Diet with Sucrose Ameliorates Solenopsis Invicta Virus 3 (Solinviviridae: Invictavirus) Infection in Solenopsis invicta (Hymenoptera: Formicidae) Worker Ants

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Source: Florida Entomologist, 104(3) : 239-242

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.104.0314

Scientific Notes

Diet with sucrose ameliorates Solenopsis invicta virus 3 (Solinviviridae: Invictavirus) infection in *Solenopsis invicta* (Hymenoptera: Formicidae) worker ants

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Solenopsis invicta Buren (Hymenoptera: Formicidae) is an invasive ant pest that was introduced into the US near Mobile, Alabama, USA, in the early 1900s (Tschinkel 2006). Control efforts and damage repair from *S. invicta* were estimated to exceed $5 billion annually in 2003 (Pereira 2003), which would exceed $8.5 billion today after adjusting for inflation. Although insecticides are highly effective at controlling this ant pest, they must be used on a regular interval to maintain control. If insecticide use is stopped, fire ant populations will invariably re-infest the area. The invasive success of *S. invicta* has been attributed to human-assisted spread, pre-adaptation for colonization of new areas (Ascunce et al. 2011), and a lack of natural enemies during founding (Porter et al. 1997).

Solenopsis invicta virus 3 (Solinviviridae: Invictavirus) has been shown to be an effective natural control agent for *S. invicta* in the laboratory (Valles et al. 2013). The virus exhibits desirable characteristics as a control agent, including species specificity, ready transmission, pathogenicity, and virulence (Porter et al. 2013; Valles et al. 2013, 2014; Valles & Oi 2014; Porter et al. 2015). The Solenopsis invicta virus 3 infection is stage-dependent; virus replication occurs only in adult ant stages. Infected adult workers stop foraging for food (Chen et al. 2012), which results in a cascade of events including starvation, a severe reduction in queen fecundity (Valles et al. 2013), and ultimate colony collapse (Valles et al. 2014). During laboratory tests involving Solenopsis invicta virus 3, it was noticed that the impact of Solenopsis invicta virus 3 on worker mortality appeared to be reduced when a sugar solution was available to the infected ant colonies as part of their diet. Therefore, the objective of this work was to test the hypothesis that the availability of a sucrose solution as part of the diet could influence the ability of *S. invicta* workers to better tolerate Solenopsis invicta virus 3 infection.

Two polygyne *S. invicta* colonies were collected from the field in Gainesville, Florida, USA, removed from the nest soil by flooding, placed into rearing trays, and provided an ad libitum diet of crickets (*Acheta domesticus* [L.]; Orthoptera: Gryllidae), 10% sucrose, and water. A sample of worker ants ($n = 15$) from each colony was examined by microscopy (Knell & Allen 1977) for the presence of the microsporidian, *Kneallhazia solenopsae* (Knell, Allen, & Hazard) (Microsporida: Thelohaniidae) (Williams et al. 1999). Reverse Transcription-Polymerase Chain Reaction (RT-PCR) also was conducted on the sample to detect Solenopsis invicta virus 1, Solenopsis invicta virus 2, and Solenopsis invicta virus 3 (Valles et al. 2009). Both colonies were found to be free of infection by these viruses and the microsporidian.

