.S. Department of Agriculture, Agricultural Research Service (Edinburg, Texas) and Instituto Mexicano de Tecnología del Aguas (Jiutepec, Morelos, Mexico) (Martinez et al. 2017) was initiated in 2007. Additional funding was provided by the U.S. Department of Homeland Security, Customs and Border Protection because of the threat arundo posed to the operations of the U.S. Border Patrol working along the international border with Mexico. Biological control of the invasive carrizo cane with specialized insects from the areas of the native range of the weed with a similar climate, such as Mediterranean Spain, was considered to be the best long-term option for managing this highly invasive weed, because it is low-cost, sustainable and suitable for use in large areas such as the Rio Grande Basin (Seawright et al. 2010).

Two specialist biological control insect agents from the native range of carrizo cane in Spain, the arundo wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) and the arundo scale, *Rhizaspidiotus donacis* (Leonardi) (Homoptera: Diaspidae) were mass-reared, released and established in Texas and Mexico (2009 and 2012), as well as in California (2013) (Racelis et al. 2010, Goolsby et al. 2011, Goolsby et al 2014, Villarreal et al. 2016). The arundo wasp, *T. romana*, has caused significant impacts to *A. donax* along the Rio Grande between Del Rio and Brownsville, TX. By 2014, a 22% reduction in above ground biomass had occurred compared to pre-release measurements taken in 2009 (Goolsby et al., 2015). This reduction in cane density was calculated to be conserving 6000 acre-feet of water per year, valued at 4.4 million dollars (Goolsby et al., 2015). By 2016, biomass was reduced 32% from 2009, and density of the *T. romana* at field sites was positively correlated with regrowth of native riparian plants (Moran et al. 2017). Arundo wasp densities have remained high since 2011 along the Rio Grande, where releases of the European arundo wasp populations were made (Goolsby et al., 2014). *Tetramesa romana* population densities were 39 and 10-fold higher in Texas along the Rio Grande as compared to locations in the native range. This is largely explained by annual heat unit accumulation being 1.3- to 2.7-fold higher in Texas sites than the native range (Marshall et al. 2018).

The second biological control agent, the arundo armored scale, *Rhizaspidiotus donacis* (Leonardi) (Hemiptera; Diaspididae), was released in 2010 along the Rio Grande in TX and Mexico and was found to be established at multiple locations by 2011 (Goolsby et al. 2011). The arundo armored scale is host-specific to *Arundo* (Goolsby et al. 2009, Goolsby et al. 2013a) and has a significant impact on the shoot growth and rhizomes of *A. donax* in the native range of the scale in Mediterranean Europe (Cortés et al. 2011a, b). In Europe, the arundo scale was found to perform best in locations that were not subject to disturbance, especially summer mowing (Goolsby et al. 2013). The life cycle takes between 4.5 and 6 months at 27°C in a laboratory with two generations per year (Moran and Goolsby 2010). Populations of *R. donacis* released in the US were collected from *A. donax* in Rivesaltes on the Roboul River near Peripignan in southwestern France, and from Villafranqueza on the Rio Seco near Alicante, on the southeastern Mediterranean coast of Spain. Infested rhizomes were shipped to USDA-APHIS-PPQ quarantine facilities at Moore Airbase, Edinburg, TX, for rearing and processing. The arundo scale subsequently established at more than 50 sites along the Rio Grande in Texas and Mexico (Villarreal et al. 2016). Because of its lengthy life cycle, impacts of the arundo scale were expected to be slow to develop. In this paper, we measured the impacts of the arundo scale nine years post release at two of the main release sites on the Rio Grande, near Brownsville and Laredo, TX.

MATERIALS AND METHODS

Release of the arundo scale. The arundo scale *R. donacis* was released at Los Indios, TX (26.03136N, 97.7282W) and Laredo Community College, near downtown Laredo, TX (27.50361N, 99.5267W) from 2011-2013. Three release methods were used: release of neonate crawlers in gelatin capsules isolated from females in the lab, as described in Goolsby et al (2011); planting of potted arundo 'microplants' infested with adult female scales (Villarreal et al. 2016), spaced 1-5 m apart and kept watered for several months after planting; and release of adult females on cut stems with scale-infested side shoots from field-grown *A. donax* plants from a scale rearing colony at the former USDA-ARS Research Farm in Weslaco, TX. At the Los Indios and Laredo sites, 1,056,767, and 459,742 crawlers were released respectively. Freshly emerged crawler scales were taken from the laboratory to Los Indios by vehicle, whereas scale crawlers were flown to the Laredo airport in USDA-APHIS aircraft and then transported immediately to the field site by vehicle. In all cases, the crawlers were released adjacent to buds of new arundo shoots emerging from rhizomes at the soil surface. Regular sampling of the sites indicated the presence of abundant adult male scale covers on leaf collars on shoots above the points of release at each site, with subsequent spread to a distance of ca. 100 m at each site as of 2019.

