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Solenopsis invicta virus 3: Further host-specificity tests with native *Solenopsis* ants (Hymenoptera: Formicidae)

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Abstract

A thorough understanding of host specificity is essential before pathogens can be used as biopesticides or self-sustaining biocontrol agents. In order to better define the host range of *Solenopsis invicta virus 3* (SINV-3), we collected and exposed colonies of 2 native fire ants (*Solenopsis aurea* Wheeler, *Solenopsis xyloni* McCook) and 2 native thief ants (*Solenopsis carolinensis* Forel, *Solenopsis molesta* [Say]) (Hymenoptera: Formicidae) to the SINV-3 virus. Despite extreme exposure to the virus, active, replicating infections resulted only in colonies of the red imported fire ant (*Solenopsis invicta* Buren). These results, combined with a previous study of ants from 13 other genera, strongly support the proposition that the SINV-3 virus can be used safely as either a biopesticide or a self-sustaining biocontrol agent in parts of California, the Caribbean, and other regions of the world where this virus does yet not occur.

Key Words: *Solenopsis invicta*; biocontrol; host range; thief ant; native fire ant; SINV-3

Resumen

Una comprensión completa de la especificidad de hospederos es esencial para poder utilizar patógenos como bioplaguicidas o agentes de biocontrol autosostenibles. Para determinar el rango de hospederos del virus Solenospsis invicta 3 (SINV-3), colectamos y expusimos el virus SINV-3 a colonias de 2 especies de hormigas de fuego nativas de EE.UU (*Solenopsis aurea* Wheeler, *Solenopsis xyloni* McCook) y 2 especies nativas de hormigas ladronas (*Solenopsis carolinensis* Forel, *Solenopsis molesta* [Say]) (Hymenoptera: Formicidae). A pesar de la exposición extrema al virus, sólo se observaron infecciones activas y replicantes en las colonias de la hormiga de fuego importada (*Solenopsis invicta* Buren). Estos resultados, combinados con los de un estudio previo de otros 13 géneros de hormigas, apoyan firmemente la propuesta de que el virus SINV-3 puede ser utilizado con seguridad como bioplaguicida o agente de biocontrol autosostenible en áreas de California, el Caribe y otras regiones del mundo donde este virus no se encuentra todavía.

Palabras Claves: *Solenopsis invicta*; control biológico; rango de hospederos; hormigas ladronas; hormigas de fuego nativas de EE.UU.; SINV-3

Three RNA viruses have been found in the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae): SINV-1, SINV-2, and SINV-3 (Valles 2012). *Solenopsis invicta virus 3* is the most virulent of these three, often causing considerable worker mortality and the cessation of brood production in laboratory colonies (Porter et al. 2013; Valles et al. 2013). SINV-3 has potential for use as a biopesticide if an effective bait formulation can be developed (Valles et al. 2013). This virus could also be a classical or self-sustaining biocontrol agent if it can be safely introduced into areas where it is not yet found, including Australia, China, the Caribbean, and parts of California (Ascunce et al. 2011; SMV, Wetterer, and SDP, unpublished data). A previous study showed that SINV-3 is highly host specific—only infecting *Solenopsis* fire ants from South America (Porter et al. 2013). The objective of this study was to expand host specificity evaluations for SINV-3 to include a 2nd and 3rd native fire ant species (*Solenospis aurea* Wheeler, *Solenopsis xyloni* McCook) and 2 species of native thief ants also in the genus *Solenopsis* (*S. carolinensis* Forel, *S. molesta* [Say]).

Materials and Methods

WHOLE COLONY INFECTION TEST

In our first test, whole *S. invicta* colonies were used as infection standards. Eight colonies were raised from founding queens collected in the Gainesville, Florida, area (N 29° 39' W 82° 19') using rearing procedures designed to eliminate viral infections (Valles & Porter 2013). Each *S. invicta* colony (Table 1) was adjusted so it weighed about 3 g including a queen, workers (1,500 to 2,200), and brood (~ 50% by weight). Colonies

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Table 1. Whole colony SINV-3 infection tests in queen-right colonies of native desert fire ants, native thief ants, and imported fire ants, as detected by the presence of the VP2 capsid protein.

