Survey for Imported Fire Ant Virus and Microsporidian Pathogens in Tennessee and Efforts to Inoculate *Solenopsis invicta* Virus 3 From Florida into Tennessee Fire Ant Populations

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Introduction

- Red (*Solenopsis invicta* Buren) (RIFA) and black (*Solenopsis richteri* Forel) (BIFA) imported fire ant and their hybrid (HIFA) now infest >148 million hectares in the U.S.

Fig. 1. Federal Fire Ant Quarantine (Red area). Image from USDA)

- In Tennessee, 66 out of 95 counties are now infested mostly by HIFA and some BIFA in west Tennessee (Fig. 2).

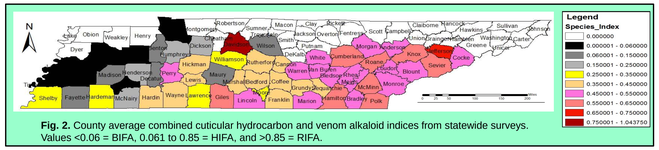


Fig. 2. County average combined cuticular hydrocarbon and venom alkaloid indices from statewide surveys. Values <0.06 = BIFA, 0.061 to 0.85 = HIFA, and >0.85 = RIFA.

- In the last two decades, there has been increased interest in the discovery, characterization, and use of imported fire ant (IFA) microbial pathogens (2, 7-14), (Fig. 3), which could reduce the fitness and spread of IFA populations, especially during periods of stress.

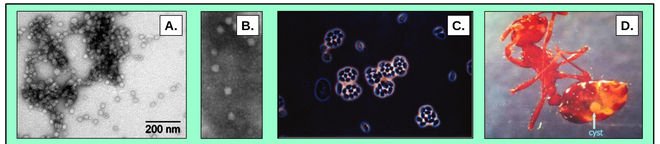


Fig. 3. A) and B) *Solenopsis invicta* virus, C) *Kneallhazia solenopsae* octospores, and D) *K. solenopsae* infected ant.

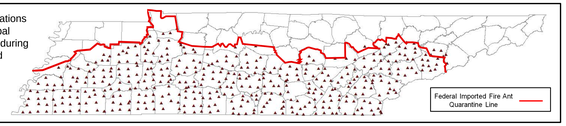
- The objectives of this study were to determine:
  1) The geographic distribution and incidence in Tennessee IFA populations of three positive sense, single-strand RNA viruses (*Solenopsis invicta*-1, -2, and -3 [SINV-1, SINV-2, and SINV-3]) and *Kneallhazia solenopsae* Knell Alan Hazard (Microsporidia: Burenellidae),
  2) If these pathogens occur in BIFA and HIFA populations
  3) The feasibility of relocating/transmitting virus to BIFA and/or HIFA locations to form the framework for a larger virus redistribution effort in Tennessee or other states.

Materials and Methods

Objectives 1 and 2: Pathogen Distribution and BIFA/HIFA Incidence

- Tennessee locations inside the Federal IFA Quarantine were divided into 14 by 14 km grids (Fig. 4) and a single fire ant colony was sampled (when possible) near the center of each grid during the summers of 2015 and 2016 (n=440). During winter 2016, ever other grid was sampled (n=227).

Fig. 4. Sample locations mapped with a global positioning system during Summers 2015 and 2016.



Materials and Methods (Continued)

Objectives 1 and 2: Pathogen Distribution and BIFA/HIFA Incidence

- At each location, a bucket and stick were used to collect a sample of fire ant workers (Fig. 5). Each colony location was mapped with a global positioning system.

Fig. 5. Top (left to right): Topping a mound with a trowel, collecting ants with a board, a powder-lined bucket with worker ants, a bucket with ants tapped to one side for removal, GPS mapping a mound, collecting ants at a park, and at a picnic area. Bottom (left to right): Collecting ants at various locations (mostly roadside).

- About 100 workers were placed in 95% ethanol for SINV and *K. solenopsae* assessment by Dr. Steven Valles (USDA-ARS CMAVE). About 30-50 workers were kept alive for subsequent hexane processing for venom alkaloid and cuticular hydrocarbon determination of ant species by GC-MS by Dr. Karla Addesso (TSU) (5, 15). Extra workers were frozen (–80ºC) for backup and other uses (3, 4).

Objective 3: Relocation of SINV-3 into BIFA or HIFA Locations

- From 2017 to 2018, SINV-3 inoculations were made at pasture, fairground, and roadside sites in Giles, Hamilton, and Lawrence Counties (Table 1). The Lawrence Co. site had 0.1 hectare plots (6 reps) to monitor SINV-3 incidence and IFA colony densities in plots with and without inoculations.

