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ABSTRACT

Ant collection, identification, and control experiments were conducted to improve the establishment of the arundo scale, *Rhizaspidiotus donacis*, a biological control agent of *Arundo donax*, an invasive weed in the riparian habitats along the Rio Grande in Texas. Observational studies indicated *R. donacis* immatures are preyed upon by a variety of predator insects, especially ants. A survey of the principle ant species was made at sites along the Rio Grande with *Arundo donax* to help direct biological control strategies. We conclude that uses of ant baits can effectively control the common ant species found in these habitats and improve the establishment of *R. donacis*.

Additional Index Words: Giant reed, carrizo cane, biotic resistance, biological control

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Arundo donax L. (Poales: Poaceae), also known as giant reed or carrizo cane is native to the Mediterranean coast of Europe and North Africa to south Asia. *Arundo donax* is an invasive weed of riparian habitats of the southwestern U.S. (DiTomaso and Healy, 2003, Yang et al. 2009, 2011). Classified as an invasive perennial species it has spread widely in riparian zones of Texas, where it has altered wildlife habitats, created fire hazards, compromised water conservation efforts, affected flood control, reduced visibility for law enforcement officers along the international border with Mexico, and facilitates cattle fever tick, *Rhipicephalus* (=*Boophilus*) spp. invasion into the permanent quarantine zone along the Rio Grande between Del Rio and Brownsville, TX (Goolsby et al. 2010, Moran and Goosby2010, Racelis et al. 2012, Seawright et al. 2010).

The arundo scale has established in Texas and field impact studies are in progress (Goolsby et al. 2010). The objective of this study was to evaluate ant diversity and the role of ant predation on the establishment of R. donacis. In 2010, the field release of the arundo scale, *Rhizaspidiotus donacis* (Leonardi) (Hemiptera: Diaspididae), as a biological control

agent of *A. donax* was approved for use in the U.S. (USDA-APHIS 2010). The arundo scale, feeds on rhizomes and lateral shoots reducing biomass and stem growth (Cortés Mendoza et al. 2011a,b). *R. donacis* is released as a mobile, first instar crawler scale at the base of the arundo plant where ant predations occurs (Goolsby et al. 2001). Ant predation was observed to be an impediment of the initial establishment of crawlers at a field site (Fig. 1).

Fig. 1. *Solenopsis invicta*, imported fire ant, attacking scales used for giant reed biological control (left). Arundo scale, *Rhizaspidiotus donacis*, a biological control agent of *Arundo donax* (right).

MATERIALS AND METHODS

Ant Sampling: Ant samples were collected from three Texas *A. donax* research sites, Del Rio, Laredo, and Los Indios (Fig. 2). Ants were sampled using pitfall traps of two designs, "A" (standard) and "B" covered (Fig. 3). Trap A was a 470 ml polypropylene container (reditainer.com) 7.6 x 11.7 x 8.4 cm (height x top diameter x bottom diameter). A plywood shelter (30.5 x 30.5 cm) was supported ≈ 1.3 cm above the trap. Trap B was the same as trap A with the addition of a lid on the container and 20 evenly spaced holes (6 mm diam) circumscribing the upper side of the container (Fig. 3). Each trap contained a 50/50 mixture of propylene glycol and water. Trapping intervals were 48-72 h. Two traps (type A and B) were located within each of the treated bio-control scale release plots, and six traps (3 A and 3 B) were located in non-treated areas outside of the treated plots.

Fig. 2. Map indicating 3 study sites along the Texas-Mexico border (North to south: Del Rio, Laredo, Los Indios).

Pitfall trap collections were conducted in 2013 at the Del Rio and Laredo sites five times in February, seven times in March, and twice in each of April, May, and June. At the Los Indios site, collections occurred twice in May and twice in June. Ant identification was conducted with a stereoscopic microscope following guidelines of ID Guide Publication B-6138 07-03, "The Common Ant Genera of Texas," AgriLife Extension Texas A&M System and The Mueller Lab's, "Ant Identification Key," University of Texas at Austin.

Ant Baits: Ant control treatments at *R. donacis* release sites consisted of broadcast applications of two granular baits, Amdro™ Fire Ant Bait (0.73% hydramethylnon) and Maxforce™ Granular Insect Bait (1% hydramethylnon), as needed, at rates of about 0.2 g/M2. At the beginning of this study, only Amdro Fire Ant Bait was applied for several weeks then alternated with Maxforce Granular Insect Bait.

Fig. 3. Standard pitfall trap (top), vertical-hole pitfall trap (bottom).

Qualitative Observations of Ant Predation of Scales: Laboratory observations of ant predatory behaviors were obtained as follows. Groups of 15 Solenopsis invicta Buren (four replicates) were held in Petri dishes (9 cm diam) with R. donacis scales alone or with scales and 15 granules of Amdro Fire Ant Bait or Maxforce Granular Insect Bait. Field observations of ant behaviors were obtained at scale release sites with and without ant baits present (eight replicates of each; no treatment, Amdro Fire Ant Bait, or Maxforce Granular Insect Bait). Scales were observed for 30 min and ant predation noted.