Solenopsis species (no. colonies)	Type	Initial weights (g)	Final weights (g)	SINV-3 capsid protein
S. aurea (3)	Treated	3.0, 3.0, 1.5	7.1, 10.9, 4.8	Negative
S. aurea (2)	Control	3.0, 1.5	19.7, 4.8[c]	Negative
S. molesta (2)	Treated	0.36, 0.39	0.24[a], 0.38	Negative
S. carolinensis (1)	Treated	0.045	0.080	Negative
S. invicta (4)	Treated	3.0, 3.1, 3.0, 3.0	4.6[b], 3.0[a], 2.9[a], 4.4[a]	**Positive**
S. invicta (4)	Control	3.2, 3.1, 3.2, 3.0	15.1, 4.0[a], 26.6[c], 15.7	Negative

[a]No brood production
[b]Trace brood production
[c]Not tested for capsid protein due to space limits

of the test species *S. aurea*, *S. carolinensis*, and *S. molesta* were variable in their sizes (Table 1). A colony of the desert fire ant (*S. aurea*) was collected in Indio, California (N 33° 43' W 116° 13'), and another colony was collected in Mecca, California (N 33° 34' W 116° 5'). The Indio colony contained 7 queens and was split into 4 colonies. Three of these colonies each contained 2 queens, 3 g of workers (1,500 to 2,200) and brood (~50% by weight), and the 4th colony contained 1 queen and 1.5 g of workers and brood that matched the size of the Mecca colony. Two colonies of the thief ant *S. molesta* (Table 1) were collected under sidewalks in Urbana, Illinois (N 40° 6' W 88° 12'). They each contained queens (1 and 3, respectively), several thousand workers, and ample brood. A colony of the thief ant *S. carolinensis* (Table 1) was collected from a pine stump in the Austin Cary Forest about 7 km NE of Gainesville, Florida. At the start of the test, it contained a queen and several hundred workers and brood. All colonies were maintained at 27 to 28 °C in nests and foraging trays appropriate to their size. Colonies were fed domestic crickets (*Acheta domesticus* [L.]; Orthoptera: Gryllidae) and 1.5 M sugar water (Gavilanez-Slone & Porter 2013).

Four of the *S. invicta* colonies, 3 of the *S. aurea* colonies (including the Mecca colony), and all of the thief ant colonies (*S. molesta*, *S. carolinensis*) (Table 1) were repeatedly exposed to massive doses of SINV-3 (see below) over a period of about a month, following a protocol similar to that of Porter et al. (2013). The remaining 4 *S. invicta* colonies and 2 *S. aurea* colonies were maintained as controls (Table 1). The treatment colonies were exposed by ad libitum access to SINV-3–laced sugar water bait 5 times at 0, 5, 12, 19, and 35 d. This bait contained > 10^6 genome equivalents of the virus per microliter and was formulated as a slurry with filtered extract from SINV-3–infected ants (~ 1 g of crushed dead worker ants per 20 mL of 15% sugar water; Valles et al. 2013). Treatment colonies were also given ad libitum access to a SINV-3–laced cricket mash 3 times during the same month (1, 4, 11 d), formulated as a 1:7 ratio of infected ants (see above) and crickets (dry: wet weight). Food was withheld from test colonies for 2 to 3 d before introducing the sugar or cricket baits. At the start of the test, treatment colonies also received several grams of cracked wheat ground together with dead infected ants (10:1 wheat: ants, dry weight). Additionally, several thousand dry crushed SINV-3–infected *S. invicta* workers were scattered across the foraging trays of test colonies at the start of the test.

Seven weeks after the start of the test, samples were collected from the fire ant colonies (15 workers per colony), the *S. molesta* colonies (~ 40 workers per colony), and the *S. carolinensis* colony (~ 60 workers) for western blot analysis to detect replication of the virus by production of the VP2 capsid protein of SINV-3 by the method described previously (Valles et al. 2014a).

WORKER INFECTION TEST

A second SINV-3 host-specificity test was conducted 5 mo later with test *Solenopsis xyloni* McCook workers from Tempe, Arizona (N 33° 24' W 111° 54'), and *S. aurea* workers from the colonies used above. We used workers because whole *S. xyloni* colonies were not available. These workers were compared to *S. invicta* workers from Gainesville, Florida (included as infection standards). We used 4 groups of *S. xyloni* workers, 5 groups of *S. aurea* workers, and 8 groups of *S. invicta* workers (Table 2). Each group was from a separate colony. Test workers were collected by placing a card into a disturbed field nest (*S. xyloni*, *S. invicta*) or a laboratory colony (*S. aurea*), permitting workers to run up the card, and then tapping them off into a collection tray. The field-collected *S. invicta* workers were pre-tested for SINV-3 virus (Valles et al. 2009) and all proved to be free of this pathogen.

