Chapter 31 Biological Control of Arundo, an Invasive Grass Threatening Water Resources and National Security

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NON-TECHNICAL SUMMARY

A giant bamboo-like grass, known as arundo, giant reed, or carrizo cane in the United States, and scientifically as *Arundo donax* (Poaceae), was introduced from Mediterranean Europe for use in fences, roof construction, and erosion control. It has invaded riparian habitats (areas close to water) along rivers, creeks, arroyos, and lakes in the dry southwestern United States. Arundo consumes and wastes billions of gallons of water per year in the Lower Rio Grande Basin of Texas and Mexico, as well as in the Central Valley of California. Arundo also displaces native plants and animals, harbors crop and veterinary pests, blocks flood control systems, and fuels wildfires in the United States, as well as hindering border enforcement in Texas and thus threatening national security. A biological control program directed by the U.S. Department of Agriculture (USDA), Agricultural Research Service has led to the release and establishment of two insects against arundo. The first is called the arundo gall wasp, *Tetramesa romana* (Hymenoptera: Eurytomidae), a small black stingless wasp harmless to humans that lays its eggs in arundo shoot tips, resulting in galls inside which the wasp's larvae develop. Released wasps have established at over 25 sites along the Rio Grande River on the Texas-Mexico border, in central Mexico, and at a few sites in northern California. By 2014 (five years after release), feeding by the arundo gall wasp had reduced live arundo shoot weight an average of 22% across 10 sites, and this impact increased by an additional 32% (total of 54%) at five sites checked again in 2016 (seven years post release), allowing a 1.8-fold increase in plant diversity along the Rio Grande and potentially saving water valued at up to $10 million per year. The other released biological control agent, the arundo armored scale, *Rhizaspidiotus donacis* (Hemiptera: Diaspididae), is a tiny, mostly immobile insect that feeds on plant sap inside the tuber-like rhizomes of arundo. It was first released in 2011, and by 2020 it had established at over 25 sites in Texas and at seven sites in northern California. In 2018 (seven years after release), plots with both arundo scale and arundo gall wasp at two sites along the Rio Grande had live shoot weights that were only 45% that of neighboring plots with only the arundo gall wasp present. In addition to the above two agents, a tiny fly, *Lasioptera donacis* (Diptera: Cecidomyiidae), that mines arundo leaves has been approved for release, and studies on how to mass rear it are underway. The arundo biological control

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program has led to beneficial reductions of arundo reed stands, enhancing protection of water resources and improving visibility and national security along the U.S.-Mexico border. Further increases in arundo control over expanding areas are expected as this project continues.

HISTORY OF INVASION AND NATURE OF PROBLEM

The Species Invasion

Arundo, *Arundo donax* (Poaceae: Arundinoideae), is a giant grass that can be easily recognized by its large stature (typically 1–6 m or 3–20 ft; and up to 9 m or 30 ft) (Perdue, 1958) (**Fig. 1a**), its stiff, upright, plume-like flower stalks (inflorescences or panicles) (**Fig. 1b**), and its thick, solid, scaly, tuber-like rhizomes found on or just below the soil surface (**Fig. 1c**). It is native to western Mediterranean Europe, eastward through the Middle East and the Persian Gulf Region, on to India and Nepal (Hardion et al., 2014; Jimenez-Ruiz et al., 2021).

Hollow arundo shoots were and still are used to make woodwind musical instruments, for which the plant is still cultivated in France (Perdue, 1958; Tracy and DeLoach, 1999). As early as the 1500s, arundo was introduced to North America for use in roof construction and fencing (Dunmire, 2004). Arundo was also planted for erosion control on canal and river levees in the United States until the mid-1900s (Perdue, 1958; Frandsen, 1996; Tracy and DeLoach, 1999). This giant grass was already widespread in the Los Angeles Basin in southern California by the early 1800s (Bell, 1997; Dudley, 2000). Arundo spread rapidly from past places of cultivation into natural riparian habitats (areas adjacent to rivers, creeks/arroyos and lakes) and other wetlands in the arid southwestern United States. It now occupies at least 10,756 ha (26,579 acres) in the Lower Rio Grande Basin of Texas and northern Mexico (Yang et al., 2009, 2011) and 4,095 ha (10,120 acres) in California (Cal-IPC, 2020), with additional major infestations in the Upper Rio Grande Basin of New Mexico and along other rivers in New Mexico, Colorado, Arizona, Utah, and Nevada (**Fig. 2**).

The source of most of the invasive arundo in the southwestern United States was the Mediterranean coast of Spain (Tarin et al., 2013). In contrast, arundo in the southeastern United States came from the