- At all sites, preliminary worker ant samples were collected to determine existing SINV-3 infection status (Table 1). Polk and Sequatchie Co. were abandoned due to existing SINV-3 infections. At other sites, SINV-3 infected ants received from Dr. Valles were macerated with a mortar and pestle, mixed in 100 ml of 10% table sugar water, and poured into the inoculated colony (Fig. 6). Colonies were subsequently sampled at various intervals to determine SINV-3 infection (Table 1).

Fig. 6. From top to bottom and left to right: Grinding SINV-3 infected ants with mortar and pestle, macerated ants, adding 10% sugar water, pouring ground ant slurry into vial, adding more slurry and sugar water, pouring mixture into a fire ant colony, and evaluating fire ant colony densities at a test site by forming a pivot line. Ant colonies were sampled for SINV-3 along pivot line and mapped by distance and cardinal direction.

Table 1. Sample sites and activity dates.

Sampling Periods	Polk Co. Pasture	Lawrence Co. Pasture	Giles Co. Pasture	Giles Co. Fairgrounds	Sequatchie Co. Roadside	Hamilton Co. Roadside
Preliminary Sampling	Apr. 17, 2017 (A)	June 19, 2017 (B)	July 13, 2017 (D)	July 13, 2017 (D)	May 16, 2018 (K)	May 16, 2018 (K)
SINV-3 Inoculation	-----	July 13, 2017 (C)	July 13, 2017 (D)	July 13, 2017 (D)	-----	June 5 & 12, 2018 (L)
Post-Sampling (1 MAT)	-----	-----	-----	-----	-----	July 16, 2018 (M)
Post-Sampling (2 MAT)	-----	Sept. 18, 2017 (E)	Sept. 18, 2017 (E)	Sept. 18, 2017 (E)	-----	Aug. 16, 2018 (M)
Post-Sampling (3 MAT)	-----	-----	-----	-----	-----	Sept. 11, 2018 (M)
Post-Sampling (4 MAT)	-----	Nov. 13, 2017 (F)	Nov. 13, 2017 (F)	Nov. 13, 2017 (F)	-----	-----
Post-Sampling (6 MAT)	-----	Jan. 25, 2018 (G)	Jan. 25, 2018 (G)	Jan. 25, 2018 (G)	-----	Dec. 3, 2018 (M)
Post-Sampling (9 MAT)	-----	Apr. 3, 2018 (H)	Apr. 3, 2018 (H)	Apr. 3, 2018 (H)	-----	-----
Post-Sampling (12 MAT)	-----	July 17, 2018 (I)	July 17, 2018 (I)	July 17, 2018 (I)	-----	June 27, 2019 (N)
Post-Sampling (24 MAT)	-----	June 26, 2019 (J)	June 26, 2019 (J)	June 26, 2019 (J)	-----	-----
Hybrid SINV-3 Relocation	Feb. 3, 2020 (O)	-----	-----	-----	-----	Feb. 3, 2020 (P)

MAT = Months After Treatment with SINV-3
Highlight = SINV-3 Detected (see below)

(A) 15 colonies pre-sampled; 6 had SINV-3 hybrid variant (site was abandoned)
(B) 12 plots with 36-m diameter circles (1/4 acre) set up. Plot center mounds all negative for SINV-3, but 4 plots had other colonies with weak SINV-3
(C) 6 mounds in center of plots inoculated and 6 mounds not inoculated.
(D) 12 mounds sampled just before inoculation with SINV-3. Infection status at time of inoculation unknown.
(E) Two center plots had SINV-3 infections and one Giles-Fairground colony. Issue with denaturant in ethanol affecting viral RNA analysis.
(F) SINV-3 assessments were weak an inconclusive. Issue with denaturant in ethanol affecting viral RNA analysis.
(G) SINV-3 not found in any sample. SINV-1 detected at Lawrence Co. New SINV-4 virus found at Giles Pasture site. Issue with denaturant in ethanol.
(H) Ants shipped live to CMAVE to avoid ethanol denaturant. All samples negative for SINV-3. Lawrence landowner converted site to row crops.
(I) Ants shipped live to CMAVE. Lawrence site sampled along roadside. One Giles pasture colony had SINV-3 infection.
(J) Ants shipped live to CMAVE. One colony along roadside at Lawrence site had SINV-3.
(K) 20 colonies pre-sampled per county site (10 control and 10 inoculation areas). SINV-3 found at Sequatchie (abandoned). Hamilton site all negative.
(L) Both dates, 10 treated area colonies received 40 ml sugar water (4.5×10^{16} virus particles) and adjacent 10-ml bait tubes (~1.79×10^{17} virus particles).
(M) Ants shipped live to CMAVE. All samples on these dates were negative for SINV-3.
(N) Ants shipped live to CMAVE. Two colonies in the control area had SINV-3 infections. Nine colonies had SINV-1 and 18 had SINV-4 infections.
(O) 16 colonies pre-sample for SINV-3 hybrid variant for relocation attempt to Hamilton Co. site. All were negative for SINV-3.
(P) 10 colonies sampled (5 former SINV-3 inoculated and 5 control areas). All were negative on this date (~ 20 MAT).