The ant colonies were permitted to acclimate to the laboratory for 2 wk. The *S. invicta* colonies were divided equally, and the queens removed. One sub-colony from each parent colony was infected with Solenopsis invicta virus 3 and the other sub-colonies were retained as control groups. A homogenate prepared from Solenopsis invicta virus 3-infected *S. invicta* colonies was used as the source of Solenopsis invicta virus 3 inoculum, which can successfully transmit the virus to uninfected ant colonies in the laboratory (Valles & Hashimoto 2009). Solenopsis invicta virus 3-infected worker ants (about 20 g) were blended with 40 mL of 10% (w/v) sucrose prepared with deionized water for 1 min at high speed. The homogenate was filtered through 3 layers of cheesecloth and then filtered by vacuum in a Buchner funnel (ThermoFisher Scientific Company, Waltham, Massachusetts, USA) through a number 1 Whatman paper (SigmaAldrich, St. Louis, Missouri, USA). The homogenate (10 mL) was mixed with 10 freeze-killed adult house crickets (*A. domesticus*) that were pulverized with a mortar and pestle to create a crude paste. Each of the 2 sub-colonies received 5 mL of homogenate paste for 24 h, after which the remaining homogenate paste was removed. A control group homogenate paste was prepared identically using Solenopsis invicta virus 3-uninfected worker ants, and the control sub-colonies were exposed in identical fashion. After 5 d, 10 individual worker ants were tested by quantitative polymerase chain reaction (qPCR) for the presence of Solenopsis invicta virus 3 to verify successful infection transmission (Valles & Hashimoto 2009). The sub-colonies were shown to have a weak, but established, Solenopsis invicta virus 3 infection. All 10 individual workers (100%) sampled from each sub-colony were shown to be infected. The mean (± standard deviation) Solenopsis invicta virus 3 titer in each worker of colony 1 and colony 2 was $8.95 \times 10^2 \pm 5.31 \times 10^2$ and $3.72 \times 10^3 \pm 8.75 \times 10^3$ Solenopsis invicta virus 3 genome equivalents per ng RNA, respectively.

Fragment colonies were prepared from each of the Solenopsis invicta virus 3-infected and -uninfected sub-colonies as experimental units. Each fragment was comprised of 1 mL of brood and 2 mL of workers. All colony fragments received a diet of a cricket every other d and water in a cotton-stoppered test tube. The treatment groups were distinguished by the addition, or not, of a 10% (w/v) sugar solution as

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part of the diet, and included Solenopsis invicta virus 3-infected fragment colonies provided a 10% sugar solution (n = 6; 3 replicates per sub-colony), Solenopsis invicta virus 3-infected fragment colony without a 10% sugar solution (n = 6; 3 replicates per sub-colony), Solenopsis invicta virus 3-uninfected fragment colonies provided a 10% sugar solution (n = 3; 3 replicates per sub-colony), Solenopsis invicta virus 3-uninfected fragment colonies without a 10% sugar solution (n = 3; 3 replicates per sub-colony). One of the control groups became infected with Solenopsis invicta virus 3 during the course of the study and was not used. Worker mortality was recorded 1, 3, 5, 10, 12, 14, 17, 19, and 21 d after the introduction of specific diets, and was expressed as cumulative mortality over the duration of the study. Solenopsis invicta virus 3 was quantified in the ants that died. Total RNA was extracted from the pooled dead worker ants (pooled by d and replicate) and used as a template for reverse transcription and subsequent qPCR (Valles & Hashimoto 2009). Solenopsis invicta virus-3 also was quantified in live and dead workers from each colony fragment on d 24.

Cumulative mortality among groups (Solenopsis invicta virus-infected with sugar supplement; Solenopsis invicta virus-infected without sugar supplement; Solenopsis invicta virus-uninfected with sugar supplement; Solenopsis invicta virus-uninfected without sugar supplementation) on d 21 was compared by Analysis of Variance followed by Scheffe's mean separation test (SAS 2009). Mortality by d (1, 3, 5, 10, 12, 14, 17, 19, and 21) for each treatment (Solenopsis invicta virus-infected with sugar supplement; Solenopsis invicta virus-infected without sugar supplement; Solenopsis invicta virus-uninfected with sugar supplement; Solenopsis invicta virus-uninfected without sugar supplement) was compared by Analysis of Variance followed by Scheffe's mean separation test (SAS 2009). Student's t-test (SAS 2009) was used to compare the quantity of Solenopsis invicta virus 3 in Solenopsis invicta virus-infected with sugar supplement and Solenopsis invicta virus-infected without sugar supplement groups for each d (Solenopsis invicta virus 3 was not present in the control groups). Student's t-test also was used to compare the quantity of Solenopsis invicta virus 3 detected in dead workers (Solenopsis invicta virus-infected with sugar supplement versus Solenopsis invicta virus-infected without sugar supplement) and live workers (Solenopsis invicta virus-infected with sugar supplement versus Solenopsis invicta virus-infected without sugar supplement) collected on d 24.

