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Horticultural technique for rearing and redistribution of the sessile biological control agent, *Rhizaspidiotus donacis* on its host plant, *Arundo donax*

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ABSTRACT

Arundo donax, giant reed is an invasive weed in the riparian habitats of the Rio Grande Basin. A biological control program using specialist insects from the native range in Mediterranean Europe, including the arundo scale, *Rhizaspidiotus donacis* has been implemented. The arundo scale is a sessile insect that spends most of its life cycle affixed to its host plant, thus creating challenges for its rearing and redistribution to field sites. A horticultural technique was developed which allowed for rearing of the scale on small, potted *A. donax* microplants, which could later be transplanted to field sites. Female scale reach maturity on the microplants and produce mobile crawler scale, which move to adjacent uninfested *A. donax* plants. Arundo scale were established at 48 sites on the Rio Grande using microplants. This horticultural technique allows for movement of the arundo scale at any stage of its life cycle to field sites to conduct biological control programs for *A. donax* where it is invasive.

Additional Index Words: : biological control of weeds, armored scale, carrizo cane, Rio Grande Basin

Arundo donax L. (Poaceae: Arundinoideae Arundineae) giant reed or carrizo cane is a tall, (2-10 m) perennial reed grass native from the western Mediterranean to India (Hardion et al. 2014). It was likely to introduced into North America from the Iberian Peninsula about 500 years ago (Tarin et al. 2013) and is now a widespread, invasive weed along the Rio Grande Basin in Texas, northern Mexico and the southwestern U.S (Yang et al. 2009, Yang et al. 2011). In Texas, large stands of giant reed cause serious ecological impacts by displacing native flora and fauna, and facilitating the invasion of cattle fever ticks from Mexico (Racelis et al. 2012). Giant reed also interferes with law enforcement activities along the international border and competes for scarce water resources in an arid region which experiences frequent droughts, and potential changes in rainfall patterns from climate change (Seawright et al. 2009). Classical biological control may be the most cost-effective and sustainable option for management of this weed over large areas such as the bi-national Rio Grande Basin. A biological control program was initiated in 2005, and two insects, a stem-galling wasp, *Tetramesa romana* Walker (Hymenoptera: Eurytomidae) and an armored scale *Rhizaspidiotus donacis* Leonardi (Hemiptera: Diaspididae), have been established (Racelis et al. 2010, Goolsby et al. 2012, Goolsby et al. 2015). Studies of the arundo scale, *R. donacis*, in Europe showed that this insect had a significant effect on growth of side shoots and weight of rhizomes. *Rhizaspidiotus donacis* occurred on *A. donax* in all soil types and had the greatest impact at undisturbed sites (Cortes et al. 2011a, Cortes et al. 2011b, Goolsby et al. 2013).

The life cycle of *R. donacis* females takes 6 months and follows the general biology for sexually-reproducing, viviparous species (Moran and Goolsby

2010). Females produce live crawlers which emerge from the edge of the female's waxy scale covering and are mobile for 12-24 hours. Except for the winged adult males, only crawlers have capacity of movement, thus they represent the only dispersive life stage. Males complete the crawler and one additional nymphal stage, a brief pupal stage, and emerge as adults within 6 weeks of birth as crawlers. The short-lived males mate with newly-molted adult females. Females complete the same two nymphal stages, then molt to the adult stage, and spend the following 3-5 months feeding before they reach maturity and start producing crawlers.

The predominantly sessile lifestyle of the arundo scale presented challenges for its rearing and redistribution. A horticultural technique was developed for both the rearing and the field release of the arundo scale, which consists of infesting small arundo plants (here after referred to as "microplants") with first instar arundo scale (crawlers) and planting these microplants at designated field locations allowing female arundo scale to reach maturity, reproduce and disperse crawlers to adjacent uninfested *A. donax* plants.

