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SUCCESSFUL TRANSMISSION OF SOLENOPSIS INVICTA VIRUS 3 TO FIELD COLONIES OF *SOLENOPSIS INVICTA* (HYMENOPTERA: FORMICIDAE)

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Solenopsis invicta virus 3 (SINV-3) is a positive sense, single stranded RNA virus that infects fire ants in the fire ant *saevissima* complex, including *Solenopsis invicta* Buren (Porter et al. 2013). The virus was associated with dying fire ant colonies, which suggested its potential as a fire ant control agent either as a biopesticide and/or classical biological control agent against fire ants in locations (e.g., California, Taiwan) where the virus is not found (Oi & Valles 2009; Valles & Hashimoto 2009). Viruses are recognized as important components of insect biological control programs (Lacey et al. 2001). Formal laboratory tests have shown that the virus causes significant mortality among infected colonies (Valles et al. 2013) to a degree that is reminiscent of colony collapse disorder in honeybees (Cox-Foster et al. 2007). SINV-3 is readily transmitted to uninfected colonies in water- and oil-based bait formulations in the laboratory, demonstrating it potential as a biopesticide (Valles et al. 2013). However, transmission to fire ant colonies in the field has not been demonstrated, which is required to utilize SINV-3 as a classical biological control agent. Thus, the objective of this research was to evaluate whether SINV-3 could be transmitted to field colonies of *S. invicta*.

Fire ant colonies were surveyed along Fred Bear Road in Gainesville, Florida. Twenty colonies were sampled by plunging a scintillation vial into the mound and collecting worker ants that fell into the vial. RNA and DNA were extracted from a pooled group of 10 worker ants from each mound as described previously (Allen et al. 2011). DNA was used as template to conduct PCR to determine the social form of the ants by genotyping the *Gp-9* locus (Valles & Porter 2003), and to detect the presence of *Kneallhazia solenopsae* (Valles et al. 2002) and *Pseudacteon* decapitating parasitic flies (Oi et al. 2009). RNA from each sample was evaluated by RT-PCR for the presence of Solenopsis invicta virus 1 (SINV-1), SINV-2, and SINV-3 (Valles et al. 2009).

Ten colonies grouped within a 30 m diam along the southern end of Fred Bear Road (GPS coordinates: N 29.614057 -W 82.384458) served as the control group and 10 colonies approximately 200 m to the southwest also grouped within a 30 m diam (N 29.607789 –W 82.379007) were treated with SINV-3. SINV-3 was prepared as a crude solution in which 42 g of workers and larvae from a laboratory-infected colony were blended (2 min at high setting) in 1 L of 5% (w/v) sucrose. Quantitative PCR (Valles & Hashimoto 2009) revealed that the homogenate contained $9.63 \pm 1.09 \times 10^{10}$ genome copies of SINV-3/mL. Each mound was drenched with 50 mL of 5% sucrose (control group) or SINV-3 homogenate in 5% sucrose (treatment group). In addition, a cotton-stopped 50 mL plastic centrifuge tube filled to capacity with either 5% sucrose or 5% sucrose + SINV-3 was placed in contact with the mound.

Mound locations in both groups were marked with a vinyl flag and corresponding number. Worker ants were sampled from all mounds 14, 28, 43, and 56 days after treatment. RT-PCR (Valles & Hashimoto 2009) was conducted to determine the presence or absence of SINV-3 in each of the colonies.

Pre-evaluation of all fire ant colonies sampled revealed that they were all homozygous (B allele) at the *Gp-9* locus indicating that they were all monogyne (Table 1 Valles & Porter 2003). Thus, comparisons between treatment and control groups would not be influenced by social form differences. SINV-1 was detected in 50% of the control colonies and 30% of the treatment colonies. Neither SINV-2 nor SINV-3 was detected in either group. SINV-3 was not detected in any of the control colonies for the duration of the experiment (56 days). However, SINV-3 was detected in 2 colonies on day 28 and 6 colonies on day 56. Detection of residue of the inoculating dose in the nests was unlikely because only live fire ants were sampled after inoculations and SINV-3 was only detected at the latter sampling dates. Had the inoculating dose posed a contamination issue, positive detection should have occurred at the earlier sampling dates. Failure to detect virus at 43 days was not expected. However, we do not yet understand the dynamics of the virus development or intra-colonial transmission in the field. Hence, failure to detect virus at this time point may be the result of inadequate sampling, limitations in detection by RT-PCR, or simply natural pathogenesis in the field. Despite these peculiarities, the data provide evidence that SINV-3 can be successfully transmitted to field colonies of *S. invicta*.