Cumulative mortality increased significantly over the 3-wk monitoring period in the Solenopsis invicta virus 3-infected colonies with (F = 16.7; df = 8,45; P < 0.0001) and without (F = 11.6; df = 8,45; P < 0.0001) sugar supplementation and the Solenopsis invicta virus 3-uninfected colonies without sugar supplementation (F = 11.6; df = 8,18; P < 0.0001) (Fig. 1). There was no significant difference (F = 2.24; df =

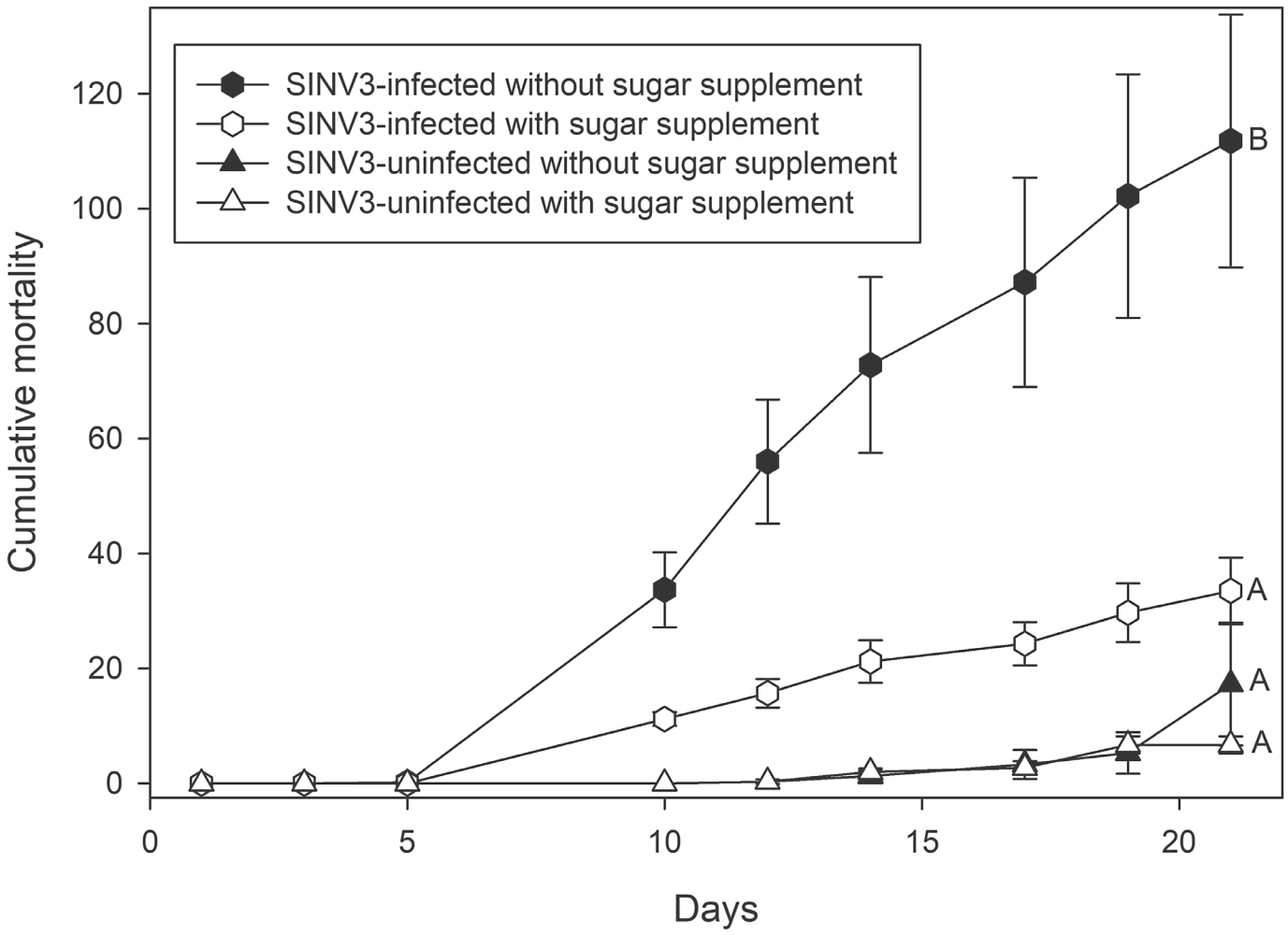

Fig. 1. Cumulative *Solenopsis invicta* worker ant mortality among Solenopsis invicta virus 3-infected and -uninfected colonies provided a diet of crickets (*Acheta domesticus*) and either supplemented (open symbols) or not supplemented (solid symbols) with a 10% sucrose solution. Analysis of Variance by treatment was conducted for d 21 values and found to be significant (F = 10.0; df = 3,14; P < 0.0009). Scheffe's multiple comparison procedure was used to separate the means. Symbols with the same letter are not statistically different.

8,18; $P < 0.074$) in cumulative mortality between the Solenopsis invicta virus 3-uninfected colonies with and without sugar supplementation. Mortality on d 21 reached a mean of 111.8 ± 22.0 worker ants in Solenopsis invicta virus 3-treated colonies without sugar supplementation, which was significantly ($F = 10.0$; df = 3,14; $P < 0.0009$) greater than the Solenopsis invicta virus 3-infected colonies with sugar supplementation (33.5 ± 5.8), Solenopsis invicta virus 3-uninfected colonies without sugar supplementation (17.3 ± 10.7), and Solenopsis invicta virus 3-uninfected colonies with sugar supplementation (6.7 ± 2.5).