MATERIALS AND METHODS

Microplant production was conducted in greenhouses at the Texas A&M Kingsville Citrus Center - South Farm in Weslaco, Texas. Main stems of mature giant reed were manually harvested using loppers from populations of mature first-year canes located near the rearing facility (Fig. 1). Stems were cut into billets (stem pieces; avg ~ 25 cm), each containing 2 nodes. Billets had side shoots removed using hand pruners before they were placed in 150 liter plastic livestock tubs filled with water kept in the greenhouse at approximately 27°C. The billets remained in the tub for 2 -4 weeks or until they had 2-3 lateral shoots (10-20 cm in length) with roots. Once new growth was clearly visible and roots were at least 8 cm long, they were then individually potted into 10 cm pots, using 80% peat moss soil with perlite (Sunshine Mix No. 1, Sun-Gro Horticulture, Bellevue, WA) as the growing medium. Billets were placed into the pot upright with new growth exposed (base of lateral shoot at least 2.5 cm above the rim of the pot to keep the infestation area dry) and roots bent gently down and planted in the soil media. Potted billets were then placed on raised benches in a greenhouse maintained at 28° ± 3°C with natural photoperiod (400 -1000 μM m^{-2} s^{-1} photosynthetically active radiation), and watered twice a day for 5 minutes by drip irrigation. Pots were fertilized monthly with 4 grams of calcium nitrate fertilizer (Hi-Yield, VPG, Bonham, TX). When needed, a foliar insecticide (Radiant© SC, Dow Agrosciences, Indianapolis, IN) was used to control greenhouse pests, including thrips and aphids.

After 2-3 weeks, when roots were firmly established in the soil media, microplants were then infested with first instar scale crawlers isolated from colonies at the USDA-Animal and Plant Health Inspection Service, Center for Plant Health Science and Technology, Quarantine Facility at Moore Air Base in Edinburg, Texas (see Moran and Goolsby 2010 for procedures for isolation and production of crawlers). Scale crawlers were isolated in groups of ~200 in 1.5-cm gelatin capsules (gelcaps, size 0, Torpac, Fairfield, NJ), and released on microplants using a passive transfer method that included pinning the open gelcap to the base of the microplant lateral side shoot. Watering was reduced to 2 minutes once a day by drip irrigation. Microplants were then monitored under greenhouse conditions for 5 1/2 months until scale were mature and ready to reproduce. At this point, the infested microplants were transferred from the greenhouse and transplanted at designated field sites by removing the pot and transferring the rooted microplant into the ground immediately adjacent to a local *A. donax* rhizome with emerging buds. Holes were created (8 cm in diameter with a depth of 12 - 17 cm) using an 18-volt cordless drill (Ryobi 18-V cordless drill, One World Technologies, Anderson, SC) with a 7.5 by 17 cm bulb planting auger (Tanaka-Hitachi Koki USA, Ltd. Braselton, GA) to minimize planting time at field site (full battery = 100 holes). Optimally, the microplant was positioned so that it was touching an uninfested rhizome. Microplants were watered at planting to increase establishment. An inverted milk jug, filled with water and with a hole (made with a straight sewing pin, size 20-24) in the cap and base to allow for slow drip of water, was placed in a shallow hole next to the microplant to improve establishment. Due to time constraints, the jugs were not always checked later or refilled although that would improve microplant survival. The microplant technique was tested at 48 locations in TX and Mexico.

RESULTS AND DISCUSSION

Soon after transplanting (1-3 weeks), second generation (F1) crawlers emerged and passively transfered to nearby field populations of giant reed. Subsequent visits to these field sites revealed successful transfer to nearby plants, evidenced by small "whitecaps" visible in the leaf collars of previously uninfested resident giant reed, indicative of settled first-instar scales (Moran and Goolsby 2010).

The arundo scale, *R. donacis*, is the first armored scale insect (Diaspididae) that has been used in classical biological control of weeds. Diaspid scales can be specialists, including species in the genus *Haliaspis*

