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SOLENOPSIS INVICTA VIRUS (SINV-1) INFECTION AND INSECTICIDE INTERACTIONS IN THE RED IMPORTED FIRE ANT (HYMENOPTERA: FORMICIDAE)

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Controlling invasive species is a growing concern; however, pesticides can be detrimental for non-target organisms. The red imported fire ant (*Solenopsis invicta* Buren; Hymenoptera: Formicidae) has aggressively invaded ~138 million ha in the USA and causes over $6 billion in damage and control efforts annually (Valles 2011). Myriad research studies have been conducted to discover safe biological control agents to manage these invasive pests (Valles et al. 2004; Milks et al. 2008; Oi et al. 2009; Yang et al. 2009; Wang et al. 2010; Callcott et al. 2011; Porter et al. 2011; Tufts et al. 2011). Viruses may be lethal due to modifications of cellular processes and induction of defense responses or may produce distinct survival outcomes depending on species (i.e. ascoviruses) (Stasiak et al. 2005). The *Solenopsis invicta* virus (SINV-1) is a positive sense, single-stranded RNA virus, which can only infect the genus *Solenopsis* at all stages of development, and is vertically-transmitted within a colony (Valles et al. 2004; Valles 2012).

We determined the sensitivity of SINV-1 infected ants to commercial insecticides: Amdro Fire Ant Bait (5-dimethyl-2(1*H*)-pyrimidinone[3-[4(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromthyl)phenyl]etheny]-2-propenylidene] hydrazone) and Over n'Out (O&O) Fire Ant Killer ((*RS*)-5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-(trifluoromethylsulfinyl)-1H-pyrazole-3-carbonitrile). Amdro (0.73% hydramethylnon) functions as a metabolic inhibitor and interrupts cellular respiration by impeding the electron transport chain within mitochondria (Hollingshaus 1987). Intoxicated individuals become lethargic, unable to feed/groom, and die within 3-4 days (Bacey 2000). O&O (0.0103% Fipronil) targets the central nervous system (CNS) blocking the movement of chloride through the GABA receptor and glutamate-gated chloride channels causing hyperexcitation and death within ~3 days (Raymond-Delpech et al. 2005). We hypothesized that virus infection would potentiate the toxicity of these insecticides eliciting individual mortality.

Polygyne *S. invicta* colonies were collected (Smith and Cherokee Counties, Texas) in 2009 and maintained under standard laboratory conditions. Colonies were tested (50 individuals) for SINV-1 using Reverse Transcriptase PCR and specific primers (Valles & Strong 2005). Positive colonies were subjected to whole virus extractions (Tufts et al. 2010). Virus concentration was estimated on protein levels as 82.7 ng/µL using a NanoDrop 1000 (Thermo Fisher Scientific Inc., Waltham, Massachusetts). Non-infected colonies were used for subsequent experiments. From a single non-infected colony, 100 ants were used in each of 6 treatment groups. Each group was composed of 10 Petri dishes, 10 ants were placed in each dish with Whatman© filter-paper wetted with 500 µL of one treatment: Control (nanopure water); SINV; Amdro; O&O; 50:50 SINV + Amdro combination; or 50:50 SINV + O&O combination. Insecticide formulations were evaluated at producer recommended field rates. Mortality was recorded daily and the experiment was repeated with a second non-infected colony.

SINV-infected individuals experienced the lowest mortality (Fig. 1A) in both trials. Because our data was categorical and followed a binomial distribution we used a repeated measures generalized linear mixed model (GLMM) adjusted for multiple comparisons using Tukey-Kramer. No evidence of overdispersion was detected. After Day 3 virus-infected individuals had significantly lower mortality than non-infected individuals, regardless of chemical treatment (Table 1). Individuals from each treatment group (n = 40) were subsequently tested for the presence of SINV-1. In all cases individuals from the SINV groups were infected and those from the chemical groups were not.

