Examining the prevalence of Solenopsis invicta virus 3 (Solinviviridae: Invictavirus) in *Solenopsis invicta* (Hymenoptera: Formicidae) alates collected in North Florida

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Abstract

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), originally is from South America and currently infests over 128 million ha in the US. Its presence has caused significant social, environmental, and economic impacts. Over the decades, chemical insecticides have controlled these pest ants successfully. However, this method is costly and unsustainable because red imported fire ant re-establishes colonies quickly after chemical application. Thus, it is important to develop additional strategies for managing the red imported fire ant in the US. Solenopsis invicta virus 3 is a positive-sense, single-stranded RNA virus specific for *S. invicta* that offers promise as a classical biological control agent or biopesticide for control of *S. invicta*. Surveys were conducted to determine the prevalence of Solenopsis invicta virus 3 in alates of *S. invicta* collected from 5 urban areas (Tallahassee, Pensacola, Jacksonville, Gainesville, and Panama City) and 5 adjacent rural areas (Quincy, Jay, Macclenny, Lake City, and Blountstown) of North Florida, USA, using the reverse transcription polymerase chain reaction technique. The prevalence of Solenopsis invicta virus 3 varied widely from city to city. No statistically significant differences in alate infection rate was found between urban and rural cities sampled. Areas in which no infections of Solenopsis invicta virus 3 were detected may be good candidates for the introduction of this virus as a biological control agent to help manage this pest locally.

Key Words: red imported fire ants; biological control; Solenopsis invicta virus 3

Resumen

La hormiga roja de fuego importada, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), es de origen Sudaméricano y actualmente infesta más de 128 millones de hectáreas en los Estados Unidos. Su presencia ha provocado importantes impactos sociales, ambientales y económicos. Durante décadas, los insecticidas químicos han controlado estas plagas de hormigas con éxito. Sin embargo, este método es costoso e insostenible porque la hormiga roja de fuego importada restablece las colonias rápidamente después de la aplicación de productos químicos. Por lo tanto, es importante desarrollar estrategias adicionales para el manejo de la hormiga roja de fuego importada en los EE. UU. Solenopsis invicta 3 es un virus de ARN monocatenario de sentido positivo específico para *S. invicta* que es prometedor como agente de control biológico clásico o biopesticida para el control de *S. invicta*. Se realizó un sondeo para determinar la prevalencia del virus Solenopsis invicta 3 en individuos alados de *S. invicta* recolectados de 5 áreas urbanas (Tallahassee, Pensacola, Jacksonville, Gainesville, y Ciudad de Panamá) y 5 áreas rurales adyacentes (Quincy, Jay, Macclenny, Lake City, y Blountstown) del norte de Florida, EE. UU., utilizando la técnica de reacción en cadena de la polimerasa con transcripción inversa. La prevalencia del virus Solenopsis invicta 3 varió ampliamente de una ciudad a otra. No se encontraron diferencias estadísticamente significativas en la tasa de infección de los individuos alados entre las ciudades rurales y urbanas muestreadas. Las áreas en las que no se detectaron infecciones por el virus Solenopsis invicta 3 pueden ser buenas candidatas para la introducción de este virus como agente de control biológico para ayudar a manejar esta plaga localmente.

Palabras Clave: hormiga roja de fuego importada; control biológico; virus de Solenopsis invicta 3

Solenopsis invicta Buren (Hymenoptera: Formicidae), commonly referred to as the red imported fire ant, was introduced from South America to the US in the early 1900s at the port of Mobile, Alabama, USA. *Solenopsis invicta* has spread across the US since then (Tschinkel 2006), and currently occupies over 128 million ha from Virginia, USA, to South Florida, and west to California, USA (Williams et al. 2001). *Solenopsis invicta* has become a serious pest in the US (Tschinkel 1998). These ants thrive in disturbed habitats and may cause serious medical (anaphylaxis from envenomation) and agricultural problems in the US (Porter & Savignano 1990). Costs to manage, prevent, repair damage, and otherwise attempt to mitigate the effects of the pest exceed an estimated $6 billion annually (Pereira 2003). Insecticides provide effective control; however, this can be costly because continuous applica-

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tion is necessary because fire ants establish new colonies once the use of insecticides is discontinued (Caldera et al. 2008).