Statistical Analysis: Census of the mean number of ants, by species captured in pitfall traps for a specific location, was compared using ANOVA and means were separated with the Tukey Test. Mean number of ants, by species captured in pitfall traps A or B for a specific location, was compared using ANOVA and means were separated with the Tukey Test. Mean number of ants, by species captured in pitfall traps in treated or untreated area for a specific location, was compared using ANOVA and means were separated with the Tukey Test.

RESULTS AND DISCUSSION

After five mo a total of 292 ants representing nine genera were captured (Table 1).

Solenopsis invicta Buren was numerically the most

the only ants collected from all three sites.

With some exceptions, trap design B captured numerically more ants at Del Rio and Laredo then the standard trap A, which was reversed at Los Indios (Table 2). Generally, non-baited areas had numerically higher ant captures compared to baited areas with the

Table 1. Census of ants captured in pitfall traps in study areas

Ant species	Del Rio		Laredo		Los Indios	
	Mean (± SE)	Total	Mean (± SE)	Total	Mean (± SE)	Total
Solenopsis invicta	11.3 ± 2.3a	47	13.6 ± 7.4a	136	2.3 ± 2.3a	9
Paratrechina terricola	1.0 ± 0.7b	4	2.5 ± 1.5a	25	none	none
Monomorium minimum	0.8 ± 0.5b	3	none	none	none	none
Atta texana	4.8 ± 2.8ab	20	none	none	none	none
Crematogaster laeviuscula	none	none	0.6 ± 0.3a	6	0.3 ± 0.3a	1
Camponotus texanus	none	none	none	none	1.3 ± 0.8a	13
Labidus coecus	--	5	--	none	--	1
Leptogenys sp.	--	3	--	8	--	3
Pogonomyrmex sp.	--	5	--	3	--	none
	F = 6.922		*F* = 2.604		*F* = 0.527	
	df = 3, 15		df = 2, 29		df = 2, 9	
	P = 0.006		*P* = 0.092		*P* = 0.607	

Means in a column followed by the same letter are not significantly different, protected Tukey Test (P < 0.05)

Table 2 (in part). Mean number of ants (± SE) in Trap A (standard pitfall) and Trap B (covered pitfall)

Location	Ants						
	Solenopsis	*Paratrechina*	*Monomorium*	*Atta*	*Crematogaster*	*Camponotus*	Combined
Del Rio							
Trap A	3.0 ± 1.1a	0.8 ± 0.6a	0.4 ± 0.4a	0.6 ± 0.4a	none	none	1.1 ± 0.4a
Trap B	6.0 ± 2.3a	0.0 ± 0.0a	0.8 ± 0.8a	3.2 ± 2.7a	none	none	2.6 ± 1.1a
	F = 1.406	*F* = 1.882	*F* = 0.200	*F* = 0.889	--	--	*F* = 1.630
	df = 1, 9	df = 1, 9	df = 1, 9	df = 1, 9	--	--	df = 1, 39
	P = 0.270	*P* = 0.207	*P* = 0.667	*P* = 0.373	--	--	*P* = 0.209
Laredo							
Trap A	4.2 ± 2.0a	0.2 ± 0.2a	none	none	0.8 ± 0.5a	none	7.3 ± 4.9a
Trap B	23.0 ± 12.9a	4.8 ± 2.6a	none	none	0.2 ± 0.2a	none	48.3 ± 35.9a
	F = 2.070	*F* = 3.076	--	--	*F* = 0.400	--	*F* = 1.280
	df = 1, 9	df = 1, 9	--	--	df = 1, 9	--	df = 1, 5
	P = 0.188	*P* = 0.118	--	--	*P* = 0.545	--	*P* = 0.321

Means in a column followed by the same letter are not significantly different, protected Tukey Test (P < 0.05)

abundant species. *Leptogenys* sp. and *S. invicta* were

exception of Camponotus texanus Wheeler at Los In-

dios (Table 3 and 4).

It was obvious through observations that ant baits

pression of fire ant foraging activity requires the treat-ment of a buffer zone of 35-40 m wide (Martin et al.

Table 2 (in part). Mean number of ants (± SE) in Trap A (standard pitfall) and Trap B (covered pitfall)

Location	Ants						
	Solenopsis	*Paratrechina*	*Monomorium*	*Atta*	*Crematogaster*	*Camponotus*	Combined
Los Indios							
Trap A	4.5 ± 4.5a	none	none	none	0.5 ± 0.5a	2.5 ± 0.5a	5.0 ± 2.3a
Trap B	0.0 ± 0.0a	none	none	none	0.0 ± 0.0a	0.0 ± 0.0b	0.0 ± 0.0a
	$F = 1.000$	--	--	--	$F = 1.000$	$F = 25.000$	$F = 4.688$
	df = 1, 3	--	--	--	df = 1, 3	df = 1, 3	df = 1, 5
	$P = 0.423$	--	--	--	$P = 0.423$	$P = 0.038$	$P = 0.296$

Means in a column followed by the same letter are not significantly different, protected Tukey Test ($P < 0.05$)

were effective in reducing or eliminating ant predation on released scale crawlers in the field.