Results and Conclusions

Objectives 1 and 2: Pathogen Distribution and BIFA/HIFA Incidence

- The majority of IFA samples in the Tennessee were HIFA (Fig. 7) with BIFA restricted to west Tennessee. Our success at locating colonies to sample declined as we moved northward (Fig. 8), which related to decreasing IFA population densities in the northern part of the range.

Fig. 7. BIFA, HIFA, and RIFA sample locations from summer 2015 and 2016 and winter 2016.

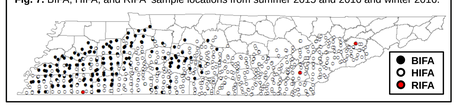
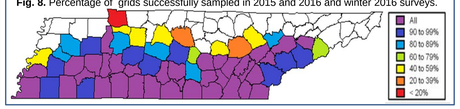
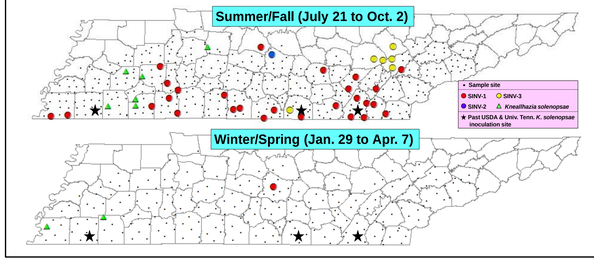


Fig. 8. Percentage of grids successfully sampled in 2015 and 2016 and winter 2016 surveys.



- All SINV viruses and *K. solenopsae* were more prevalent during summer/fall months (Fig. 9). SINV-1 was the most common and widespread pathogen, followed by SINV-3 and *K. solenopsae*. SINV-2 was rare and only found at one site in Rutherford Co. SINV-3 was mostly found in east TN and *K. solenopsae* in west TN. The west TN *K. solenopsae* sites were near a previous inoculation site at Ames Plantation by Univ. of Tenn. (Dr. Karen Vail) and USDA-ARS (Dr. Valles), which may indicate successful introduction.

Fig. 9. SINV-1, -2, and -3 virus and *K. solenopsae* positive sample sites during summer 2015 and 2016 and winter 2016 surveys.



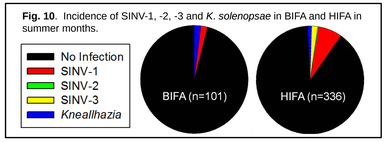
Infection rate tables by BIFA, HIFA, or RIFA.

Summer 2015 & 2016					
Infection	Black	Hybrid	Red	Total	% of Total
SINV-1	2	24	1	27	6.1
SINV-2	0	1	0	1	0.2
SINV-3	0	6	0	6	1.4
Kneallhazia	2	4	0	6	1.4
None	97	301	2	400	90.9
Total	101	336	3	440	
% of Total	23.0	76.4	0.7		

Winter 2016					
Infection	Black	Hybrid	Red	Total	% of Total
SINV-1	0	1	0	1	0.4
SINV-2	0	0	0	0	0.0
SINV-3	0	0	0	0	0.0
Kneallhazia	2	0	0	2	0.8
None	97	157	1	255	98.8
Total	99	158	1	258	
% of Total	38.4	61.2	0.4		

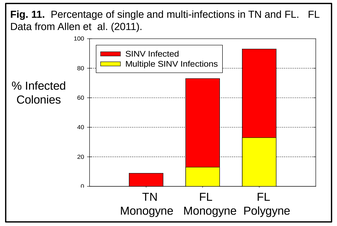
- All SINV viruses were found in HIFA populations, but only SINV-1 and *K. solenopsae* were found in BIFA populations (Fig. 10). A single RIFA colony had SINV-1 infection. A recently described SINV-4 virus (14) also was found in Giles and Henderson Co. and some HIFA colonies also were found with polygyne alleles (6) (data not shown). *Kneallhazia* appeared to be more common in BIFA and definitely infected monogyne colonies.

Fig. 10. Incidence of SINV-1, -2, -3 and *K. solenopsae* in BIFA and HIFA in summer months.



- Compared to Florida, TN pathogen infection rates were very low. Likewise, no colonies in TN had simultaneous infections of more than one SINV virus or *K. Solenopsae* (Fig. 11).

Fig. 11. Percentage of single and multi-infections in TN and FL. FL Data from Allen et al. (2011).



Objective 3: Relocation of SINV-3 into BIFA or HIFA Locations

- Efforts to relocate SINV-3 form Florida RIFA populations were unsuccessful (Table 1). Inoculation sites had BIFA and HIFA (data not shown). A more promising outcome was the location of a HIFA SINV-3 isolate (13).

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