Test worker groups that had not been fed in 2 to 3 d were exposed to 0.5 mL sugar water SINV-3 bait 3 times (day 0, 3, and 12). Sugar water bait was formulated as in the first test. Additionally, 1/4 to 1/3 of test workers were shaken in an aqueous solution of crushed SINV-3–infected ants prepared as above for the sugar water, but without sugar. After draining off the solution, these workers were returned live to their respective test groups. Finally, 0.4 ± 0.05 g (SD, wet weight) of crushed infected *S. invicta* workers were scattered in each test tray. Cricket baits were not used because brood was not present. At 18 d, 20 workers from each test group were tested for SINV-3 capsid protein using the western blot analysis as above.

Results

WHOLE COLONY INFECTION TEST

Despite repeated and extensive exposure, the desert fire ant (*S. aurea*) and the 2 species of native thief ants, also in the genus *Sole-*

Table 2. SINV-3 infection tests with workers of two species of native fire ants and the red imported fire ant, as detected by the presence of the VP2 capsid protein.

Solenopsis species (no. groups)	Type	Weight of test groups[a] (g)	SINV-3 Capsid Protein
S. xyloni (4)	Treated	1.4, 0.8, 0.3, 0.2	All negative
S. aurea (5)	Treated	0.9, 1.0, 0.9, 0.2, 0.9	All negative
S. invicta (8)	Treated	1.4, 0.8, 0.3, 0.2, 1.4, 0.8, 0.3, 0.2	**7 of 8 Positive**

[a]One gram of fire ant workers (all 3 species) equals about 1,000 to 1,500 workers

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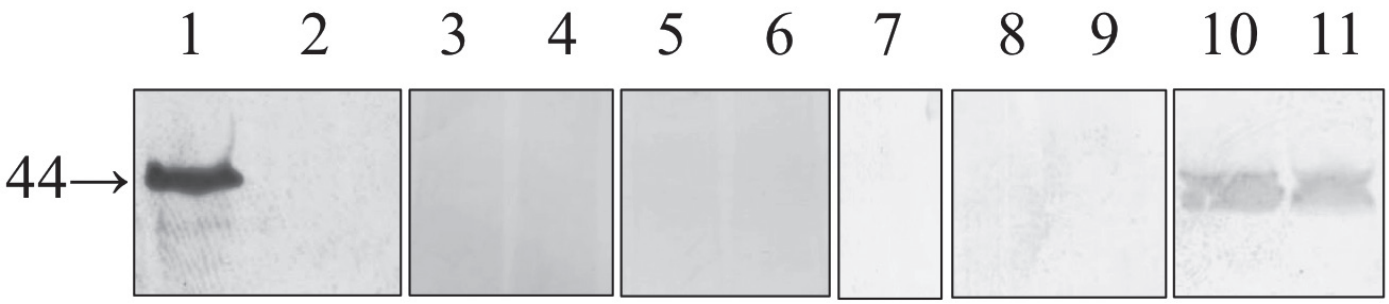


Fig. 1. Representative western blot analyses to detect the presence of SINV-3 capsid protein (VP2) in different ant species. Lane assignments are as follows: 1) purified SINV-3; 2) *S. invicta* negative control colony; 3,4) *S. aurea*; 5,6) *S. xyloni*; 7) *S. carolinensis*; 8,9) *S. molesta*; 10,11) *S. invicta* positive control colonies. Molecular retention indicated as kDa.

nopsis, did not support replication of SINV-3. Western blot analysis produced distinct bands for the viral capsid protein (VP2, see Fig. 1) in all 4 of the treated *S. invicta* colonies (Table 1) indicating that the virus was replicating. Furthermore, all 4 treated *S. invicta* colonies had stopped growing by the end of the test (Table 1) and contained either no brood (3 colonies) or only a trace of brood (1 colony). In contrast, the capsid protein (see Fig. 1) was not detected in the 3 treated *S. aurea* colonies or the 2 species of thief ants (Table 1) confirming the lack of viral replication in these ants. The capsid protein was also not found in 3 of the 4 untreated *S. invicta* colonies (see Fig. 1; the 4th colony was not tested because of space limits, Table 1). After 7 wk, 3 of the 4 *S. invicta* control colonies and all of the *S. aurea* colonies contained large amounts of brood and had at least doubled in weight (Table 1). Brood production had stopped in 1 of the untreated *S. invicta* colonies and 1 of the *S. molesta* colonies (Table 1), but neither colony contained capsid protein evidence of replicating virus. The *S. carolinensis* colony and the other *S. molesta* colony remained healthy with large amounts of brood. Four months later, all of the *S. aurea* colonies, the *S. carolinensis* colony, and the one *S. molesta* colony remained healthy.