Development of a potential biological control agent, like SINV-3, requires extensive investigation to ensure, as best as possible, the safety of the

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TABLE 1. PRE-TEST EVALUATION OF *SOLENOPSIS INVICTA* COLONIES FOR SINV-1, SINV-2, AND SINV-3, AND *GP-9* ANALYSIS FOR SOCIAL FORM. POST-TREATMENT EVALUATIONS FOR SINV-3 AT 14, 28, 43, AND 56 DAYS AFTER VIRUS EXPOSURE (ND = NOT DETECTED).

	Pre-evaluation for:				Post evaluation for SINV-3 at day:			
Colony designation	SINV-1	SINV-2	SINV3	Gp-9[a]	14	28	43	56
Control 1	ND	ND	ND	BB	ND	ND	ND	ND
Control 2	ND	ND	ND	BB	ND	ND	ND	ND
Control 3	Detected	ND	ND	BB	ND	ND	ND	ND
Control 4	ND	ND	ND	BB	ND	ND	ND	ND
Control 5	ND	ND	ND	BB	ND	ND	ND	ND
Control 6	ND	ND	ND	BB	ND	ND	ND	ND
Control 7	Detected	ND	ND	BB	ND	ND	ND	ND
Control 8	Detected	ND	ND	BB	ND	ND	ND	ND
Control 9	Detected	ND	ND	BB	ND	ND	ND	ND
Control 10	Detected	ND	ND	BB	ND	ND	ND	ND
Treatment 1	ND	ND	ND	BB	ND	ND	ND	Detected
Treatment 2	ND	ND	ND	BB	ND	ND	ND	ND
Treatment 3	Detected	ND	ND	BB	ND	ND	ND	Detected
Treatment 4	Detected	ND	ND	BB	ND	ND	ND	Detected
Treatment 5	ND	ND	ND	BB	ND	ND	ND	Detected
Treatment 6	ND	ND	ND	BB	ND	Detected	ND	Detected
Treatment 7	ND	ND	ND	BB	ND	Detected	ND	Detected
Treatment 8	Detected	ND	ND	BB	ND	ND	ND	ND
Treatment 9	ND	ND	ND	BB	ND	ND	ND	ND
Treatment 10	ND	ND	ND	BB	ND	ND	ND	ND

[a]Two alleles have been identified in North American *S. invicta*, B and b.

agent in the introduced range (Flint & Dreistadt 1998). SINV-3 has been shown to be efficacious against fire ants in the laboratory (Valles et al. 2013) and host specific for the *saevissima* complex of fire ants (Porter et al. 2013) In addition, SINV-3 has characteristics that facilitate its utilization as a microbial control agent. Unlike the microsporidium *Kneallhazia solenopsae*, SINV-3 can be disseminated via bait formulation (Valles et al. 2013). In contrast to the entomopathogenic fungus *Beauveria bassiana*, SINV-3 avoids fire ant behaviors of grooming and cadaver removal that limit the spread of infections (Oi & Valles 2009). Thus, SINV-3 appears to have satisfied some of the most important regulatory and biological requirements to be suitable as a biological control agent against *S. invicta*. The current study satisfies an additional crucial requirement for use as a classical biological control agent, field transmission.

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SUMMARY

Solenopsis invicta virus 3 (SINV-3) is a positive sense, single stranded virus that exhibits host specificity toward *saevissima* complex fire ants. The virus is being considered for release as a biological control agent in areas in which the virus is absent. This study demonstrates that field transmission is possible.

Key Words: biopesticide and/or classical biological control agent, fire ant *saevissima* complex, single stranded RNA virus

RESUMEN

El virus *Solenopsis invicta* 3 (SINV-3) es un virus de sentido positivo con cadena sencilla que muestra una especificidad del hospedero hacia el complejo *saevissima* de la hormiga de fuego. La liberación del virus está siendo considerado como un agente de control biológico en áreas en las que el virus no está presente. Este estudio demuestra que la transmisión de campo es posible.

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Palabras Clave: bioplaguicida y/o agente de control biológico clásico, complejo *saevissima* de la hormiga de fuego, virus de ARN de cadena simple

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