Additionally, insecticide use may not be environmentally suitable, nor economically feasible in large areas (i.e., wildlife and agricultural regions) where *S. invicta* has substantial impacts (Drees & Gold 2003). Because of these economic and environmental issues, it is important to explore more sustainable management strategies (Summerlin et al. 1977). *Solenopsis invicta* is not considered a threat in its native range, and this has been attributed to the presence of natural enemies (Porter et al. 1992). A minimum of 30 fire ant natural enemies have been identified in South America, but nearly all of these are absent among US populations (Porter et al. 1997). Thus, permanent sustainable management of *S. invicta* populations in the US likely will depend on self-sustaining biological control agents (Valles 2012). Past research has focused on the discovery and use of biological control agents such as entomopathogenic viruses. In the US, 3 positive-sense single-stranded RNA viruses, Solenopsis invicta virus 1 (SINV-1), Solenopsis invicta virus 2 (SINV-2), and Solenopsis invicta virus 3 (SINV-3), were discovered infecting *S. invicta* using a metagenomic/next generation sequencing approach (Valles et al. 2009; Valles 2012).

Laboratory experiments have been conducted to examine the effects of these viruses on *S. invicta* populations. Solenopsis invicta virus 3 was found to cause the highest mortality of all the viruses tested and is the best viral candidate to be used as a biological control agent in the field (Valles et al. 2004). While research has examined the distribution of the virus in Argentina, California, and Florida (Gainesville), no distribution studies have been performed for Solenopsis invicta virus 3 on *S. invicta* alates (Hashimoto & Valles 2008). Because the virus is vertically transmitted, alates may serve to disseminate and maintain the Solenopsis invicta virus 3 infection. In order to better understand the natural prevalence and potential dissemination of Solenopsis invicta virus 3, a survey was conducted to examine the presence or absence of Solenopsis invicta virus 3 in S*. invicta* alates collected from urban and rural areas in North Florida.

Materials and Methods

FIELD COLLECTION

Solenopsis invicta female alates were collected from 5 urban cities (Tallahassee, Pensacola, Jacksonville, Gainesville, and Panama City) and 5 adjacent rural cities (Quincy, Jay, Macclenny, Lake City, and Blountstown) in North Florida from Feb to Oct 2018 (Fig. 1). Cities were classified as rural or urban based on the 2010 US census classification map of an urban area. The rural cities were located within an 80.4 km radius of their paired urban cities with high infestations of *S. invicta*. Female alates were collected from 7 to 10 mounds in each city, with each mound separated from the other by at least 1.6 km. Alates were collected by hand from nests by opening the *S. invicta* nest with a shovel. Alates were placed in 20 mL scintillation vials. Each vial contained a cotton ball soaked with a 10% pure cane sugar solution to keep ants alive for transport. The vials were labelled with the date, time, and location. Forceps were sterilized with a 10% bleach solution before being used at a new mound location. Five randomly selected colonies per city were selected to be screened for Solenopsis invicta virus 3 infections.