WORKER INFECTION TEST

In the worker infection test, 7 of the 8 *S. invicta* standards tested positive for SINV-3 capsid protein demonstrating replicating viral infections (Table 2). In contrast, none of the 4 *S. xyloni* groups or the 5 *S. aurea* groups tested positive for SINV-3 capsid protein (Table 2). This test was terminated after 18 d because this is a reliable period to assess worker infections (Valles et al. 2014b) and because we were not looking at long-term effects on colony growth.

Discussion

A previous study showed that red, black, and hybrid fire ants in the *saevissima* species group from South America served as hosts for SINV-3, whereas 16 species of ants in 13 other genera did not support replication of SINV-3 (Porter et al. 2013). Importantly, Porter et al. (2013) also reported that laboratory test colonies of the native fire ant *Solenopsis geminata* (F.) were not infected, and the virus was not found in several dozen samples of miniature *Solenopsis* thief ants collected in the field.

The results reported in this paper are important because they confirm that a 2nd and 3rd native fire ant species (*S. aurea* and *S. xyloni*) and 2 species of native thief ants (*S. carolinensis* and *S. molesta*) are also not susceptible to SINV-3 viral infections (Tables 1 and 2), despite massive exposure in the laboratory (i.e., multiple exposures to baits, drenches, and crushed workers each with well over a million viral genome copies per microliter). Although only workers were tested for *S. xyloni*, rather than fully functioning colonies, it should be noted that SINV-3 is specific to *S. invicta* adults and does not appear to infect immature stages (Valles et al. 2014b); consequently, there is little reason to expect different results if whole colonies had been tested, especially considering the absence of infections in fully functioning colonies of two sister fire ant species (*S. geminata*, *S. aurea*; Table 1 and Porter et al. 2013).

Apparently, only fire ant species from the South American *saevissima* group (Fig. 2; Trager 1991) are susceptible to the virus, because 3 closely related *Solenopsis* fire ants in the North and Central American *geminata* group (i.e., *S. geminata*, *S. xyloni*, *S. aurea*; Fig. 2) were not suitable viral hosts (Tables 1 and 2 and Porter et al. 2013). Furthermore, the 2 species of more distantly related *Solenopsis* thief ants (Fig. 2) did not become infected in our tests (Table

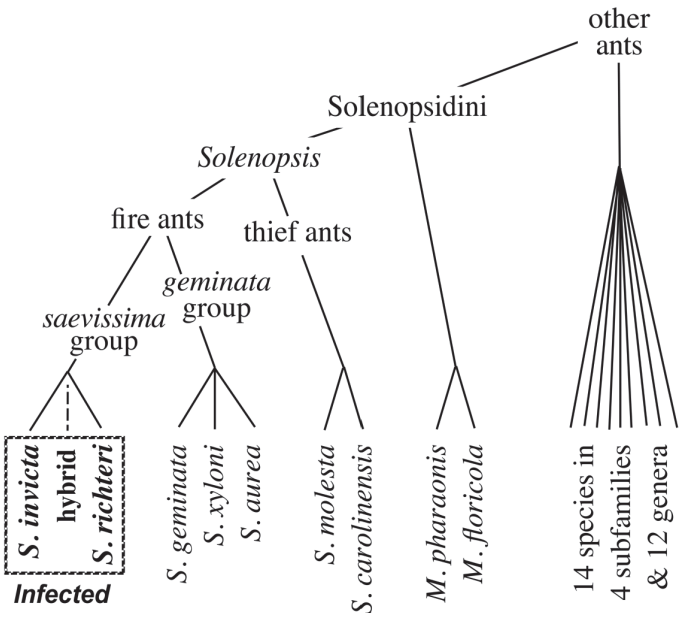


Fig. 2. *Solenopsis invicta virus 3* (SINV-3) infections are restricted to *Solenopsis* fire ants in the South American *saevissima* group (*S. invicta*, *S. richteri*, and their hybrid in the United States). North American fire ants in the *geminata* group, thief ants, two species of *Monomorium* (also tribe Solenopsidini), and 14 additional species of ants from 3 subfamilies and 12 genera were not infected in lab trials (this paper and Porter et al. 2013).

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1). Finally, 2 *Monomorium* species from the same tribe as *Solenopsis* fire ants (Fig. 2) were also not infected in previous tests (Porter et al. 2013). In short, centrifugal testing of potential hosts according to their degrees of relatedness, as recommended by Briese (2005), confirms that SINV-3 is a highly host-specific pathogen limited to closely related ants from South America (Fig. 2). Consequently, we conclude that SINV-3 can be used safely as either a biopesticide or a self-sustaining biocontrol agent without threat to native ants in the United States, the Caribbean, and nations along the Pacific Rim that are currently infested with red imported fire ants (Ascunce et al. 2011).

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