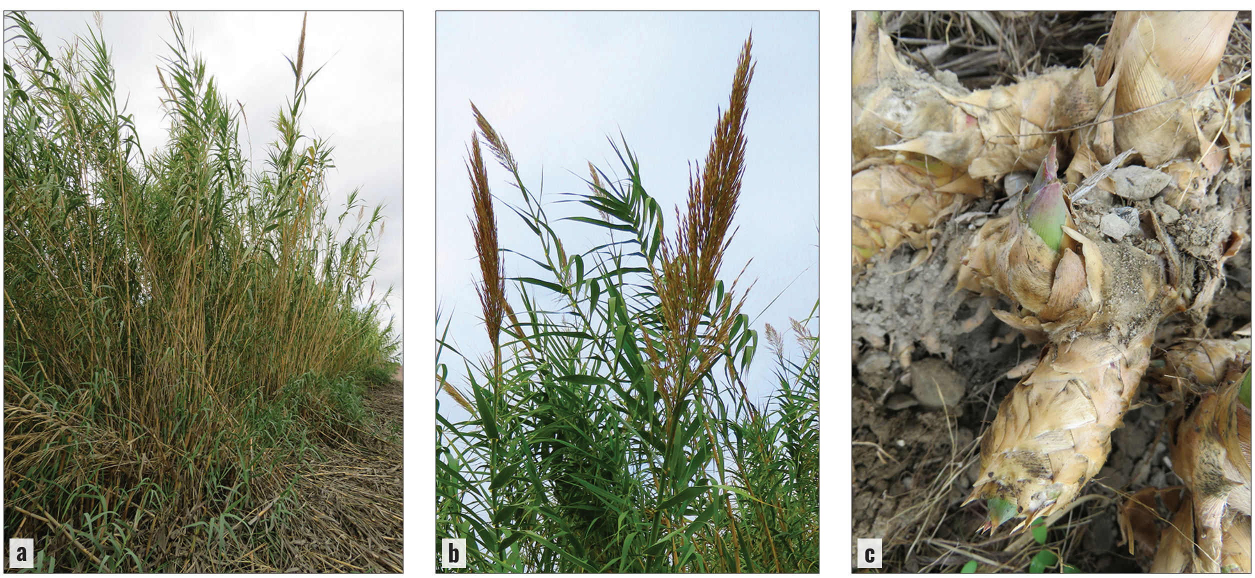

Figure 1. (a) *Arundo donax* stand on a canal bank in California. This species has characteristic (b) upright, plume-like inflorescences (panicles) and (c) thick, solid rhizomes (modified stems) that sit on or just below the soil surface and can be recognized by their dense, leafy, sharp-tipped scales and red/pink shoot buds. (a–c: K. Santa Caruz, USDA-ARS)

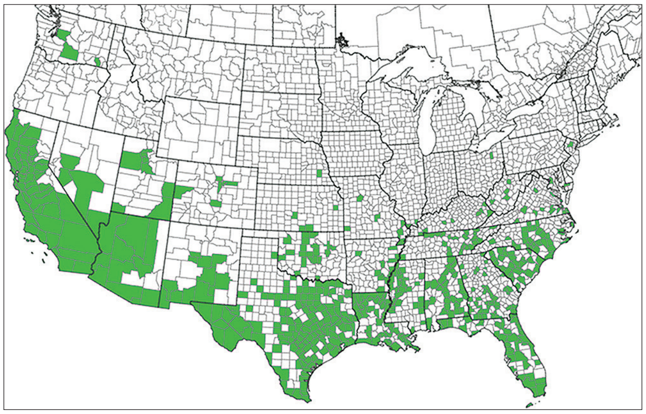

Figure 2. Occurrences of *Arundo donax* in the United States by county, showing also U.S. state boundaries. (EDDMapS, 2022)

Canary Islands (part of Spain) and the Seville area in southwestern Spain (Tarin et al., 2013). Arundo is not as invasive in the southeastern United States as in southern Texas to northern California (Tracy and DeLoach, 1999). Arundo is also found in isolated locations in states with a generally cold temperate climate (EDDMapS, 2022), including New Jersey, Maryland, Ohio, Nebraska, Colorado, Utah, and Washington (**Fig. 2**).

Arundo continues to spread through movement of vegetative propagules by water within watersheds and, between watersheds, as fragments attached to earth moving equipment (Decruyenaere and Holt, 2005; Wijte et al., 2005; Cal-IPC, 2020). Floods dislodge and move rhizomes and broken shoots. Both rhizomes and shoot pieces as short as a few cm (1 in) that bear axillary buds can quickly form roots and new shoots when deposited on moist sand, silt, or clay (Wijte et al., 2005; Boland, 2008; Mann et al., 2013) as arundo can grow in a wide range of soils (Quinn and Holt, 2008; Goolsby et al., 2013a). In the United States and beyond, arundo has been used or considered for use in wastewater treatment to remove heavy metals (Cristaldi et al., 2020) or as a biofuel (Lewandowski et al., 2003; Angelini et al., 2005; Mariani et al., 2010), and attempts to capitalize on such possibilities may contribute to further spread.

Nature of the Problem

Arundo consumes water in arid southwestern U.S. ecosystems, where water is scarce and is expected to become scarcer due to climate change (IPCC, 2021) as a result of decreased precipitation, altered water resource patterns (rainfall to snowpack ratios), and/or increased evaporation from soil and vegetation. Even in winter, arundo in the Lower Rio Grande Basin of Texas consumes and releases water to the air (evapotranspiration) at a level equivalent to corn in the summer in the southern United States (Gowda et al., 2011; Watts and Moore, 2011; Racelis et al., 2022). Indeed, water use by arundo occurs at levels that are higher than ever seen in most plants (Cal-IPC, 2020). Based on water use measured on individual plants, with instruments suspended over arundo patches, or with satellite imagery analysis, arundo has been estimated to consume an estimated 56.2 million m^3 of water (45,540 acre-ft) per year in the Lower Rio Grande Basin of Texas and Mexico (Seawright et al., 2009) and 37.8 million m^3 (30,086 acre-ft) per year in the Central Valley of California (Cal-IPC, 2020). The significant threat posed by arundo stands to water resources is recognized worldwide wherever it is invasive, including Australia (Haddadchi et al., 2013), New Zealand (Virtue et al., 2010), and South Africa (Versfeld et al., 2000; Nkuna et al., 2018).