Plot sampling. The arundo scale plus wasp sites were in areas where arundo scale had been released between 2010 to 2013 and represented the most advanced populations available for impact studies. The arundo wasp is widely established along the Rio Grande, so it was not possible to find sites with only arundo scale. All sampling for combined impact of arundo scale plus occurred in plots (3 m x 3 m) marked with PVC pipe located within 50 m of the Rio Grande. The arundo armored scale *R. donacis* is able to actively disperse only in the crawler stage over short distances (Moran and Goolsby 2010), and so sampling for combined arundo scale plus wasp impact at each site was restricted to the release areas where leaf collars with adult male scale covers, as well as side shoot bases with female and male scale covers, were readily observed in 2019. Two scale plus wasp plots were sampled at the Los Indios site, and three at Laredo (n = 5 plots total). Plots were spaced 5 to 10 m apart. The arundo wasp *T. romana* had previously spread throughout the sites (Goolsby et al. 2014; Moran et al. 2017). Three wasp-only plots at Los Indios and three at Laredo, located at similar distances from the Rio Grande as the scale plus wasp plots, but lacking evidence of arundo scale presence, were sampled (n = 6 plots total).

Plots were sampled as in Moran et al (2017) with modification to detect *R. donacis*. Three subplots, 0.5 x 0.5 m (0.25 m^2) were sampled with a PVC frame that was thrown haphazardly into the 9 m^2 sampling plot. Total live and dead main shoots were counted. All live main shoots were cut at the soil line and main shoot height (to the highest living meristem, on either the main shoot or side shoot) was determined. For each live main shoot, exit holes were counted as one sum across the main shoot and all side shoots. The total number of nodes (each containing one or more side shoots) was determined, as was the number of nodes with female scale covers, indicative of long-term reproduction and establishment of armored scale populations, and the number of dead nodes was counted (all side shoots either dead or broken off).

Data analysis. Live *A. donax* biomass per m^2 plot area was determined using an allometric equation modified from Spencer et al (2006), as used in prior studies of wasp impact (Goolsby et al 2015; Moran et al 2017). Live main shoot length was averaged and counts of live shoots summed across all three subplots per plot. The product of these two values was multiplied by 1.25 to scale to 1 m, squared to scale to 1 m^2 area, then multiplied by 14.254 and divided by 1000 to yield kg biomass per m^2. Percent main shoots per plot that were dead was determined, as was percent total side shoot nodes with scale, density of nodes with scale per m main shoot length, and proportion dead nodes. Density of arundo wasp exit holes per m main shoot length was also determined.

Data were analyzed with SAS 9.4 (SAS Institute, Cary, NC), using generalized linear models analyses of variance (ANOVAs) in SAS PROC GLIMMIX. Normality assumptions were tested with SAS PROC UNIVARIATE and were met for data for density of dead nodes and of arundo wasp exit holes. A log-normal distribution assumption was used for analyses of estimated kg biomass per m^2 to examine the impact of armored scale plus wasp vs wasp alone in 2019 on biomass. Binomial distribution assumptions were used for analyses involving percent dead main shoots and percent dead nodes. In all analyses, Type 3 least-square means were compared between wasp plus scale and wasp-only plots to examine the impact of the scale in 2019.

RESULTS

Biomass in plots with both the arundo scale plus wasp was 55% less than in plots with only the arundo wasp (Table 1). The percentage of dead main shoots and dead nodes was not significantly different in the presence of the armored scale compared to only the wasp. The mean percentage of stem nodes in per plot infested with arundo scale was 52.6% and density of infested nodes per meter length of stem was 3.9. The number of wasp exit holes differed slightly between treatments, but this difference was not significant.

Table 1. Effect of infestation by the arundo armored scale *Rhizaspidiotus donacis* in combination with the arundo wasp *Tetramesa romana*, in relation to infestation by the arundo wasp alone, on population vigor of *Arundo donax* across five (scale plus wasp) or six (wasp only) field plots at two sites adjacent to Rio Grande in south Texas. Effect of the scale measured as live aboveground biomass per m^2 (estimated from live main shoot length), percent dead main shoots and percent dead nodes (each consisting of one or more dead side shoot(s). Values for percent and density of nodes with arundo armored scale covers, and density of arundo wasp exit holes, are included as estimates of biocontrol agent population sizes. Differing letters after means indicate significant differences. Data represent means ± SE, except means ± 95% confidence interval for percentage data.

