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Introduction of fire ant biological control agents into the Coachella Valley of California

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The stinging and invasive red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), has been reported from the Coachella Valley (Riverside County) of California, USA, since 1998 and has continued to spread throughout the region (Jetter et al. 2002; Henke 2015). Fire ants generally inhabit irrigated, urban landscapes in the desert climate of the Coachella Valley. In 2014, a project was initiated to introduce the following fire ant biological control agents into the Valley: 2 species of decapitating phorid flies, *Pseudacteon curvatus* Borgmeier and *Pseudacteon obtusus* Borgmeier (Diptera: Phoridae); Solenopsis invicta virus 3 (Picornavirales: Solinviviridae) (SINV-3); and the microsporidian pathogen *Kneallhazia solenopsae* Knell, Allan, & Hazard (Microsporida: Thelohaniidae). The objectives of this project were to introduce these agents from Florida, and to determine if they could establish in fire ant populations colonizing the hot, dry, irrigated habitat of the Coachella Valley.

Four sprinkler-irrigated sites that included a golf course (Monterey Country Club) and 3 storm-water retention basins (El Dorado, Sonrisa, and Harland) were initially surveyed for fire ant biological control agents. Surveys were conducted in Mar 2014 with an additional survey site in Oct 2014 of irrigated turf between a street and parking lot (La Quinta Medical Center). Surveys were conducted by collecting worker ant samples from 8 to 25 nests per site, and using RT-PCR and PCR to detect pathogens or flies (Valles et al. 2009a, b). In addition, the social form of sampled fire ants (i.e., monogyny or polygyny) was determined by Gp-9 analysis (Valles & Porter 2003). We found that the Monterey, El Dorado, and Sonrisa sites were predominantly polygyne (85–100%), whereas Harland and La Quinta samples were 92 and 100% monogyne, respectively. *Kneallhazia solenopsae* was detected at Sonrisa (3 of 15 positive samples), Monterey (1 of 14), and La Quinta (6 of 8). SINV-3 was detected at Sonrisa (8 of 15), Monterey (4 of 14), and Harland (2 of 25). Phorid flies were not detected in the Mar 2014 samples (n = 67). The Harland site was excluded from releases because most fire ant nests were located partially under a sidewalk that would hinder inoculations.

For the decapitating phorid fly releases, a total of 64 g of fire ants (about 64,000) were collected from 13 colonies at the Monterey site, and 28 g collected from 8 colonies at the Sonrisa site on 8 to 9 May 2014. These ants were subsequently exposed to parasitism attacks from *P. curvatus* and the larger sized *P. obtusus* in Gainesville, Florida, USA, on 12 to 14 May. Attacks were conducted in the field and at the US Department of Agriculture, Animal and Plant Health Inspection Service (USDA/APHIS), Florida Department of Agriculture and Consumer Services phorid fly rearing facility. Approximately 90% of attacking females were *P. curvatus* (Porter & Calcaterra 2013). Parasitized ants were then shipped back to California, and released into their mother colonies on 15 to 16 May 2014 by Coachella Valley Mosquito and Vector Control District staff. Releases of about 5,970 and about 15,270 parasitized ants also were conducted at the Monterey and La Quinta Medical Center sites, respectively, in Nov 2014.

Six months after phorid fly releases, 2 male *P. obtusus* were collected at the Monterey site. Subsequent trapping (Puckett et al. 2007) for the flies in 2015 to 2017 resulted in a total collection of 93 *P. obtusus*, consisting of 80 males and 13 females, and 41 *P. curvatus*, with 3 males and 38 females (Table 1). In May 2016, only 4 male *P. obtusus* were trapped; however, in 2015 there was a severe drought and irrigation was restricted in 2016. Collections rebounded in May 2017 with an increase of *P. curvatus* being trapped (30 females, 3 males), in addition to 11 male and 3 female *P. obtusus* (Table 1). Flies trapped in 2017 were as far as 201 m away from the release location. Despite releasing many more ants parasitized by *P. curvatus*, the larger *P. obtusus* flies were first to establish at the Monterey site, and remained more abundant than expected even after *P. curvatus* was established (Table 1) (Porter & Calcaterra 2013). Flies were never collected at the Sonrisa or La Quinta sites after 4 samplings spanning 2 and 3 yr, respectively.