The quantity of Solenopsis invicta virus 3 in the dead worker ants generally was higher (by about 1 order of magnitude) in colonies without sugar supplementation compared with colonies with sugar supplementation (Fig. 2). However, significant differences were noted only on d 12 ($t = 2.52$; df = 9; $P < 0.033$) and 19 ($t = 2.44$; df = 9; $P < 0.037$). Solenopsis invicta virus 3 was not detected in either of the control (virus-uninfected) colonies (Fig. 2). A final comparison of Solenopsis invicta virus 3 quantity was made on d 24 among live and dead workers in the Solenopsis invicta virus 3-infected colonies with and without sugar supplementation. Among dead workers collected on that d, the mean ($2.21 \times 10^6 \pm 6.38 \times 10^6$ Solenopsis invicta virus 3 genome equivalents per ng RNA) amount of Solenopsis invicta virus 3 was greater significantly ($t = -3.14$; df = 9; $P = 0.012$) in colonies without sugar supplementation compared with colonies with sugar supplementation ($8.55 \times 10^5 \pm 6.06 \times 10^5$ Solenopsis invicta virus 3 genome equivalents per ng RNA). Among live worker ants in the Solenopsis invicta virus 3-infected colonies, there was no significant difference in the quantity of Solenopsis invicta virus 3 detected in colonies without sugar supplementation ($3.17 \times 10^6 \pm 2.14 \times 10^6$ Solenopsis invicta virus 3 genome equivalents per ng RNA) or with sugar supplementation ($2.53 \times 10^5 \pm 1.47 \times 10^5$ Solenopsis invicta virus 3 genome equivalents per ng RNA). However, the trend was higher in the colonies without sugar supplementation.

