

Rhizaspidiotus donacis* (Hemiptera: Diaspididae), an Armored Scale Released for Biological Control of Giant Reed, *Arundo donax

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Abstract

Non-native, invasive perennial grasses have not been widely targeted for classical biological control with insects, despite their global prevalence and damaging effects, in part because of a perceived paucity of host-specific insect herbivores. Armored scales (Hemiptera: Diaspididae) have not been used for biological weed control. However, over 250 armored scale species occur on grasses, of which 87% feed only on this family and 58% feed on only one grass genus, suggesting that armored scales may have unrealized biological control potential. We selected the armored scale *Rhizaspidiotus donacis* Leonardi as a candidate agent against the exotic, invasive, water-consuming grass known as giant reed (*Arundo donax* L.), based on literature records and our collections indicating host-specificity to the genus *Arundo* and its broad geographic range in the Mediterranean basin. Observations of reduced giant reed vigor at scale-infested sites in Spain and France were confirmed in native range field studies showing a 50% reduction in lateral shoot growth rate and rhizome weight on scale-infested versus non-infested *A. donax*, as well as significant reductions in photosynthesis rates in quarantine laboratory studies. Specialized procedures were developed using gelatin capsules to isolate females from host tissues and neonate crawlers from females. Based on laboratory and native range field studies, the host range of *R. donacis* is limited to *Arundo* spp., and the life cycle requires 5–6 months. A scale accession from eastern coastal Spain established larger populations on rhizomes of two invasive *A. donax* accessions from this same region than on rhizomes representing a separate, geographically isolated introduction. In 2011, *R. donacis* became the first armored scale released for biological weed control, establishing robust rearing colonies and reproductive field populations with evidence of lateral shoot deformities at the first release site along the Rio Grande in Texas, USA.

Introduction

Arundo donax L, known as arundo, giant reed, or carrizo, is native from the Mediterranean Basin to India, and invasive in North and South America, South Africa, and Australia. Arundo has colonized at least 30,000 ha along rivers, reservoirs and irrigation canals in the Lower Rio Grande Basin of south Texas and northern Mexico (Yang et al., 2009, 2011), removing economically-significant amounts of water, displacing native plants, altering flood and fire regimes, harboring cattle fever ticks, and hindering border security activities (Moran and Goolsby 2009; Racelis et al., 2011), with similar ecological impacts in California (Coffman et al., 2010; Lambert et al., 2010) and in other arid riparian ecosystems. Non-native, invasive perennial grasses (Poaceae) and sedges (Cyperaceae) are among the world's most widespread weeds (Witt and McConnachie, 2003), attaining densities in riparian and rangeland habitats that exceed the capacity of chemical and mechanical control. However, few biological control projects have targeted perennial grasses, due to a perceived paucity of genus- or species-specific insect herbivores (Evans, 1991). Pathogenic fungi have been considered for inoculative (Yobo et al., 2009; Anderson et al., 2011) or inundative releases (Yandoc et al., 2004). Surveys of herbivorous insect fauna on common reed (*Phragmites australis* (Cav.) Trin. ex. Steud.) (Tewksbury et al., 2002), giant reed (*Arundo donax*) (Tracy and DeLoach, 1998; Kirk et al., 2003), *Sporobolus* spp. (Witt and McConnachie, 2003), *Calamagrostis* spp (Dubbart et al., 1998) and multiple grass genera (Tschardtke and Greiler, 1995) have shown that oligo- and monophagous herbivores exist on grasses, but exhibit feeding patterns and taxonomic affiliations that are non-traditional in weed biological control. Examples include shoot-galling wasps (Hymenoptera: Eurytomidae), stem-boring flies (Diptera: Chloropidae), galling and non-galling leafminers (Diptera: Cecidomyiidae) and, in the case of giant reed, an armored scale (Hemiptera: Diaspididae).

Armored scales are immobile besides the short-lived neonate crawler (first instar) and adult male stages. These small, sometimes cryptic insects generate cumulative damage to perennial plants over several generations of an often prolonged life cycle (McClure, 1990). Over 200 pest species are

known, most with broad host ranges (Miller and Davidson, 1990). No armored scales have been released for weed control, although two adventive *Chionaspis* species are widespread and damaging on saltcedars (*Tamarix* spp.) in North America (Wiesenborn, 2005). Armored scales are commonly found on grasses (Evans and Hodges, 2007), with over 250 species known (<http://www.sel.barc.usda.gov/scalenet.html>), and 222 of these (87.5%) occur only on hosts in the Poaceae; 58% (128) are found on only one grass genus. Armored scales may thus have unrealized potential for biological control of perennial grasses.