In addition, arundo has other detrimental effects in the southwestern United States. It exacerbates fire hazards in riparian habitats (Frandsen, 1996), acting as a vertical fuel source and increasing fire intensity (Coffman et al., 2010). In the Central Valley of California, fire frequency in watersheds is positively correlated with the area of arundo, and in coastal watersheds in southern California, fires in arundo are known to promote fire spread to native riparian habitat (Cal-IPC, 2020). Fire aids arundo invasions because this weed recovers quickly, while native plants often recover more slowly (Coffman et al., 2010). Dense, monotypic arundo stands reduce biodiversity of plants (Bell, 1997; Quinn and Holt, 2008; Racelis et al., 2012a; Rubio et al., 2014), insects (Herrera and Dudley, 2003), and birds and reptiles (Tracy and Deloach, 1998; Dudley, 2000; Cal-IPC, 2020), as well as rare fish populations (Tracy and DeLoach, 1999), likely causing the extinction of one endemic Mexican fish species (McGaugh et al., 2006). Arundo facilitates invasion of a major veterinary pest, the cattle fever tick (*Rhipicephalus microplus*), along the Rio Grande (Racelis et al., 2012b). Arundo is considered an 'ecological transformer' species (Quinn and Holt, 2008; Racelis et al., 2012a) because it invades both human-disturbed and some undisturbed riparian habitats and converts them into habitats favoring its own further invasion. Arundo invasion alters stream geomorphology, converting shallow sandy, rocky, or silty emergent riparian floodplain habitats into narrow, deep channels with monotypic stands of arundo along their banks (Cal-IPC, 2020) (**Fig. 3**). When this process takes place in flood control channels, their ability to contain floodwaters can be reduced (Spencer et al., 2013). Arundo also harbors pathogenic fungi and viruses that can infect crop plants (Tracy and DeLoach, 1999). Finally, arundo interferes with law enforcement activities by reducing visibility along the international border between the United States and Mexico (Goolsby et al., 2017a), and it screens transient occupancy and other illegal activities along rivers in the Central Valley of California, exacerbating fire hazards (Cal-IPC, 2020).

Figure 3. Prior to biocontrol, dense *Arundo donax* stands converted sandy and silty floodplains along the Lower Rio Grande on the U.S.-Mexico border to narrow deep channels, depriving native plants and animals of habitat. (J. Goolsby, USDA-ARS)

WHY CONTROL THIS INVASIVE SPECIES?

Arundo is a deep-rooted perennial grass that remains green year-round in subtropical habitats such as those in the Rio Grande Valley of South Texas (Gowda et al., 2011), while senescing (leaves turning brown) in the winter in more temperate parts of its range, as in northern California (Spencer et al., 2005; Thornby et al., 2007; Spencer, 2012). Either with or without winter browning, first-year canes without branches sprout many lateral branches beginning in winter or early spring and throughout the second and third years. Young shoots can grow as fast as 10 cm (4 in) a day, among the fastest growth rates known in plants (Perdue, 1958; Bell, 1997). Dead canes remain standing for years, contributing further to the thickness of arundo stands. The most common methods used to control arundo are herbicide application, mechanical removal, mowing, and fire. Glyphosate and imazapyr, applied alone or in combination from the ground or air, can kill 90% or more of shoots and be temporarily effective, but regrowth occurs, requiring follow-up treatment (Spencer et al., 2009, 2011; Bell, 2011; DiTomaso et al., 2013). Reductions in arundo after chemical control can be sustained for three or more years after treatment (Lawson et al., 2005; SEC, 2019), but this method is usually cost-prohibitive for large areas. Mechanical removal and mowing also provide only temporary benefits, as they must be done repeatedly (e.g., every year for five or more years), often in hazardous, steep terrain

(Bell, 1997; Goolsby et al., 2019a). Physical control with fire is ineffective because arundo uses nutrients released by fire and re-grows rapidly (Coffman et al., 2010). None of these control methods are selective for arundo; all threaten native plants and the animals that depend on them. These methods are also very costly; over $100 million has been spent in California for control of arundo, and the expected total cost to control arundo chemically in the Central Valley is $70 million (Cal-IPC, 2020). Large-scale programs in Texas have incurred similar costs (Seawright et al., 2009; J. Goolsby, pers. obs.). Sustainable control is urgently needed; the expected benefits to water, improved riparian habitat, fire prevention, and protection of rare, threatened, or endangered species in California's Central Valley are estimated at $115 million (Cal-IPC et al., 2020).

Given the low sustainability of large-scale chemical or mechanical control activities, a biological control program against arundo was developed (Goolsby and Moran, 2009; Goolsby et al., 2009a). Arundo is the first grass species in the world targeted for classical biological control for which actual field releases of non-native, introduced insects have been made (Sutton et al., 2019). Grasses had long been thought of as being unsuitable for biological control because their 'simple' architecture would likely host few host-specific herbivorous insects (Evans, 1991). However, in recent decades this assumption has been proven wrong, based on studies of shoot-feeding insects (Tscharntke and Greiler, 1995; Tewksbury et al., 2002; Sutton et al., 2019, 2021).