Table 1. Arundo scale release locations in Texas

Brownsville	Loop Farms	25°54'23.99"N	97°22'45.94"W
Brownsville	Veterans Bridge	25°53'14.12"N	97°28'37.95"W
Brownsville	Ft. Brown	25°53'28.55"N	97°29'46.96"W
Brownsville	Ft. Brown	25°53'42.74"N	97°29'45.15"W
Brownsville	Ft. Brown	25°53'52.03"N	97°30'0.36"W
Brownsville	Gateway Bridge	25°53'54.65"N	97°29'59.32"W
Brownsville	B&M Bridge	25°53'34.57"N	97°30'16.38"W
Brownsville	B&M Bridge	25°53'30.84"N	97°30'18.68"W
Brownsville	Palm Blvd	25°53'46.59"N	97°31'21.14"W
Brownsville	Palm Blvd	25°53'44.32"N	97°31'21.15"W
Los Indios	Cable Crossing	26° 1'52.65"N	97°43'40.82"W
Bluetown	Bauer Farm	26° 3'55.72"N	97°48'27.88"W
Bluetown	Bauer Farm	26° 3'25.21"N	97°49'29.52"W
Weslaco	USDA South Farm	26° 8'14.48"N	97°57'22.58"W
Mission	Hardwicke Farm	26° 9'6.86"N	98°21'5.60"W
Mission	National Butterfly Center	26°10'9.88"N	98°22'3.42"W
Mission	National Butterfly Center	26°10'10.10"N	98°22'4.36"W
Edinburg	Moore Airbase	26°23'49.98"N	98°20'14.97"W
Rio Grande City	Sherrin Farm	26°18'59.31"N	98°43'53.13"W
Rio Grande City	Sherrin Farm	26°18'8.84"N	98°44'22.77"W
Roma	Fronton Island	26°24'8.59"N	99° 4'20.66"W
San Ygnacio	Overlook	27° 5'37.68"N	99°25'40.90"W
San Ygnacio	Overlook	27° 5'48.84"N	99°25'52.70"W
San Ygnacio	Herbst Vega	27°13'3.60"N	99°26'20.08"W
Laredo	La Azteca Island	27°30'1.92"N	99°29'56.31"W
Laredo	La Azteca Island	27°29'54.93"N	99°29'41.53"W
Nuevo Laredo	Puente Ferrocarril	27°29'51.71"N	99°30'57.08"W
Laredo	Laredo Community College	27°30'12.59"N	99°31'34.54"W
Laredo	Laredo Community College	27°30'13.03"N	99°31'35.76"W
Laredo	Laredo Community College	27°30'13.38"N	99°31'36.62"W
Laredo	La Bota Ranch	27°36'41.26"N	99°32'59.41"W
Laredo	La Bota Ranch	27°36'42.79"N	99°33'1.42"W
Laredo	Farco Mines	27°48'2.81"N	99°52'36.54"W
El Indio	El Cenizo Ranch E El Cenizo Ranch	28°16'22.57"N	100°16'59.15"W
El Indio	Rosita Valley Ranch	28°35'10.33"N	100°23'55.95"W
Eagle Pass	International Bridge	28°42'37.19"N	100°30'29.72"W
Eagle Pass	International Bridge	28°42'33.85"N	100°30'33.77"W
Eagle Pass	International Bridge	28°42'39.42"N	100°30'28.30"W
Eagle Pass	International Bridge	28°42'59.56"N	100°30'22.88"W
Eagle Pass	International Bridge	28°43'02.80"N	100°30'21.76"W
Eagle Pass	International Bridge	28°44'16.11"N	100°30'22.69"W
Eagle Pass	Hydroelectric Plant	28°49'45.22"N	100°33'15.10"W
Eagle Pass	Hydroelectric Plant	28°49'36.66"N	100°32'53.81"W
Del Rio	Sycamore Creek	29°14'36.89"N	100°47'34.55"W
Del Rio	International Bridge	29°19'35.96"N	100°55'14.91"W
Del Rio	International Bridge	29°19'38.78"N	100°55'19.21"W
Del Rio	International Bridge	29°20'03.09"N	100°55'42.42"W
Del Rio	International Bridge	29°20'52.14"N	100°57'36.02"W

that feed on grasses (Moran and Goolsby, 2010). Future classical biological control of weed programs targeting grasses should consider diaspid scales as potential agents, even though their sessile life cycle can make them difficult to rear and release. Microplants allow for long distance movement of sessile scale insects to field release sites.