A biological control program targeting *A. donax*, led by the USDA-Agricultural Research Service, is the first to release multiple non-native insect agents to control a grass weed. We selected *Rhizaspidiotus donacis* Leonardi as a candidate agent on the basis of literature records (reviewed in Goolsby et al., 2009a) and our collections indicating specificity to the grass genus *Arundo*, which has no native members in North or South America. The geographically-broad native range of this scale, as well as the thin, brittle arundo shoots observed at sites with dense scale populations, also favored its selection for further testing.

Methods and Materials

Determination of native distribution

Collections to survey insects on *A. donax* were made at over 330 sites in 19 countries between 2000 and 2007 during the spring, summer and fall. Rhizome samples were taken to the USDA-ARS European Biological Control Laboratory to detect the presence of *R. donacis* and natural enemies (Kirk et al., 2003).

Evaluations of impact

In the Province of Alicante, in southeastern coastal Spain, 15 shoots at each of five sites were treated with foliar and root drench insecticide monthly for 12 months and 15 shoots were left untreated. Growth rates were compared across the two treatments and between monthly measurements (Cortés et al., 2011a). To examine arundo scale

effects on rhizome weight, nine sites with and nine without *R. donacis* were selected in the Languedoc region of southwestern France and the Province of Alicante in Spain (Cortés et al., 2011b). In a three-month quarantine laboratory study, potted, fertilized arundo stems were infested with *R. donacis* at a rate of 158 crawlers per week, and also received arundo wasps (*Tetramesa romana* Wallker) at a rate of 18 per week. Shoot and selected leaf lengths were compared to wasp-only and control treatments (Goolsby et al., 2009b). In a six-month lab study, 500 to 700 *R. donacis* crawlers were released per stem and leaf gas exchange measured 24 weeks later to infer effects on photosynthetic processes (Moore et al., 2010). In a six-month greenhouse study, rhizomes and crowns of young arundo shoots were infested with 1,000–5,000 crawlers and shoot elongation and biomass examined (Racelis et al., this volume). In greenhouse studies, crawlers were released onto rhizomes either fertilized with urea plus slow-release nitrogen-phosphorous-potassium pellets, or given only pellets (Moran and Goolsby, this volume).

Determination of biology and host range

Adult females were shipped on arundo rhizomes from 15 sites in southwestern France and Mediterranean Spain. Females were removed from rhizomes and stored in 1.5-cm gelatin capsules at 27 °C, 60% RH, 14:10 L:D. Crawlers were collected in gelcaps, which were screened microscopically for *Aphytis acrenulatus* Rosen and DeBach ectoparasites and other contaminants. Capsules with crawlers were pinned to shoots of 2-month old arundo shoots or non-target plants. Destructive dissections at varying time points and isolation of crawlers, adult males and females were used to determine duration and survival of life stages (Moran and Goolsby 2009). For host range studies, shoots of *A. donax*, 40 other grass species, and 5 non-grasses received 200 crawlers each and were dissected three months after infestation (Goolsby et al., 2009a). Field studies were conducted in Spain as a follow up to examine laboratory non-target development on *Leptochloa* spp. and *Spartina alternifolia* Loisel grasses. Sub-specific host range was examined by infesting arundo rhizomes from two Texas sites (Austin and Laredo) that genetically match several populations in eastern and southern coastal Spain (D. Tarin, A.

Pepper, J. Goolsby, P. Moran, A. Contreras Arquieta, A. Kirk, and J. Manhart, unpublished), and from one site (Balmorhea) with a distant point of origin.

Mass-rearing and field release

A permit from the USDA-APHIS-PPQ to release the arundo armored scale into the field was received on 16 December 2010. Mass-rearing was conducted in 700-L plastic inner tubs filled to one-half depth with pea gravel and elevated 10 cm above an outer tub modified with drain holes to maintain the water level below the rooted arundo rhizomes, which were kept dry and partially exposed on the gravel surface. A similar subsurface watering system was used for rhizomes planted in outdoor and greenhouse trenches. Releases at a field site in Del Rio, Texas on the floodplain of the Rio Grande began in January 2011 (Goolsby et al., 2011).

Results

Native distribution

Our collections and literature records indicate that the geographic range of *R. donacis* includes eastern and southern Spain, extreme southern France, Italy, Crete, southern and western coastal Turkey, and coastal Algeria, with no collections in the Balearic Islands, Corsica, Sicily, Croatia, Bulgaria, Israel, Egypt, Morocco, the Canary Islands, China, Nepal, or India. Some of the most robust *R. donacis* populations, in eastern and southern Spain, were found on arundo accessions matched using microsatellites to the invasive arundo genotypes in the Lower Rio Grande Basin.