VIRUS DETECTION

Collected female *S. invicta* alates were screened for the presence or absence of Solenopsis invicta virus 3 using molecular techniques adapted from Valles et al. (2010). RNA was extracted using a Qiagen RNA easy mini kit (Qiagen Inc., Germantown, Maryland, USA) according to the manufacturer's instructions. For each of the 5 colonies selected per city, total RNA was extracted from female alates from each urban and rural colony sampled. Total RNA was quantified using a Spectrophotometer (Nanodrop 1000; Fisher Scientific, Pittsburgh, Pennsylvania, USA). Samples then were serially diluted with diethyl pyrocarbonate (DEPC)-treated water to achieve a total RNA concentration of 25 to 100 ng per µL. The cDNA was synthesized in a thermal cycler (Eppendorf, Hamburg, Germany) from the extracted RNA template using a reverse transcriptase master mix and a Solenopsis invicta virus 3 specific oligonucleotide primer (p812: 5'AATATCAGCATATTGATGATCCAAAATGCCAA3').

Polymerase chain reaction was performed subsequently on the resulting cDNA. A 10 µL polymerase chain reaction master mix was created using 1.0 µL of sample buffer, 0.8 µL of 25 mM $MgCl_2$, 0.2 µL of 10 µM dNTP mix, 0.5 µL Taq DNA polymerase, 1 µL of 4 µM oligonucleotide primers (p812) and p813 (5'AAGAGAACGTATGCCTACTCCATCAGAAGCAT3'), 2 µL of cDNA and water (variable). Samples were incubated in a thermal cycler for 1.5 h with the following temperature regime: 94 °C for 2 min, then 35 cycles at 94 °C for 15 s, next 60 °C for 15 s, followed by 72 °C for 30 s, and finally 72 °C for 5 min. Polymerase chain reaction products then were separated by electrophoresis in a 2% agarose gel and visualized using SYBR® safe dye. Solenopsis invicta virus 3-infected female alates produced an amplicon of 259 base pairs, which indicated a positive response for Solenopsis invicta virus 3 viral infection (Valles et al. 2009). A gel documentation system (Bio-Rad, Hercules, California, USA) was used to capture the image of agarose gel for analysis and interpretation. Positive and non-template controls were included in each reverse transcription-polymerase chain reaction.

STATISTICAL ANALYSIS

Fisher's exact test was used on the count data to determine if there were any statistical differences between the numbers of alates infected with Solenopsis invicta virus 3 from colonies collected in rural versus urban cities (Du-Prel et al. 2010). R statistical software version 3.5.1 was used to analyze data (R Foundation for Statistical Computing, Vienna, Austria).

Results

Reverse transcription-polymerase chain reaction analysis successfully identified Solenopsis invicta virus 3 infections in female alates (Fig. 2). Solenopsis invicta virus 3 was detected in female alates from *S. invicta* nests from 7 of the 10 collection locations in northern Florida (Fig. 1.). The infection rate ranged from 0 to 100% depending on location. The virus was not detected in alates collected from Jay, Jacksonville, or Pensacola. Solenopsis invicta virus 3 was most prevalent in Gainesville with a 100% infection rate per colony (Fig. 2). The overall mean infection rate (± standard error) for North Florida was 44.0 ± 11.9%. Solenopsis invicta virus 3 infections in rural and urban areas were similar. The mean infection rates were 40.0 ± 14.1% and 48.0 ± 21.0% for rural and urban areas, respectively. Fisher exact analysis showed no significant difference between the number of colonies infected with Solenopsis invicta virus 3 in rural and urban areas (P = 0.7761; Odd Ratio 1.38).

Discussion

The goal of this study was to examine the presence and distribution of Solenopsis invicta virus 3 among female alates of *S. invicta* in rural