To quantify and control the amount of active chemical individuals received, an experiment was performed using laboratory grade Fipronil (ChemServices Inc., West Chester, Pennsylvania). Individuals from a naturally-infected colony (experimental) and individuals from a naturally non-infected colony (control) were used. Ten ants from each colony were dipped in Fipronil solvated in ACS-grade acetone (FischerScientific, Pittsburgh, Pennsylvania). Dosage response ranges

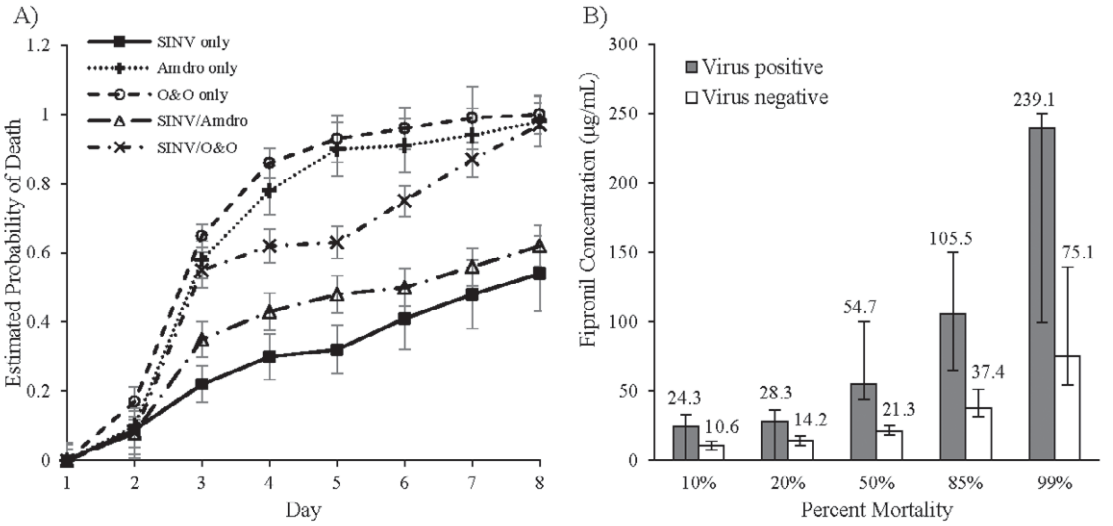

Fig. 1. A) Estimated probability of *Solenopsis invicta* death, mean treatment type by day after exposure interactions (mean ± 1 SEM). SINV alone demonstrated the lowest amount of ant mortality where Over n' Out (O&O) alone displayed the highest amount of mortality through the trial period. B) Effective concentrations of Fipronil mortality in fire ants are shown above each bar with 95% confidence intervals. In every case, more Fipronil was required to kill individuals infected with SINV than non-infected individuals and this difference became more pronounced with an increase in the percent of colony mortality.

were established (n = 720) and two concentration series were used to determine dose-response relationships in infected (n = 60) and non-infected (n = 60) ants. Mortality was assessed after 1h of exposure; a PROBIT regression was used to determine effective concentrations (µg/mL) at 10, 20, 50, 85, and 99% mortality (Fig. 2B). No mortality was observed in the control group (~100% acetone). The Fipronil contact toxicity experiment produced similar results as our previous experiment; SINV-1 infected individuals were more resilient against the chemical.

SINV-1, delivered at the rates and concentrations tested, decreases mortality in *S. invicta* exposed to certain insecticides. The underlying mechanism for this protective benefit is unknown. Viruses still have many unknown impacts on immune responses. Viruses may provide a benefit to their hosts, however, benefits imparted by them may only manifest under particular environmental circumstances (Roossinck 2011), some may also inhibit chemically induced apoptosis (Hussain & Asgari 2008). Many viruses infecting invertebrates elicit the formation of small-interfering RNAs (siRNA) that function as a defense response to viral infection and can trigger the release/production of microRNAs (miRNA) which regulate gene expression and metabolism. The actions of suppressors also influence miRNA expression, affecting host health, longevity, and immunity. siRNAs are also known to play a vital role in antiviral defense in *Drosophila* with regards to Cricket paralysis virus (CrPV) and *Drosophila* C virus (DCV) (Ping et al. 2011). Additionally, different bacteria have been found in the gut of *S. invicta* (Gunawan et al. 2008; Tufts & Bextine 2009) which may work in combination with SINV to provide protection against toxic substances (Lacey et al. 2001; Hedges et al. 2008; Roossinck 2011). ssRNA viruses may also impart protection to hosts by integrating portions of viral RNA into the host's genome (Valles 2011) which has been reported for SINV-1(TX5) (Tufts et al. 2010), although this phenomenon may not be universal (Valles & Bextine 2011).

Hydramethylnon and Fipronil intoxicate ants by different modes of action; hydramethylnon acts as a metabolic inhibitor while Fipronil targets the CNS. Fipronil induced higher mortality compared to hydramethylnon; however when ants were exposed to these toxicants, SINV-infected individuals exhibited greater survival than non-infected individuals. Future work on SINVs for biological control should investigate the broader gene expressions linked to immunity and toxicity in various metabolic pathways. SINV-3, which is ubiquitous in all tissues and at greater titers, may be more effective at causing mortality, unlike SINV-1 which produces chronic, asymptomatic infections which only manifest under certain environmental stressors (Valles 2011). Additional research on virus/ant host interactions is urgently needed to fully elucidate their potential as biological control agents for *S. invicta* populations.