Thus application of ant baits did not reduce the number of ants captured in pitfall traps but did mitigate ant predation on crawlers. These results suggest baits out

1998). Additionally, as localized baiting reduces the strength of one ant colony, other colonies readily begin foraging the same area and new incipient colonies proliferate (Apperson et al. 1984, Lofgren and Weidhass 1972, Lofgren and Williams 1985). Thus,

Table 3. Del Rio, TX: Mean number of ants (± SE) in pitfall traps in treated and untreated areas

Location	Ants				
	Solenopsis invicta	*Paratrechina terricola*	*Monomorium minimum*	*Atta texana*	Combined
Del Rio, TX					
Treated	1.2 ± 1.2a	0.0 ± 0.0a	0.4 ± 0.4a	0.0 ± 0.0a	0.4 ± 0.2a
Untreated	2.6 ± 0.7a	0.3 ± 0.2a	0.3 ± 0.2a	1.3 ± 0.9a	1.1 ± 0.3a
	$F = 1.099$	$F = 0.537$	$F = 0.148$	$F = 0.690$	$F = 2.082$
	df = 1, 19	df = 1, 19	df = 1, 19	df = 1, 19	df = 1, 107
	$P = 0.308$	$P = 0.473$	$P = 0.705$	$P = 0.417$	$P = 0.152$

Means in a column followed by the same letter are not significantly different, protected Tukey Test ($P < 0.05$)

compete scale as a food source through predator satiation and dilution effects (Ali et al. 1984, Coster-Longman et al. 2002, Karban 1982, Lachance and Cloutier 1977, Treherne and Foster 1982). Our laboratory and field observations indicated *S. invicta* would remove scale in the absence of other food sources but favored baits over the scale. High density and even distribution of bait granule ensure that foraging ants will encounter granular baits before the scale. Continued presence of ant foraging post-treatment reflects the localized nature of areas treated. Maximum sup-

foraging ants are transporting bait granules instead of searching for prey which reduces predation pressure sufficiently to increase the likelihood of successful scale establishment.

Initially in 2010, when it became apparent that ant predation affected the success of the field release of the arundo scale biological control agent, only Amdro™ Fire Ant Bait was applied to mitigate the dominant predator, *S. invicta*. As *S. invicta* predation pressure was reduced, scale predation by other ant species was still problematic, consistent with the find-

Table 4. Mean number of ants (± SE) in pitfall traps in treated and untreated areas

Location	Ants				
	Solenopsis invicta	*Paratrechina terricola*	*Crematogaster sp.*	*Camponotus texanus*	Combined
Laredo, TX					
Treated	3.2 ± 1.5a	0.0 ± 0.0a	0.2 ± 0.2a	none	5.7 ± 5.2a
Untreated	3.4 ± 1.4a	5.0 ±2.5a	1.0 ± 0.5a	none	50.0 ± 35.5a
	$F = 2.2586$	$F = 3.906$	$F = 2.667$	--	$F = 1.529$
	df = 1, 9	df = 1, 9	df = 1, 9	--	df = 1, 5
	$P = 0.171$	$P = 0.084$	$P = 0.141$	--	$P = 0.284$
Los Indios, TX					
Treated	0.0 ± 0.0	none	0.5 ± 0.5	2.5 ± 0.5A	2.0 ± 1.5
Untreated	4.5 ± 4.5	none	0.0 ± 0.0	0.0 ± 0.0B	3.0 ± 3.0
	$F = 1.000$	--	$F = 1.000$	$F = 25.000$	$F = 0.0882$
	df = 1, 19	--	df = 1, 3	df = 1, 3	df = 1, 5
	$P = 0.423$	--	$P = 0.423$	$P = 0.038$	$P = 0.781$

Means in a column followed by the same letter are not significantly different, protected Tukey Test (P < 0.05) proceeded by a significant ANOVA

ings of Apperson et al. (1984) and Way and Khoo (1992). Addition of Maxforce™ Granular Insect Bait to protocol for field release of the arundo scale solved the ant predation problem. Different ant species have different food preferences with the soybean oil of Amdro readily consumed by fire ants but not by some of the other ant species which prefer the silkworm moth protein found in Maxforce (Krushelnycky and Reimer 1998, Stanley and Robinson 2007, Tripp et al. 2000). Similarly, ant predation had also been reported to interfere with arthropod based biological control efforts for invasive weeds (Ciomperlik et al. 1992, Robertson 1985).

Pitfall trapping of ants has been recognized as an effective monitoring technique (Borgelt and New 2005, Calizto et al. 2007, Pendola and New 2007). Increased sampling effort with a higher pitfall trap density and the incorporation of multiple sampling techniques such as baits, hand collection, and litter extraction with Berlese funnels may add to the number of ants and species sampled (King and Porter 2005). The social nature of ants promotes clumping, making the determination of abundance challenging and may require sophisticated analysis of incidence data (King and Porter 2005, Morrison and Porter 2003).

This is the first study attempting to evaluate the effect of ant predation on *R. donacis* establishment. Expansion of research in this area can improve release strategies for this biological control agent which is a key component of the *Arundo donax* biological control program.

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