Adequate nutrition is essential for normal insect development and propagation (Dadd 1973), but it also influences other aspects of survival, including resistance to pathogen infection (Muturi et al. 2011; Di Pasquale et al. 2013). Solenopsis invicta virus 3-treated colonies without a sugar supplement to their diet exhibited significantly higher mortality rates and generally higher Solenopsis invicta virus 3 titers than their counterparts that were provided a sugar supplement. During virus development, especially in the chronic to acute phase of pathogenesis, glucose demands may increase significantly to support the energetic and anabolic demands of virus replication (Wang et al. 2019). These increased metabolic demands often are detrimental to the insect host. Honey bees infected with various RNA viruses (similar to Solenopsis invicta virus 3) exhibit higher mortality with poor qual-

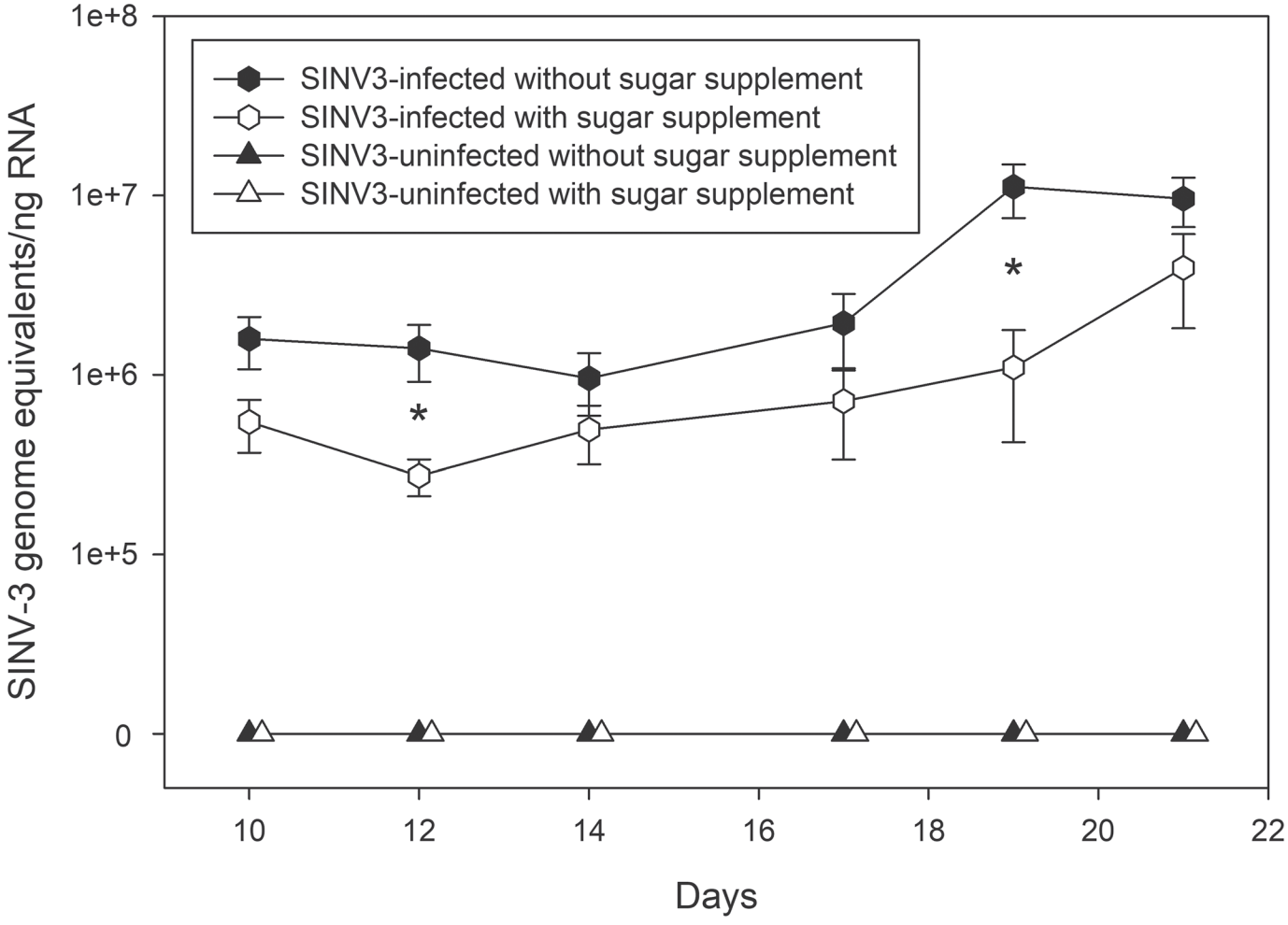

Fig. 2. Solenopsis invicta virus 3 genome equivalents per ng RNA from dead *Solenopsis invicta* worker ants among Solenopsis invicta virus 3-infected and -uninfected colonies provided a diet of crickets (*Acheta domesticus*) and either supplemented (open symbols) or not supplemented (solid symbols) with a 10% sucrose solution. Solenopsis invicta virus 3 was not detected in the Solenopsis invicta virus 3-uninfected group. Student's *t*-test was conducted to compare the virus quantity in colonies with and without the sucrose supplement by d. Solenopsis invicta virus 3 genome equivalents per ng RNA was greater significantly in colonies without sugar supplementation on d 12 ($t = 2.5$; df = 9; $P < 0.033$) and 19 ($t = 2.4$; df = 9; $P < 0.037$).

ity diets (Schmidt et al. 1987; Dolezal et al. 2019). Poorly nourished bees mount a weaker immune response (Alaux et al. 2010) and exhibit higher virus titers (DeGrandi-Hoffman et al. 2010). Sucrose supplementation during virus infection may help to alleviate the impact of greater biosynthetic material demands from the host by a virus in the active replication phase, as observed with Solenopsis