Successful biological control of arundo was projected to potentially save water valued at $3.5–5 million per year in the Lower Rio Grande Basin of Texas, in 2009 dollars (Seawright et al., 2009), and successful control using chemical and mechanical methods in the Central Valley of California was projected to save at least 46,700 m^3 of water per ha (or 15.4 acre-ft per year per acre of arundo). The goal of this biological control project was to reduce the ability of new arundo shoot tips to survive, thus reducing stand density, and to reduce the ability of rhizomes to spread the plant vegetatively by producing new shoots. A key anticipated outcome was reduction of arundo stands to facilitate recovery of native vegetation, as studies near Laredo, Texas that simulated live shoot reduction in plots by hand removal, led to the emergence of native plants from seed banks (Racelis et al., 2012a; Rubio et al., 2014). Additional desired outcomes included reduction of water use by arundo and improved visibility along the U.S-Mexico border for law enforcement.

PROJECT HISTORY THROUGH AGENT ESTABLISHMENT

Efforts to control arundo biologically began in the 1990s with a review of its known herbivores (Tracy and DeLoach, 1999). Tall perennial grasses like arundo are often hosts for many internal leaf-mining, shoot-boring, or root-boring flies, sawflies, gall wasps, or beetles. Known external feeders include leaf- or stem-chewing or sucking insects such as aphids, scales, and true bugs (Hemiptera), as well as various beetles and moths (Tewksbury et al., 2002; Lambert et al., 2010). Surveys of arundo in the United States found very few herbivores (Tracy and DeLoach, 1999). An adventive (meaning self-introduced at an unknown time; in this case, probably accidentally-introduced) aphid, *Melanaphis donacis* (Hemiptera: Aphididae), is present in California (Dudley et al., 2008; Moran, 2020), but not in the Lower Rio Grande Basin. It is unsuitable for biological control, as it is known to feed on common reed, *Phragmites australis*, in its native range (Tracy and DeLoach, 1999) as well as plants in the rose family (Blackman and Eastop, 2018). It is widely distributed in its native range (Amin et al., 2019) and in other areas invaded by arundo, including Argentina (Ortego et al., 2004) and Chile (Undurraga et al., 2020). Shoot tip-borers have been observed in California (Dudley et al., 2008), and the native fly *Chaetopsis massyla* (Diptera: Ulidiidae) was observed in both greenhouses and less commonly in the field in Texas (Goolsby and Mangan, 2010).

For the purposes of this project, the native range of arundo was considered to run from India and Nepal through the Persian Gulf and Middle East to western Mediterranean Europe and the northern coast of Africa (Moran and Goolsby, 2009). Some authors, based on genetic evidence, restrict the native range to India, Nepal, Pakistan, and Afghanistan (Jimenez-Ruiz et al., 2021), but we found insect diversity on arundo to be highest in the Mediterranean region. A literature survey (Tracy and DeLoach, 1999) found only one potentially host-specific (monophagous) insect feeding on arundo in its native range, the shoot-tip-galling wasp *Tetramesa romana* (Hymenoptera: Eurytomidae), which had been reported from Europe. Field surveys in India and Nepal were conducted in 2000 (Stelljes, 2001) by R. Carruthers, A. Kirk, and T. Widmer, of the USDA and T. Dudley of the University of California, Santa Barbara. Kirk surveyed many other areas in both the native range and invaded Asian, South African, and Australian ranges. Climate match modeling indicated that the climate of Valencia in Mediterranean Spain showed a strong match to the climate of the Lower Rio Grande Basin (Goolsby and Moran, 2009), and genetic analyses indicated that the origin of most southwestern U.S. populations of arundo was the southern or eastern coast of Spain (Tarin et al., 2013). Intensive surveys were, therefore, carried out in this region, which led to the importation and testing of several candidate biological control agents in quarantine laboratories in the United States between 2007 and 2013.

Tetramesa romana, a Shoot Tip-Galling Wasp

Tetramesa romana (Hymenoptera: Eurytomidae) is a shoot-tip galling wasp on arundo, and it is referred to here as the arundo wasp. This wasp is a small (5–7 mm or ⅛–¼ in) (Moran and Goolsby, 2009) insect (**Fig. 4**). The arundo wasp was found in Spain, southern France, Italy (including Sicily), Morocco, Egypt, Greece (including Crete), Turkey, and Bulgaria in the native range (Goolsby and Moran, 2009). Adventive arundo wasps were also found as localized populations in Laredo, Texas along the Rio Grande (Racelis et al., 2009); Austin, in central Texas (Goolsby et al., 2009a); southern California (Dudley et al., 2008); South Africa (Canavan et al., 2019); and China (Goolsby and Moran, 2009). The genus *Tetramesa* includes over 200 species in Europe, Asia, Africa, Australia, and the Americas, most (or all) of which feed on one or just a few grass species (Claridge, 1961; Al-Barrak, 2006). The adventive species *Tetramesa phragmitidis* is present in the United States on common reed and appears to feed only on this grass (Tewksbury et al., 2002). Two *Tetramesa* species are under evaluation in South Africa for control of giant rat's tail grass (*Sporobulus pyramidalis*) in Australia (Sutton et al., 2021), and a new *Tetramesa* species was recently described from medusahead (*Taeniatherum caput-medusae*) in Greece (Lotfalizadeh et al., 2021).