SINV-3 was released at the El Dorado site on 11 Jun 2014, and at the La Quinta Medical Center site on 26 Jan and 22 Oct 2015, using methods adapted from Valles and Oi (2014). At El Dorado, inocula consisted of 3 g of dead, SINV-3 infected fire ant workers and brood homogenized in a 5% (wt/vol) sucrose solution. The homogenate contained 1.65 ± 9.31 × 10^7 genome copies of SINV-3 per ng of RNA. The SINV-3 slurry was applied as a 50 mL drench on individual fire ant nests, or as a bait by inserting into a nest a plastic centrifuge tube (15 mL) plugged with cotton, that contained 12 mL of the slurry. A total of 20 nests were inoculated with an estimated 1.11 × 10^{13} virus particles per nest. Unlike the El Dorado inoculations where nests were small and difficult to find, fire ant nests inoculated at the La Quinta Medical Center were discrete and easily detected. For the Jan 2015 inoculation, SINV-3 (10^{15} virus particles) was mixed with 25% sucrose solution as the bait attractant, and dispensed in either plastic centrifuge tubes inserted into 12 nests, or poured atop 12 nests as a drench. In Oct 2015 another inoculation was conducted at the La Quinta site where 18 baits and 18 drenches (SINV-3 in 10% sucrose solution) were applied to new and previously inoculated fire ant nests.

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Table 1. Fire ant decapitating phorid fly release dates, species, and gender of flies collected in the Coachella Valley, California (2014-2017).

Site	Release dates	Survey dates						
		11 Jun 2014	28–29 Oct 2014	27 Jan 2015	13–14 May 2015	21 Oct 2015	4–5 May 2016	8 May 2017
Monterey Country Club	16 May 2014 6 Nov 2014	0	*P. obtusus:* 2 ♂	*P. obtusus:* 1 ♀	*P. obtusus:* 19 ♂, 5 ♀ *P. curvatus:* 1 ♀	*P. obtusus:* 46 ♂, 4 ♀ *P. curvatus:* 7 ♀	*P. obtusus:* 4♂	*P. obtusus:* 11 ♂, 3 ♀ *P. curvatus:* 30 ♀, 3 ♂
Sonrisa	15 May 2014	0	0	0	0		—[a]	—[a]
La Quinta Medical Center	7 Nov 2014	NA[b]	NA	0	0		0	0

[a]monitoring discontinued due to consistent absence of phorid flies and low fire ant nest densities.
[b]NA, not applicable; releases not made until Nov 2014.

Samples of foraging worker ants collected along transects in the vicinity of the treated nests at El Dorado, and worker ants collected directly from inoculated and non-inoculated nests at La Quinta, revealed that the virus had established at both sites. SINV-3 prevalence at El Dorado fluctuated between 9 and 48% from Jul 2014 to May 2017. At the La Quinta site, SINV-3 prevalence ranged from 13 to 68% from Feb 2015 to May 2017 (Table 2). Infected samples were obtained as far as 103 m and 127 m beyond the inoculated areas at El Dorado and La Quinta, respectively, that indicated SINV-3 was spreading.

Kneallhazia solenopsae was released on 8 May 2014 at Monterey by introducing live, infected brood into 16 colonies following the procedures described in Oi and Valles (2009). Approximately 5 g of brood with an infection rate of 42.8% were introduced into each colony. Although initial *K. solenopsae* prevalence was considered low (7.1%) prior to inoculations, infection prevalence in fire ants increased to 100, 70, and 71% in samples collected after inoculation (Table 2). Infections were detected from pupal samples several mo after inoculations that indicated the pathogen had established, because infections were occurring in progeny not from pupae contained in the inocula. It should be noted that infections persisted despite irrigation being reduced at the site when landscapers tried to establish drought-tolerant grass.