Variable	Armored scale + Wasp	Wasp Only	F[1]	P
Live biomass (kg per m^2)	26.4 ± 6.1 b	58.7 ± 11.6 a	6.20	0.0345
Percent dead main shoots (%)	56.4 ± 18.1	51.4 ± 19.3	0.03	0.8737
Percent dead nodes (%)	20.7 ± 4.0	19.2 ± 5.6	0.00	0.9519
Percent of nodes with scale (%)	52.6 ± 10.4	0	-	-
Density of nodes with scale per m main shoot length	3.9 ± 0.3	0	-	-
Density of wasp exit holes per m main shoot length	2.1 ± 0.5	3.9 ± 0.8	3.34	0.1008

[1]Results from analyses of variance; df = 1,9.

DISCUSSION

The arundo scale, *R. donacis* caused significant additional suppression of *A. donax* biomass, as compared to plots with only infestations of the arundo wasp, *T. romana*. Given the substantial impact of the arundo wasp (Goolsby et al 2015; Moran et al 2017), the combination of the two agents should produce significant long-term impacts to *A. donax* along the Rio Grande over time. The limiting factor for the arundo scale is its slow dispersal and lengthy life cycle. These two factors could be overcome by additional rearing and redistribution of the agents. Rearing and release of scale-infested microplants or cut stems of infested *A. donax* could be used to accelerate the impacts of this agent. Both approaches are successful, but the farm-raised or field-collected cut stem approach is less labor-intensive and has been shown to lead to armored scale establishment (Villarreal et al 2016; J. Goolsby, pers. obs.).

Our data on impact of the arundo scale from sites along the Rio Grande confirm studies conducted in the native range in Mediterranean Europe. In Europe, the scale reduced side shoot length by 50% compared to insecticide-protected plants (Cortes et al. 2011a). In Mediterranean Spain and southwestern France, scale-infested rhizomes were found to weigh significantly (about 50%) less than uninfested rhizomes (Cortes et al. 2011b). In Texas, Moran et al. (2017) found that density of the arundo wasp was correlated with higher biodiversity of the native riparian vegetation along the Rio Grande. We expect that the arundo scale is having the same effect on rhizomes in Texas which contributes to the reduced above ground biomass and should allow for more rapid decline of *A. donax* and revegetation by the native riparian plant suite along the Rio Grande. Further decline of *A. donax* on the Rio Grande will lead to additional water conservation, reduce habitat for invasive cattle fever ticks, and increase visibility of the international border for law enforcement.

At the field sites near Los Indios, and Laredo More than 52% of the stem nodes were infested, as compared to 82-100% of nodes at sites in Mediterranean Spain (Cortes et al. 2011b). This indicates the density of the arundo scale has not yet reached its maximum in Texas. In Europe we determined that arundo scale populations were highest in undisturbed stands (Goolsby et al. 2013). Summer tail-water from irrigation, herbicide applications and mowing were human -caused disturbances that reduced populations of the arundo scale and increased biomass of *A. donax* in Europe. Therefore, stands of arundo scale-infested *A. donax* in the Rio Grande Basin and other arundo- invaded regions should be left undisturbed to allow for maximum impact. In Texas, as well as in other invaded regions such as California (Giessow et al 2011), the arundo scale will likely perform best during years with dry summers (dry soil), as occurs in the climate of Mediterranean Europe, as crawlers and females are at soil level. Wet summers are not common in Texas, but when they do occur could lead to a surge in growth of *A. donax* without scale infestations. Multiple accessions of the armored scale from Spain and France were released, increasing the likelihood of adaptation to regional variations in climate. Similarly, the arundo wasp, also from Mediterranean Spain, is also negatively affected by prolonged periods of rain (Racelis et al 2009; Goolsby et al. 2015). Human induced disturbances, such as ground level mowing may destroy above ground populations of the arundo armored scale and stimulate new growth during a time of year when scale populations are sessile and not able to infest the new growth. Herbicide applications are likely to eliminate local arundo armored scale populations. Sites on the Rio Grande with scale populations (Villarreal et al. 2016) should be conserved for long-term survival and dispersal of this important biological control agent. The combination of the arundo wasp, *T. romana* and arundo scale, *R. donacis* will produce significant long-term benefits for agriculture, the environment and law enforcement.

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