Figure 4. The arundo wasp (*Tetramesa romana*) adult female injects eggs into an arundo shoot tip. (J. Goolsby, USDA-ARS)

Tetramesa romana females emerge with developed eggs which are deposited into shoot tips, and so they can reproduce without mating (Goolsby et al., 2014). Each female can produce an average of 12–25 offspring on arundo (Goolsby et al., 2009; Moran and Goolsby, 2009). Limited reproduction (7–14% of the level found on arundo) occurs on *Arundo formosana* (a non-native species used rarely as an ornamental plant in the United States). The arundo wasp does not develop on common reed or 26 other grasses and 8 non-grass, habitat-associate plants tested (Goolsby et al., 2009a). After release in Texas and Mexico, no wasp exit holes were found on common reed, 10 other wild grasses (both native and non-native species), or two economic grasses (corn, sugarcane) growing adjacent to arundo (Goolsby et al., 2020).

Gall-forming insects insert their eggs into suitable plant tissues, such as shoot tips, and either adult females or eggs release plant hormones that cause the plant to produce an abnormal tumor-like growth, inside which the next generation of immature gall-makers feed (Raman et al., 2005). Gall-formers are known for their high host specificity and hence have high utility in biological weed control (Muniappan and McFadyen, 2005). Several shoot-galling insects have been found to be effective biocontrol agents (Dhileepan, 2004; Aigbedion-Atalor et al., 2019).

Female arundo wasps insert eggs into the tips of young stems, and each egg forms its own gall. Galls are often clustered near the shoot tip, resembling one large gall (**Fig. 5a**). Galled main shoots become bent and distorted (**Fig. 5b**). Galls are most often found on lateral shoots on mature main shoots. Larvae feed, develop, and pupate inside the gall. Adult emergence holes (**Fig. 5c**) on green or brown shoots allow observers to spot wasp presence, and by counting them it is possible to estimate wasp density (Marshall et al., 2018a), as each wasp makes its own exit hole (Moran and Goolsby, 2009). Wasps enter a quiescent (hibernating) state as final-stage larvae or as pupae under cold winter conditions or drought (P. Moran and J. Goolsby, pers. obs.), but otherwise can complete several generations per year (Racelis et al., 2010; Goolsby et al., 2014). Abundant sunlight, water, and nitrogen enhance the population growth rate of the arundo wasp by increasing the output per female and/or shortening total development time (Moran et al., 2014; Moran and Goolsby, 2014: Moran, 2015).

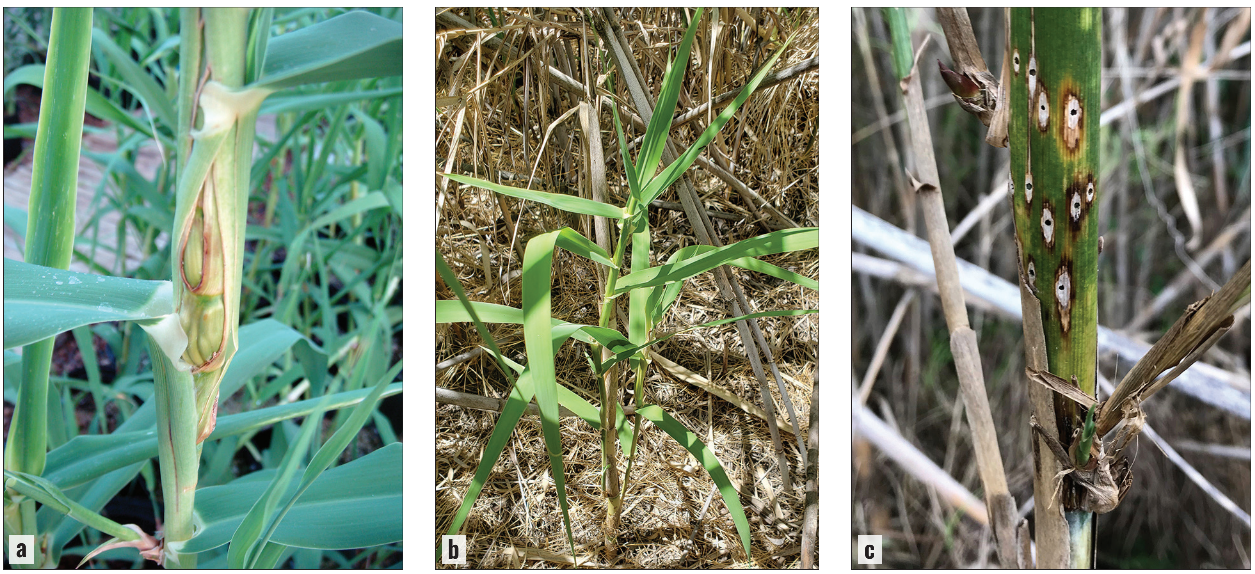

Figure 5. (a) Galls made by the arundo wasp on a young main shoot; (b) young main shoot of *Arundo donax* galled by the arundo wasp (note the characteristic bent and distorted appearance of the shoot; galling increases the likelihood of shoot breakage and death); (c) exit holes made by emerging arundo wasps. (a,b: P. Moran, USDA-ARS; c: J. Goolsby, USDA-ARS)