We collected *P. obtusus*, *P. curvatus*, SINV-3, and *K. solenopsae* 2 to 3 yr after their introductions in the Coachella Valley. The extreme heat of this region was illustrated by average daily maximum temperatures that ranged from 36.4 to 43.2 °C (97.5–109.7 °F) for the months of Jun to Sep during 2014 to 2016. In addition, daily maxima were > 32.2 °C (90 °F) for 184 to 200 d per yr in 2014 to 2017. Annual precipitation for 2014 to 2017 was 5.1 to 18.2 cm (2.1 to 7.2 in) (NCEI/NOAA 2018, Palm Springs station). Despite these extreme environmental conditions, the biocontrol agents we released increased in prevalence or spread from their original release locations. Our study represents the first establishment of fire ant decapitating phorid flies in California. We also documented that SINV-3 and *K. solenopsae* were naturally present in the Valley. In fact, *K. solenopsae* was found in 6 of 8 nests sampled (Oct 2014) at the La Quinta Medical Center site, over 11.2 km away from the Monterey inoculation site. In addition, we demonstrated human mediated establishment of SINV-3 and *K. solenopsae* in hot, dry, but irrigated habitat. Unfortunately, fire ant populations were not suppressed based on the number and size of fire ant nests still present at sites where the biocontrol agents had established. This observation was expected due to the limited duration of establishment, and the subtle impacts of these agents on fire ant field populations (Oi et al. 2015). However, desert climate and unirrigated landscapes inhospitable to fire ants may serve to isolate these populations from natural re-infestation, and thus may provide an opportunity for biocontrol impacts to accumulate over time.

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Summary

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a stinging, invasive ant from South America that has plagued the southern US since the 1930s. It is currently established in parts of California, including the Coachella Valley. We introduced and

Table 2. Inoculation dates and percent (n) prevalence of Solenopsis invicta virus 3 (SINV-3) and of the microsporidian pathogen *Kneallhazia solenopsae* on specified sampling dates in Coachella Valley, California (2014–2017).

Site [Pathogen]	Inoculation Date	Survey Year 2014	2014	2014	2015	2015	2016	2017
El Dorado [SINV-3]	11 Jun 2014	1 Jul 25 (40)	1 Aug 9 (33)	19 Nov 48 (40)	—[a]	24 Nov 20 (35)	5 May 33 (15)	8 May 24 (33)
La Quinta [SINV-3]	26 Jan & 22 Oct 2015	NA[b]	NA	NA	20 Feb 20 (30)	13 May 41 (22)	5 May 68 (19)	8 May 13 (15)
Monterey [*K. solenopsae*]	8 May 2014	31 Jul 100 (16)	—[a]	29 Oct 70 (24)	—[a]	21 Oct 71 (14)	—[c]	—[c]

[a]no sampling
[b]NA, not applicable; inoculations not made until Jan 2015.
[c]monitoring discontinued.

established the fire ant decapitating phorid flies, *Pseudacteon curvatus* Borgmeier and *Pseudacteon obtusus* Borgmeier (Diptera: Phoridae); Solenopsis invicta virus 3 (Picornavirales: Solinviviridae) (SINV-3); and the microsporidian fire ant pathogen *Kneallhazia solenopsae* Knell, Allan, & Hazard (Microsporida: Thelohaniidae) for the biological control of fire ants in this region of California. This is the first establishment of fire ant decapitating phorid flies in California. In addition, we demonstrated human-mediated establishment of SINV-3 and *K. solenopsae* in hot, dry, but irrigated, urban desert habitat.

Key Words: *Solenopsis invicta*; *Pseudacteon*; *Kneallhazia solenopsae*; Solenopsis invicta virus 3; pathogen

Sumario

La hormiga roja de fuego importada, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), es una hormiga invasora de América del Sur que pica y que ha plagado el sur de los EE. UU. desde la década de los 1930. Actualmente, está establecida en partes de California, incluido el Valle de Coachella. Introdujimos y establecimos las moscas fóridas, *Pseudacteon curvatus* Borgmeier y *Pseudacteon obtusus* Borgmeier (Diptera: Phoridae) que decapitan a la hormiga de fuego; *Solenopsis invicta* virus 3 (Picornavirales: Solinviviridae) (SINV-3); y el patógeno microsporidian hormiga de fuego *Kneallhazia solenopsae* Knell, Allan, & Hazard (Microsporida: Thelohaniidae) para el control biológico de las hormigas de fuego en esta región de California. Este es el primer establecimiento de las moscas fóridas que decapitan las hormigas de fuego en la California. Además, demostramos el establecimiento de SINV-3 y *K. solenopsae* por medio de la interacción humana en un hábitat desértico urbano, seco, pero irrigado.

Palabras Clave: *Solenopsis invicta*; *Pseudacteon*; *Kneallhazia solenopsae*; *Solenopsis invicta* virus 3; patógeno

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