Impact on target weed

Infestation by *R. donacis* reduced both lateral shoot growth and rhizome weight of *A. donax* by 50% in the native range (Table 1). In quarantine, the arundo armored scale slightly enhanced the negative effect of the arundo wasp on main shoot growth, and independently reduced the ability of the plant to absorb light energy to convert nitrogen into protein by over 60% (Table 1). In a 6-month greenhouse study, high levels of arundo scale infestation (5,000 crawlers)

reduced main shoot growth by 50% or more (Table 1). Urea fertilization increased female reproduction and settling of second-generation crawlers, but results were inconsistent across two rearing environments (Moran and Goolsby, this volume).

Biology

At 26 °C, arundo scale crawlers lived less than two days without food, or, on arundo shoots, settled to an immobile 'whitecap' phase to complete the first instar within 10–13 days of emergence. Males completed second-instar development within an additional 20 days and emerged as adults by the 40th day after emergence. Non-feeding winged males lived less than two days. Females completed the second instar

by the 50th day post-emergence, with a brief period beforehand during which adult females had eclosed but were still inside the second instar nymphal cuticle. Adult females fertilized by males required a total of 170 total days from emergence as crawlers to reproductive maturity. Survival to adulthood (both sexes combined) was 20–25%. Each reproductive female produced an average of 85 crawlers. More details may be found in Moran and Goolsby (2010).

Host range

The arundo scale readily completed development on *A. donax*, with significantly less development on *A. formosana*, and none on closely related common reed, *Phragmites australis* or 37 other

Table 1. Evaluations of impact of the arundo armored scale *Rhizaspidiotus donacis* on giant reed (*Arundo donax*), based on pre-release observational and manipulative field studies in Mediterranean Europe, quarantine laboratory studies, and a post-release greenhouse study in Texas, USA.

Study Environment	Length of Time of Scale Infestation	Variable	Reduction Associated with Scale Infestation	Reference
Field-Spain	12 months	Daily rate of shoot growth (cm day ⁻¹)	61%	Cortés et al., 2011a
Field-France and Spain	Unknown	Rhizome weight (g)	46%	Cortés et al., 2011b
Laboratory	3 months	Stem and leaf length ¹	5–10%	Goolsby et al., 2009b
Laboratory	6 months	Maximum rate of electron transport ²	61%	Moore et al., 2010
Non-quarantine greenhouse	6 months	Shoot elongation rate and above-ground biomass	50%–60%	Racelis et al., this volume

¹Effect of scale over and beyond that of the arundo wasp *Tetramesa romana*. Differences between arundo wasp infestation alone and wasp+scale were not significant.

²Measure of efficiency of conversion of light energy in photosynthesis.

native grasses. Very limited development to adult occurred on three species in the genus *Leptochloa* and on *Spartina alterniflora* in quarantine, but no settling by *R. donacis* was observed in dissections of these grasses when found in populations sympatric with *A. donax* in Spain and France, even when potted plants were maintained in stands of heavily scale-infested arundo for 6 months. More details may be found in Goolsby et al. (2009a). Sub-specific host specificity tests in quarantine indicated development of significantly larger second-generation populations on invasive Texas rhizomes matched to Spanish locations from which crawlers were obtained than on Texas rhizomes representing a geographically distinct source of introduction (J. Goolsby, E. Cortés, P. Moran, J. Adamczyk, M. Marcos García and A. Kirk unpublished).

Mass-rearing and field release

Mass-rearing of *R. donacis* began in December 2010 and open field releases in January 2011. On 20 July 2011, whitecaps indicative of reproduction in the field were observed at the Del Rio, Texas site. Reproductive females and a new generation of immatures were found on rhizomes at this site in August 2011 (Goolsby et al., 2011). Colony-reared females produced similar numbers of crawlers as did females collected in Europe (P. Moran, unpublished).

Discussion

The arundo armored scale *R. donacis* exhibits a broad native geographic range with substantial, measurable impacts on the growth of giant reed, but narrow fidelity to the target genus *Arundo*, with full population growth potential only on *A. donax*. The biological attributes of this scale are suitable and adaptable for mass rearing, given sufficient attention to geographic matching of scale accessions from the native range to the known geographic sources of the giant reed populations that are invasive. After only one generation in greenhouse studies, the arundo scale reduced lateral and main shoot growth and alters photosynthetic processes. Feeding by multiple generations is likely necessary to reduce rhizome weight and shoot recruitment in the field. Improvements in visibility through dense giant reed

stands along the Rio Grande and decreases in water consumption by giant reed are key benchmarks in this biological control program, and are being examined as arundo armored scale populations develop.

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