ETHANOL PRESERVATION OF FIRE ANTS ALLOWS RETROSPECTIVE SCREENING FOR SOLENOPSIS INVICTA VIRUS-1

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Solenopsis invicta virus-1 (SINV-1) is a positive-strand RNA virus assigned to the Dicistroviridae (Mayo 2002) that appears to infect ants only in the *Solenopsis* genus (Valles et al. 2007). SINV-1 infection has been associated with brood death among colonies of *Solenopsis invicta*, but whether it is the causative agent of mortality is not known (Valles et al. 2004). Thus, it is hoped that SINV-1 may be able to be exploited for use as a microbial control agent, or as a mode of delivering genes or inhibitors to study functional genomics in this ant pest. Because the virus was only recently discovered and it is the only currently known virus to infect *S. invicta*, basic knowledge about its biology is lacking. Epidemiologic and phylogenetic studies would certainly benefit if the virus could be examined in archived samples. To address this question, we archived SINV-1-positive *S. invicta* worker ants in 95% ethanol and tested the ants for the presence of SINV-1 on an irregular basis over a 2-year period. In addition, we examined 2-propanol- and ethanol-archived *S. invicta* samples from 1999 and 2001, respectively, for the presence of SINV-1.

In February 2005, 2 groups of approximately 300 worker ants from a colony of *S. invicta* previously determined to be SINV-1-positive (collected from Gainesville, FL) were placed into a 7-ml scintillation vial containing 5 ml of 95% ethanol and stored at room temperature. On an irregular basis over the next 2 years, RNA was extracted from a group of 20 workers from each vial. RNA was extracted by the Trizol (Invitrogen, Carlsbad, CA) method as described previously (Valles & Strong 2005). cDNA was synthesized and subsequently amplified by the One-Step RT-PCR kit (Invitrogen) with oligonucleotide primers p114 (5' CTTGATCGGGCAGGACAAATTC) and p116 (5' GAACGCTGATAACCAATGAGCC). Samples were considered positive for virus when a visible amplicon of anticipated size (646 nt) was present after separation on 1.2% agarose gel containing ethidium bromide. Positive and negative controls were included for each time point. RT-PCR was conducted in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 45°C for 30 min, 1 cycle at 94°C for 2 min, 35 cycles of 94°C for 15 s, 54°C for 15 s, 68°C for 30 s, followed by a final elongation step of 68°C for 5 min. Additional alcohol-archived *S. invicta* ant samples from previous surveys of Florida were examined for the presence of SINV-1 by RT-PCR. Samples from 2001 were archived in 95% ethanol, and those from 1999 were archived in 70% 2-propanol.

Table 1 summarizes the results of ethanol preservation on SINV-1 detection in *S. invicta*. At every time point over the course of the 2-year

TABLE 1. EVALUATION OF RNA PURIFIED FROM ETHANOL-ARCHIVED *SOLENOPSIS INVICTA* WORKERS FOR SINV-1 BY RT-PCR ANALYSIS OVER A 2-YEAR PERIOD.

		Amplification (+/-)	
Date of analysis	Days after placement in ethanol	Tube 1	Tube 2
28 Feb 2005	0	+	+
1 Mar 2005	1	+	+
7 Mar 2005	7	+	+
14 Mar 2005	14	+	+
31 Mar 2005	30	+	+
27 Apr 2005	57	+	+
31 May 2005	91	+	+
28 Jun 2005	119	+	–
26 Jul 2005	147	+	+
26 Sep 2005	209	–	+
1 Dec 2005	277	+	+
6 Feb 2006	344	+	+
9 Mar 2006	374	+	+
8 Sep 2006	535	+	+
7 Mar 2007	723	+	–

study, SINV-1 was detected by RT-PCR. Only 3 preparations, 119, 147, and 723 days in ethanol, failed to produce an amplicon. SINV-1 RNA in those samples was most likely degraded to a point that precluded cDNA synthesis and subsequent amplification, or the ants sampled at that time were not infected with SINV-1; the frequency of the infection in the colony of ants chosen for the study was never determined. However, viral load, the RNA extraction procedure, and extraction proficiency also could have had an influence on successful detection of SINV-1 by RT-PCR (Krafft et al. 2005).

The results were corroborated by successful detection of SINV-1 in ethanol-archived samples of *S. invicta* collected in 2001. Therefore, SINV-1 can be detected for at least 6 years in ethanol-archived ants. Conversely, attempts (n = 40) at RT-PCR detection of SINV-1 in *S. invicta* workers and queens archived in 2-propanol failed. This failure was most likely from RNA degradation accelerated by the high water content (>30%) in those samples. RNA is more susceptible to hydrolysis than DNA because of the presence of the 2'-hydroxyl group on ribose (Lindahl 1993). Thus, exclusion of water appears to be critical to successful preservation of nucleic acids (Fukatsu 1999).

Although freezing below -80°C is considered the gold standard for preservation of tissues (Perlmutter et al. 2004), ethanol has been shown to provide sufficient preservation of viral nucleic acids for amplification by RT-PCR and PCR (Fukatsu 1999; Whittier et al. 2004; Krafft et al. 2004; Perlmutter et al. 2004). However, many of these studies also indicated a significant number of false negatives compared with other preservation techniques, such as freezing, especially as the duration of storage increased. It is, therefore, highly likely that ant samples stored in ethanol would not present a completely accurate reflection of the infection rate of SINV-1 based on an increased incidence of false negatives. Indeed, the 3.3% infection rate of SINV-1 among the 6-year-old ethanol-archived fire ant samples was less than anticipated based on previous infection rates collected during the same time (Valles et al. 2004, 2007). Regardless, the ability to examine ant samples retrospectively provides a unique opportunity that could facilitate epidemiologic and phylogenetic studies.

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