Microplants can be transplanted at any point in the life cycle of the scale. With fully developed root systems, microplants will establish and allow for maturation of female scale and subsequent dispersal of the crawler scale provided they are irrigated and/or receive adequate rainfall. Dispersal of the scale from

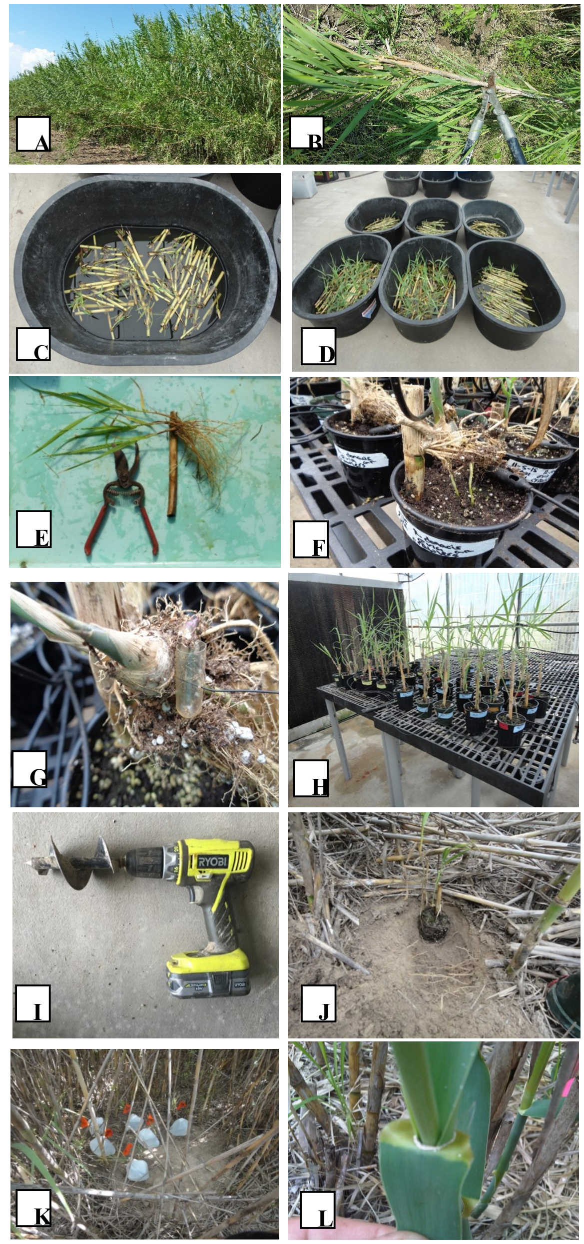

Fig 1. (A) Stand of *Arundo donax* used for harvesting of plant material, (B) mature stems used for harvesting of billets, (C) tub with freshly harvested billets, (D) billets with emerging roots from nodes, (E) two-node billet with roots ready for planting, (F) potted microplant on dripper system with developing side shoot, (G) gelatin capsule with crawler arundo scale pinned on side shoot of microplant, (H) greenhouse bench with infested microplants, (I) drill and auger used for planting microplants, (J) microplant transplanted to stand of *A. donax*, (K) watering jugs inserted next to transplanted microplants, (L) scale white caps in leaf collar indicating successful transfer of scale from microplants to field stand.

microplants has advantages over direct release of fragile, short-lived crawlers. Crawlers emerging from mature mother scales on microplants can disperse under optimal conditions, which is likely at night when humidity is highest. Crawlers that are hand released may encounter adverse conditions associated with day time releases. The microplant technique also allows for release of crawler scales on to uninfested plants under cool, shaded greenhouse conditions which increases settling and survival of crawlers.

Further studies are needed to optimize production of microplants including application of nitrogen and micronutrients. The application of fertilizer may shorten the life cycle of R. donacis and increase fecundity of females (Moran and Goolsby 2014). Nitrogen application may also increase the quality of parenchymal tissues at the base of the side shoots which influences feeding of the immature scales. Microplants allow for standardized testing of horticultural techniques which may affect scale development.

The horticultural technique presented here provides an efficient method for rearing and field release of this novel biological control agent. This technique may be useful for biological control programs directed at *A. donax* and other invasive weeds using diaspidid scales.

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