invicta virus 3-infected *S. invicta*. This knowledge may prove important when developing Solenopsis invicta virus 3 as a natural control agent against *S. invicta*. It is known that the prevalence of Solenopsis invicta virus 3 among *S. invicta* is greater during the winter months (Valles et al. 2010). Scarcity of carbohydrate sources (for example, honeydew [Helms & Vinson 2002]) during the winter may make *S. invicta* less able to resist Solenopsis invicta virus 3 infection.

Summary

Mortality and virus titer were monitored in *Solenopsis invicta* colony fragments to examine the impact of diet sucrose supplementation. Mortality on d 21 reached a mean of 111.8 ± 22.0 worker ants in Solenopsis invicta virus 3-treated colonies without sugar supplementation, which was significantly greater (F = 10.0; df = 3,14; P < 0.0009) than the Solenopsis invicta virus 3-infected colonies with sugar supplementation (33.5 ± 5.8), Solenopsis invicta virus 3-uninfected colonies without sugar supplementation (17.3 ± 10.7), and Solenopsis invicta virus 3-uninfected colonies with sugar supplementation (6.7 ± 2.5).

Key Words: nutrition; RNA virus; fire ant; virus replication; virus pathogenesis

Sumario

Se monitorearon la mortalidad y la toxicidad del virus en fragmentos de colonias de *Solenopsis invicta* para examinar el impacto de la suplementación con sacarosa en la dieta. La mortalidad en el día 21 alcanzó un promedio de 111,8 ± 22,0 en hormigas obreras en colonias tratadas con Solenopsis invicta virus 3 sin suplementos de azúcar, que fue significativamente mayor (F = 10,0; gl = 3,14; P < 0,0009) que el Solenopsis invicta virus 3-colonias infectadas con suplementación con azúcar (33,5 ± 5,8), colonias no infectadas con el virus Solenopsis invicta 3-colonias no infectadas sin suplementación con azúcar (17,3 ± 10,7), y colonias no infectadas con el virus Solenopsis invicta 3-con suplemento con azúcar (6,7 ± 2,5).

Palabras Clave: nutrición; Virus de ARN; hormiga de fuego; replicación de virus; patogenia del virus

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