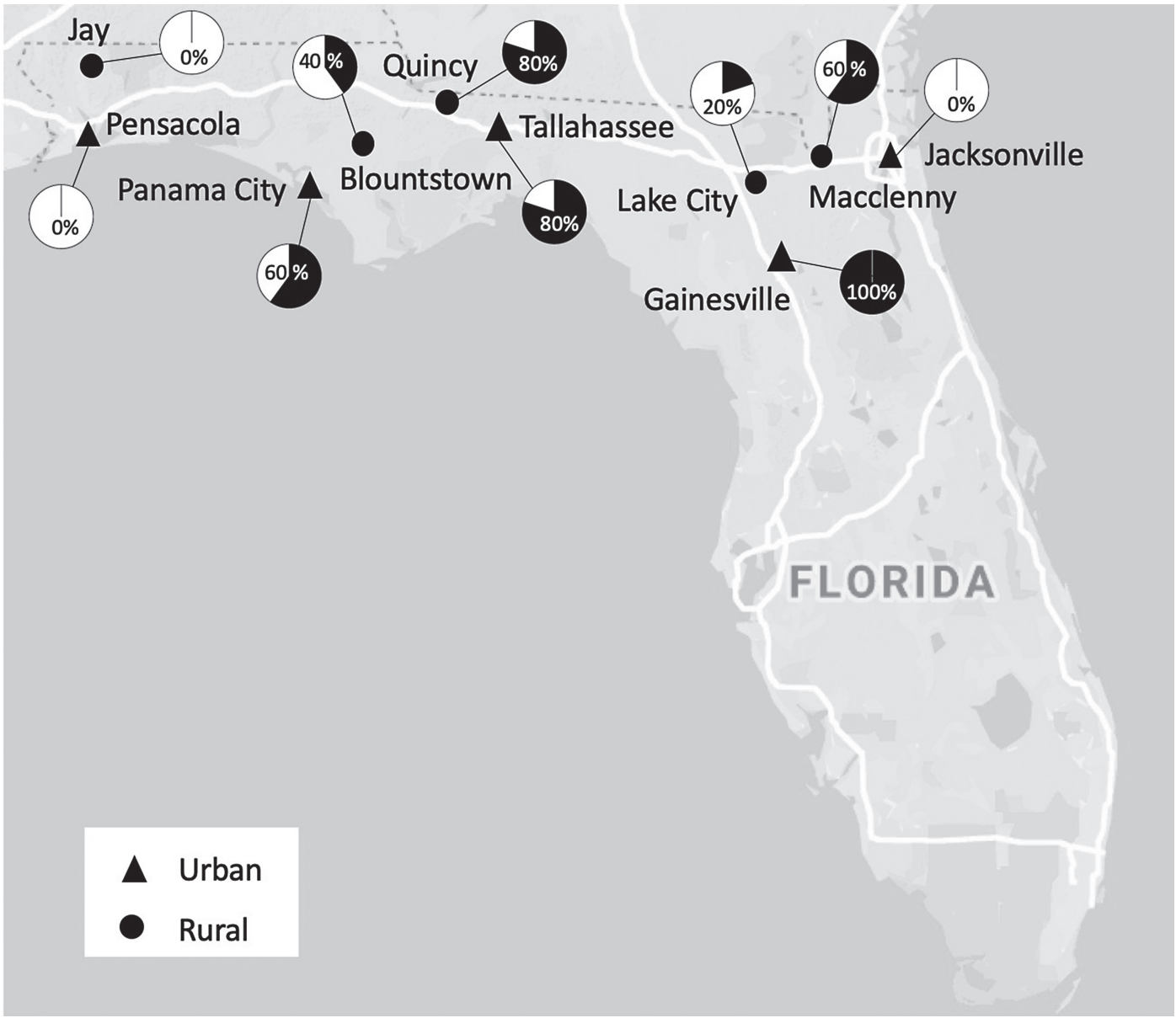

Fig. 1. Map of Florida illustrating the collection locations and distribution of Solenopsis invicta virus 3 infection rate of female alates of *Solenopsis invicta* colonies in urban and rural cities of North Florida. Black portions of the pie charts represent the proportion of alates infected with Solenopsis invicta virus 3.

