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Scavenging crickets (Orthoptera: Gryllidae) transmit Solenopsis invicta virus 3 to red imported fire ant (Hymenoptera: Formicidae) colonies

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Solenopsis invicta virus 3 (SINV-3) is a positive-sense, single-stranded RNA virus with similarities to Nylanderia fulva virus 1 and Kelp fly virus in the newly proposed family Solinviviridae (Valles et al. 2014a, 2016). SINV-3 may have value as a self-sustaining biocontrol agent because it is highly pathogenic in laboratory-reared colonies (Valles et al. 2013; Valles & Porter 2015) and specific to South American fire ants in the *Solenopsis saevissima* group (Hymenoptera: Formicidae) (Porter et al. 2013, 2015b). In the field, it has been reported to occur in about 11% of *Solenopsis invicta* Buren colonies near Gainesville, Florida (Valles et al. 2010) and 10% in Argentina (Valles et al. 2009). SINV-3 can be transmitted quickly among laboratory-reared colonies of this species by mechanical transfer of workers and/or refuse among colonies during feeding and cleaning (Valles & Porter 2013). Intra-colony transmission can occur by trophallaxis because SINV-3 is easily transmitted in liquid baits (Valles et al. 2013); however, social grooming or handling of feces may also play a role (de Miranda & Genersch 2010). Inter-colony transfer among polygyne colonies likely occurs by the exchange of infected workers and perhaps the adoption of infected queens. However, the natural mode of SINV-3 transmission among fire ants in the field is still unknown. SINV-3 does not occur more commonly in polygyne than in monogyne colonies (Valles et al. 2010). Thus, the objective of this study was to test the hypothesis that SINV-3 can be transmitted among monogyne and polygyne colonies by consumption of scavengers that have themselves consumed dead fire ant workers infected with SINV-3.

We tested this hypothesis with the house cricket, *Acheta domesticus* L. (Orthoptera: Gryllidae), because fire ants readily consume dead house crickets (Porter et al. 2015a) and omnivorous house crickets (Wineriter & Walker 1988) consume dead fire ants. In fact, we observed fire ant legs, antennae, pieces of exoskeleton, and even whole head capsules in the feces of crickets with access to dead fire ant workers.

Each day for 2 wk, 6 adult or nymphal crickets were removed from a cricket colony and held for 24 h without food before holding them another 24 h with 0.1 g of freeze-killed fire ant workers (~100) infected with SINV-3 (~5×10^9 viral genome copies per ant). This quantity of ants always exceeded what the crickets would eat. These crickets were then frozen, cleaned of all dead ants and detritus, and fed to the 6 treatment colonies. Each colony received 1 cricket. Six other fire ant colonies in an adjacent rack were fed frozen crickets that had not consumed infected fire ants. These colonies served as a control for the possibility that colonies might become infected by dust in the air or poor rearing hygiene (Valles & Porter 2013). Colonies were randomly assigned to treatment and control groups and were each about 3 to 4 g in size including brood. We reared colonies at 27 ± 1 °C in small boxes with test tube nests as described previously (Porter et al. 2015a). Prior to the test, 15 workers were collected from each of the 12 test colonies and preserved in 95% ethanol. Twenty-four days after colonies received the first test crickets, we collected 2 vials of 15 workers from each of the 12 colonies. The 1st vial was tested for the presence of SINV-3 by reverse transcriptase polymerase chain reaction (RT-PCR) as previously detailed (see Valles & Hashimoto 2009; Valles et al. 2009) and the 2nd vial was tested for the presence of SINV-3 capsid protein VP2 by Western blot analysis (for methods see Valles et al. 2014b).

To determine if crickets could be infected by SINV-3, we placed about 20 adult and late instar crickets in a rearing jar for 3 d without food. These crickets were then given access to 0.4 g of the freeze-killed fire ants infected with SINV-3 (see description above) for 3 d, after which they were transferred to a clean rearing container with ample food (dry dog food and fresh lettuce). Nineteen days later, 18 crickets were cut in half sagittally and tested for SINV-3 with half of each cricket used for RT-PCR and the other half used for Western blot analysis as noted above.

At the conclusion of the study, all 6 of the worker samples from the treatment colonies were positive for SINV-3 by RT-PCR tests, but none of the samples from the control colonies were positive. Four of the 6 samples from the treatment colonies were also positive for the viral capsid protein, but none of the samples from the control colonies were positive (1-tailed Fisher exact test, $P = 0.03$). RT-PCR tests also showed that none of the treatment fire ant colonies had been positive for SINV-3 prior to the commencement of the study.

Three of 18 crickets tested were weakly positive using RT-PCR for detecting SINV-3, 22 d after having had access to freeze-killed infected ants. However, capsid proteins were not detected in any of the crickets by Western blotting. Fire ant body parts were common in feces collected 3 d after initial access to the ants, demonstrating that the crickets were actually consuming the ants. In a side test, cricket feces did not appear to be attractive to the fire ants so a fecal route does not seem likely with house crickets although this route cannot be ruled out for other scavengers (de Miranda & Genersch 2010).

The finding that the virus-treated fire ant colonies were positive for SINV-3 was not surprising considering that the RT-PCR test is extremely sensitive to the presence of even a few hundred viral genome copies

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(Valles et al. 2009). However, the detection of the capsid protein in 4 of the 6 treatment colonies demonstrated that the virus was replicating in these colonies. The absence of both viral RNA and capsid protein in the control samples confirmed that positive results with the treatment samples were not simply due to poor rearing hygiene.

The absence of the capsid protein in 18 crickets that had consumed infected fire ants showed that the virus did not replicate in these crickets. We expected that SINV-3 would not replicate in the crickets because this virus is host specific to South American fire ants (Porter et al. 2015b). Extensive tests with native North American fire ants, ants from 13 other genera, and honey bees were all negative for SINV-3 infections (Porter et al. 2013, 2015b, 2016).

These results confirm our hypothesis that SINV-3 can be transmitted between fire ant colonies by scavengers like *A. domesticus* that consume dead infected fire ants and are then, in turn, consumed by fire ants. SINV-3 was most likely mechanically vectored in the gut of the cricket because the crickets themselves did not serve as host for the virus and cricket feces do not seem attractive to the ants. The results of this study are important because they explain a likely route of SINV-3 infection among both monogyne and polygyne fire ant colonies in the field.

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Summary

This study demonstrated that Solenopsis invicta virus 3 (SINV-3) can be mechanically transmitted among colonies of fire ants (Hymenoptera: Formicidae) by scavengers like the house cricket (Orthoptera: Gryllidae), which readily eat dead infected fire ant workers without becoming infected themselves.

Key Words: *Solenopsis invicta*; house cricket; *Acheta domesticus*; inter-colony transmission; SINV-3

Sumario

Este studio demostró que el virus Solenopsis invicta 3 (SINV-3) puede transmitirse mecánicamente entre las colonias de las hormigas bravas por medio de carroñeros como el grillo doméstico, el cual come fácilmente hormigas trabajadoras muertas infectadas sin infectarse a sí mismos.

Palabras Claves: *Solenopsis invicta*; grillo doméstico; *Acheta domesticus*; transmisión entre colonias; SINV-3

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