Arundo wasps from eastern coastal Spain and France were first released in 2009 in the Lower Rio Grande Basin, specifically the far southern tip of Texas along the Rio Grande. Over 1.2 million total arundo wasps, from three Spanish and three French locations, and from the adventive Laredo, Texas population, were mass-reared between 2009 and 2011 (Moran et al., 2014). They were released from the ground or, in remote areas, dropped from airplanes (with wasps inside custom-designed cardboard boxes) (Racelis et al., 2010), at 20 sites between Brownsville and Del Rio, Texas. Surveys in 2013 found established *T. romana* populations at 25 sites in this region (Goolsby et al., 2014). Dispersal occurred to sites 32 km (20 mi) from the nearest release area. Genetic marker analysis indicated that 390 of 409 arundo wasps were of a genetic form (haplotype) that matched the three Spanish populations but was mixed with, and indistinguishable from, the Laredo population at the Rio Grande sites; the Spanish populations were present separately at a few sites; and the French wasps did not establish (Goolsby et al., 2014). In subsequent years, wasp galls from the Rio Grande sites were reared in plots of arundo at a Texas A & M University research farm in Weslaco, Texas, and cut stems or galls were placed at field sites in the spring, summer, or fall to further distribute this gall wasp. Mowing or 'topping' of dense arundo patches before release to induce prolific side shoot regrowth was found to increase subsequent arundo wasp density after release (Racelis et al., 2012c). This release technique led to establishment in Big Bend National Park, much further west on the Rio Grande (Goolsby et al., 2019a). The arundo wasp was also released and established in Mexico in Nuevo Laredo in the state of Tamaulipas on the Rio Grande, and near Jiutepec in the state of Morelos in central Mexico (Martínez Jiménez et al., 2017). In California, galls from Texas were held in cardboard barrels (Moran et al., 2014) for adult emergence. Approximately 12,000 adult wasps were released at nine field sites between the northern Sacramento River watershed to the southern San Joaquin River watershed between 2013 and 2017, and the arundo wasp established at two sites, one in each watershed (Moran, 2020). Reduced climate suitability may be the reason why establishment has not been as successful, in terms of the number of sites, in dry, temperate northern California as in the humid, subtropical Lower Rio Grande Basin of Texas, where wasp populations are 10 to 39-fold higher than in the wasp's native Mediterranean range (Marshall et al., 2018a).

Rhizaspidiotus donacis, a Rhizome-Feeding Armored Scale

The armored scale *Rhizaspidiotus donacis* (Hemiptera: Diaspididae) is referred to here as the arundo scale. It is the first and, to date, only armored scale in the world to be intentionally released for biological weed control (Winston et al., 2021). The scale occurs in France, Spain, Italy, Turkey, and Algeria (Goolsby et al., 2009a). Insects for host-range testing were collected in southwestern France and the eastern Mediterranean coast of Spain, and additional collections were later made in Greece and Italy to examine the scale's occurrence in a broader range of habitats (Goolsby et al., 2013a). The scale's life cycle begins with the birth of live 'crawlers' and lasts two months until the production of short-lived winged adult males that leave their scale covers behind, or six months when immobile females, which are black or brown and measure about 1.2 mm (1/20 in), mature (**Fig. 6a,b**) (Moran and Goolsby, 2010). In the field in Spain (Cortés et al., 2012) and in southern California in an adventive population (Braman et al., 2021), there is one scale generation per year, and possibly two in subtropical field settings such as the Lower Rio Grande Basin (J. Goolsby, pers. obs.). In laboratory tests with 200 crawlers released per plant, 21 ± 4 females were produced per arundo plant across 29 plants tested, and only 14% as many adult females were produced on the related, non-native plant species *A. formosana* (3 ± 2 females across three plants tested). The scale produced 3 or 0 females on two specimens of native sprangletop grass (*Leptochloa virgata*). No adult females were produced on any of 32 other grass species or 9 non-grass plants, including species that often grow close to arundo (Goolsby et al.,

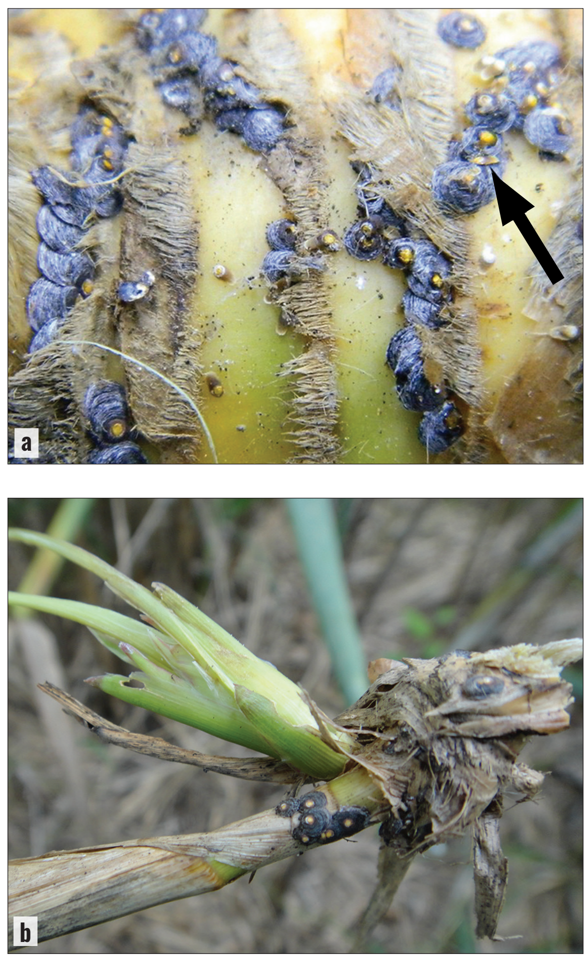

Figure 6. The arundo armored scale, *Rhizaspidiotus donacis*, (a) showing both black, round adult females and much smaller brown, oyster-shaped adult male scale cover (arrow), on arundo rhizomes in Greece; (b) adult females on a side shoot of arundo near Brownsville, Texas, showing 'witches' broom' shoot distortion. (a,b: J. Goolsby, USDA-ARS)