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TABLE 1. VIRUS-INFECTION EXPRESSED AS AN OVERVIEW OF THE SIMPLE EFFECT COMPARISONS OF TREATMENT TYPE BY DAY AFTER EXPOSURE (DAE) INTERACTIONS. ONLY SIGNIFICANT VALUES ARE REPORTED (ADJ. P).

DAE	Treatment	Treatment	Std Error	Adj. *P*
3	SINV	O&O	0.722	0.0266
4	SINV	Amdro	0.953	0.0222
4	SINV	O&O	0.749	<0.0001
4	SINV/Amdro	O&O	0.664	0.0011
5	SINV	Amdro	1.011	0.0024
5	SINV	O&O	0.910	<0.0001
5	SINV/Amdro	Amdro	0.935	0.0276
5	SINV/Amdro	O&O	0.832	0.0016
5	SINV/O&O	O&O	0.804	0.0346
6	SINV	Amdro	1.012	0.0082
6	SINV	SINV/O&O	0.768	0.0306
6	SINV	O&O	0.874	<0.0001
6	SINV/Amdro	Amdro	0.945	0.0426
6	SINV/Amdro	O&O	0.801	<0.0001
6	SINV/O&O	O&O	0.750	0.0324
7	SINV	Amdro	1.024	0.0086
7	SINV	SINV/O&O	0.814	0.0016
7	SINV	O&O	1.112	<0.0001
7	SINV/Amdro	Amdro	0.953	0.0352
7	SINV/Amdro	SINV/O&O	0.730	0.0088
7	SINV/Amdro	O&O	1.054	<0.0001
8	SINV	Amdro	1.001	0.0012
8	SINV	SINV/O&O	0.917	0.0002
8	SINV	O&O	0.755	<0.0001
8	SINV/Amdro	Amdro	0.928	0.0055
8	SINV/Amdro	SINV/O&O	0.843	0.0010
8	SINV/Amdro	O&O	0.665	<0.0001
8	Amdro	O&O	0.801	<0.0001
8	SINV/O&O	O&O	0.703	<0.0001

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SUMMARY

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is of great concern because of its destructive nature to endemic wildlife, livestock, and people. Various methods for managing this pest are currently being developed, including the use of viruses as biological control agents. In this study, the effectiveness of the *Solenopsis invicta* virus (SINV-1), (a positive sense, single-stranded RNA virus in the *Dicistroviridae* family (Genus: *Aparavirus*) which only infects the Genus *Solenopsis*) as an effective biological control agent against *S. invicta* infestation in combination with commonly used insecticides was investigated. Surprisingly, ants treated with the virus experienced significantly greater survival rates than non-infected but chemically treated individuals. SINV-1 might provide some unidentified benefit to aid individual ant survival, however at this point, without fully understanding the virus-ant interaction, the use of SINV-1 as a biological control agent requires further investigation.

Key Words: antagonist, biological control, Fipronil, Hydramethylnon, virus-ant interaction

RESUMEN

La hormiga de fuego roja importada, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), causa una gran preocupación debido a su naturaleza destructiva para la vida silvestre endémica, el ganado y las personas. Se están desarrollando actualmente varios métodos para el manejo de esta plaga, incluyendo el uso de virus como agentes de control biológico. En este estudio, se investigó la

eficacia del virus de *Solenopsis invicta* (SINV-1), (un virus ARN monocatenario de sentido positivo de la familia de Dicistroviridae (Género: Aparavirus) que sólo infecta el género *Solenopsis*) como un agente eficaz de control biológico contra infestaciones de *S. invicta* en combinación con insecticidas de uso común. Sorprendentemente, las hormigas tratadas con el virus experimentaron significativamente mayores tasas de sobrevivencia que las no infectadas, pero tratadas químicamente. El SINV-1 podría proveer un beneficio no identificado para ayudar a la sobrevivencia de hormigas individuales, sin embargo, en este punto, sin comprender claramente la interacción virus-hormiga, el uso de SINV-1 como agente de control biológico requiere mayor investigación.

Palabras Clave: antagonista, control biológico, Fipronil, Hidrametilnona, interacción virus-hormiga

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