and urban areas of North Florida. The Solenopsis invicta virus 3 infection rate was higher considerably in female alates compared with earlier studies that examined the infection rate in worker ants (Valles et al. 2009). Female alates were chosen for this study because this life stage had not been surveyed extensively previously, and female alates may portend future establishment of this virus in new areas because the virus is vertically transmitted. Surveys of workers would indicate only the current infection status for an area. The Solenopsis invicta virus 3 infection rate was higher than anticipated based on previous surveys. The infection rate of Solenopsis invicta virus 3 in *S. invicta* colonies (based on the interrogation of workers) typically is under 10% (Valles et al. 2010). However, pockets of high infection rates (20–50%) have been reported in the US (Valles et al. 2010) and Argentina (Valles et al. 2009). Several factors may contribute to higher Solenopsis invicta virus 3 infection rates, including social form and temperature. Higher infection rates typically are observed in polygyne colonies compared with monogyne colonies (Valles et al. 2010). However, we did not examine the social form of the female alates in this study. Solenopsis invicta virus 3 infection exhibits a negative relationship with temperature and is more prevalent during winter mo in Florida (Valles et al. 2010).

While several of these cities had high infection rates (Tallahassee, Quincy, and Gainesville), some cities exhibited low infection rates; surprisingly Jacksonville, Pensacola, and Jay had no detectable infection. We fully expected that large cities such as Pensacola and Jacksonville would have Solenopsis invicta virus 3 present given the frequent travel of people transporting all sorts of materials between these cities and cities infected with Solenopsis invicta virus 3. The absence of Solenopsis invicta virus 3 in these cities may be attributed to the small number of colonies sampled. Interestingly, in the paired cities Pensacola (urban) and Jay (rural), Solenopsis invicta virus 3 was not detected in female alates, which may represent an actual absence of the virus in these areas.

Knowing the geographic Solenopsis invicta virus 3 incidence affords the opportunity to conduct future research comparing the health of

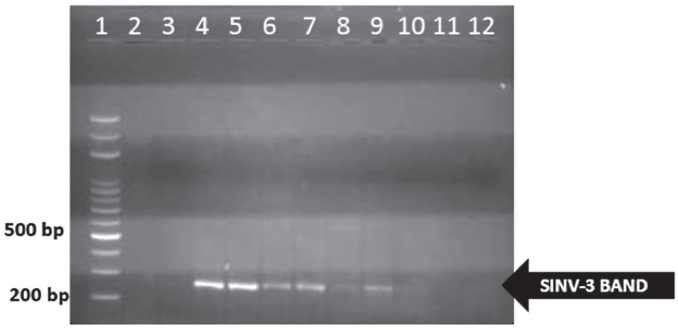

Fig. 2. Representative agarose gel depicting detection of Solenopsis invicta virus 3 as determined by polymerase chain reaction using cDNA generated from RNA purified from female alates of *Solenopsis invicta*. Arrow represents the 258 bp amplicon from Solenopsis invicta virus 3. Lane 1 is a 1 kb DNA ladder and lane 12 is non-template control.

S. invicta colonies in cities that have high infection rates versus those that had no infection rate. One could also examine the health of *S. invicta* colonies in cities without the virus such as Jay, Pensacola, or Jacksonville, and then introduce the virus to those cities. One could then compare the before and after health changes of colonies in the field afflicted by Solenopsis invicta virus 3. The introductions could follow protocols used by studies that introduced the virus to Gainesville (Valles et al. 2009) and California (Oi et al. 2019).

In conclusion, the results of this study indicate that Solenopsis invicta virus 3 is well distributed in female alates in North Florida. Thus, it is likely that Solenopsis invicta virus 3 is being disseminated and maintained in *S. invicta* by this life stage. However, female alates from some areas were devoid of Solenopsis invicta virus 3, suggesting that they may be good areas for augmentative release of the virus as a natural control agent.

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The dataset may be found here: https://data.nal.usda.gov/dataset/data-examining-prevalence-solenopsis-invicta-virus-3-solinviviridae-invictavirus-solenopsis-invicta-hymenoptera-formicidae-alates-collected-north-florida (last accessed 16 Dec 2020).

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