2009b). In follow-up laboratory tests with 1,000 crawlers per plant, 130 adult females were produced per plant on *A. donax*, 5 per plant on *L. virgata*, and 1 or less on smooth cordgrass, *Spartina alterniflora*. Field surveys in the native range in Spain found no infestation by this scale on *Leptochloa* or *Spartina* species (Goolsby et al., 2009b). Post-release surveys in Texas found the arundo scale only on arundo, and it was not found on common reed or other neighboring grasses (Goolsby et al., 2020). In greenhouse tests, arundo scales from an adventive southern California population (and a French population used for comparison) were able to produce adult female offspring on native accessions of common reed, but at levels of only 1–2% (1–6 females) of that produced on arundo (105–221 females) (Braman et al., 2021). The arundo armored scale poses little to no risk to plants other than arundo in the field.

Greenhouse studies confirmed that *R. donacis* from eastern and southern Spain can produce large numbers of progeny on arundo from the Rio Grande Basin (Goolsby et al., 2013b). The arundo scale was first released in January 2011 at two sites: an experimental plot of arundo at the USDA-APHIS facility in Edinburg, Texas (147,000 crawlers) and in a patch of arundo in Del Rio, Texas on the Rio Grande River (635,000 crawlers). By July 2011, a new generation of settled scales was observed at both sites, indicating establishment (Goolsby et al., 2011). Methods were developed to rear the arundo armored scale on potted arundo 'microplants' (Villarreal et al., 2016) derived from rooted shoot cuttings. Five and a half months after infesting microplants with scales, they were examined to verify the presence of mature females. Plants were removed from pots and planted in holes drilled into rhizome beds in dense arundo patches. Use of this technique resulted in the arundo scale's establishment at all 48 locations where they were released along the Rio Grande River from Brownsville to Del Rio, Texas (Villarreal et al., 2016; Goolsby and Moran, 2019). In northern California, releases were made at nine sites in the San Joaquin and Sacramento River watersheds, and establishment was confirmed at seven sites two years later (Moran, 2020).

HOW WELL DID IT WORK

Before release of the arundo wasp, its likely impact was estimated in greenhouse cage studies in which 6 wasps per stem per week were applied for 12 weeks to potted arundo plants. This treatment reduced shoot growth by 92% over the trial period and induced the formation of lateral shoots (Goolsby et al., 2009c). Addition of the arundo scale to the treatment did not further reduce plant growth, due to the scale's much longer life cycle. In separate greenhouse trials, infestation of arundo plants by *T. romana* at similar high densities (as above) for 12 weeks reduced photosynthetic carbon fixation by 32%, and *R. donacis* infestation for one generation reduced photosynthetic carbon assimilation by over 67% (either with or without the arundo wasp as a co-treatment) (Moore et al., 2010). Pre-release evaluations of the arundo scale in Spain, made by comparing side shoot growth on insecticide-protected and unprotected plants over one year, indicated that the presence of the scale reduced average side shoot growth by 61% (mean ± standard error, 2.2 ± 0.4 cm [0.9 in] compared to 5.7 ± 0.6 cm [2.2 in] of growth on protected plants) (Cortés et al., 2011a). In a comparison of sites with or without the scale in Spain, average rhizome biomass was 46% lower at nine sites with arundo scale (388.0 ± 16.5 g [0.9 lbs]) than at nine sites without the scale (716.7 ± 30.9 g [1.6 lbs]) (Cortés et al., 2011b).

Post-release evaluations of the arundo wasp have been conducted in the Lower Rio Grande Basin in south Texas (**Table 1**). Five years after first release of the arundo wasp in 2009, live aboveground biomass (main shoots + side shoots + leaves) per m^2, as inferred from live main shoot length (Spencer et al., 2006), was 22% lower after releases (mean ± standard error, 86.9 ± 12.6 kg/m^2 [17.8 lb/ft^2]) compared to before releases (111.0 ± 14.2 kg/m^2 [22.7 lb/ft^2]), and the proportion of main shoots that were alive (per 9 m^2 [97 ft^2] plot) was 18% lower than before the release program began (Goolsby et al., 2016). Across 11,403 side shoots examined, 2,010 had galls and 1,336 (66%) of those were dead, while among 9,393 ungalled side shoots, 4,610 (49%) were dead; galling by the arundo wasp thus increased the likelihood of side shoot death by 17% (**Table 1**). Seven years after agents were released (2016), estimated aboveground biomass had decreased to 54% of the level before biological control, to 61.3 kg/m^2 (12.6 lb/ft^2) across five sites (Moran et al., 2017). These findings indicate that *T. romana* reduced the ability of arundo shoots to grow and survive, a key objective of the project.

Because the arundo scale female is immobile for most of its life, impact studies have been limited to release plots. In a 2019 study at two sites, eight years after initiation of crawler releases and out-planting of scale-infested microplants, arundo aboveground live biomass was 55% lower in plots with both the arundo scale and arundo wasp (26.4 ± 6.1 kg/m^2 [5.4 lb/ft^2]), compared to plots with the wasp alone (58.7 ± 11.6 kg/m^2 [12.0 lb/ft^2], **Table 1**) (Goolsby and Moran, 2019). At this point in time, only about half (52%) of the side shoots of arundo in the scale plots had scales at their bases, compared to 82–100% in Spain (Cortes et al., 2011b), suggesting that arundo scale populations were still increasing, and so their full impact had not yet become apparent.

Table 1. Key measures of impact of the arundo biological control program in the Lower Rio Grande Basin of south Texas.

BIOLOGICAL CONTROL AGENT	MEASURE	YEARS POST-RELEASE[1]	IMPACT (CHANGE FROM PRE-BIOLOGICAL CONTROL)	REFERENCE
Arundo wasp	Live aboveground biomass	5	22% decrease[2]	Goolsby et al., 2016
	Proportion live shoots per plot	5	18% decrease	
	Proportion of dead side shoots	5	17% increase	
	Live aboveground biomass	7	32% decrease from 2014[3]	Moran et al., 2017
	Plant diversity	7	1.8-fold increase[4]	
Arundo armored scale + Arundo wasp	Live aboveground biomass	8	55% decrease compared to arundo wasp alone[5]	Goolsby and Moran, 2019

[1] From the start of release program for the agent indicated.
[2] Average decrease across 10 sites.
[3] Additional decline over two years at five sites measured both five (2014) and seven (2016) years after arundo wasp release.
[4] Relative to dense stand of arundo with no other species in plot (Simpson's D = 1).
[5] Average across two sites, total of five pairs of plots with scale + wasp compared to wasp alone.

BENEFITS OF BIOLOGICAL CONTROL OF ARUNDO

A key benefit expected from a reduction of arundo's live aboveground biomass was reduction of water consumption. On the U.S. side (southern- and eastern-most portion) of the Lower Rio Grande Valley (LRGV), a 22% reduction in live arundo biomass five years after release of the arundo wasp resulted in annual savings estimated at 269 ha-m (2,183 acre-ft) of water per year (Goolsby et al., 2016), based on models developed in Seawright et al. (2009). The estimated value of the water saved was $303,000 in 2016 at a water value per acre-ft of $139 for water across both sides of the U.S.-Mexico border, or up to $4.4 million when the water value was estimated at $2,000 per acre-ft for use in irrigated crops on the U.S. side of the LRGV. Assuming a further 32% decrease in biomass (i.e., total of 54% after seven years, Moran et al., 2017) an additional 391 ha-m (3,175 acre-ft) was expected to be saved in the U.S. portion of the LRGV annually, with a value (depending on the price of water) of $441,000 to $6.4 million. The total water savings were thus valued at up to $10.8 million per year by 2017. The projected benefit-to-cost ratio of the arundo biological control program in the Lower Rio Grande Basin is 4.3 dollars of water saved for each dollar spent on research, agent release, and monitoring (Seawright et al., 2009). In the first post-release study, a 24% decrease in water use between 2014 and 2015 coincided with increases of up to 53% in wasp density (Racelis et al., 2022). The arundo scale has not yet dispersed sufficiently to add its impact to the estimates of water savings attributed to the release of the arundo wasp.

A key realized ecological benefit of biological control of arundo in the Lower Rio Grande Basin of Texas is increased plant biodiversity. Seven years after arundo wasp release, 44 total plant species were found across 21 wasp-infested arundo plots examined (three plots per site at seven sites) in the Rio Grande Valley, including 38 native species (1 grass, 19 forbs or herbs, 8 vines, 4 shrubs, and 6 trees). Overall plant diversity (Simpson's D) per plot increased 1.8-fold relative to the baseline condition of dense arundo with no other plants (D = 1) (Moran et al., 2017). These results are consistent with pre-biocontrol studies near Laredo, Texas that found that cutting of arundo shoots to simulate biological control led to emergence of diverse plant species from the soil seedbank (Racelis et al., 2012a; Rubio et al., 2014). Studies on a U.S. Fish and Wildlife Refuge in this region suggested that mechanical topping followed by biological control further increased plant diversity by allowing sunlight to penetrate the arundo canopy more quickly than biological control alone (Goolsby et al., 2019b).

Stakeholders along the U.S. side of the Rio Grande are reporting reduced efforts and cost to control arundo locally with herbicides and mowing (J. Goolsby, pers. obs.). Law enforcement personnel now have greater visibility (>30 m or 98 ft) and ease of access into arundo-infested areas along the Rio Grande (Goolsby et al., 2017a).

REMAINING WORK

The arundo biocontrol project is not yet over, as the following evaluations or introductions would be useful:

(1) Additional post-release field assessments of arundo water use in the Lower Rio Grande Basin are needed to verify the expected water savings noted above.

(2) An assessment of the impact of the arundo wasp on arundo in northern California will begin in 2022, five years after wasp release.

(3) The leaf-mining midge *Lasioptera donacis* (Diptera: Cecidomyiidae) was found in France, Spain, Italy, and Greece, mining the leaf sheaths of arundo. The leaf sheaths rapidly turn brown or black due to the presence of a fungus (Bon et al., 2018), and the larvae feed on both plant and fungal material (Botti et al., 2019). The presence of the leafminer accelerated leaf death in the field in Greece (Marshall et al., 2018b). This midge can infest only arundo (Goolsby et al., 2017b), and was permitted for release in the U.S. in December 2016. Additional research is needed to develop practical rearing and field release procedures.

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