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PSEUDACTEON DECAPITATING FLIES: POTENTIAL VECTORS OF A FIRE ANT VIRUS?

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Solenopsis invicta virus (SINV-1) is a positive-stranded RNA virus recently found to infect all stages of the red imported fire ant, *Solenopsis invicta* (Valles et al. 2004; Valles & Strong 2005). SINV-1 and a second genotype have been tentatively assigned to the *Dicistroviridae* (Mayo 2002). Infected individuals or colonies did not exhibit any immediate, discernible symptoms in the field. However, under stress from introduction into the laboratory, brood death was often observed among infected colonies, ultimately leading to the death of the entire colony (Valles et al. 2004). These characteristics are consistent with other insect-infecting positive-stranded RNA viruses. They often persist as inapparent, asymptomatic infections that, under certain conditions, induce replication within the host, resulting in observable symptoms and often death (Christian & Scotti 1998; Fernandez et al. 2002). The SINV infection rate among colonies was reported to be around 25% in Gainesville, Florida (Valles et al. 2004; Valles & Strong 2005). SINV vertical and horizontal transmission were inferred based on RT-PCR detection of virus genome in eggs and successful colony to colony transfer under lab conditions (Valles et al. 2004). However, the exact mechanisms by which the virus is spread from nest to nest in the field are unknown.

Phorid flies in the genus *Pseudacteon* are a common natural enemy of *S. invicta* in South America (Porter 1998) and these flies have been released as a biological control in the U.S. Because egg laying by female flies is an intrusive and repetitive action and flies develop within the ant host while consuming internal tissues, we were interested to know whether the *Pseudacteon* parasitoids that oviposited or developed in SINV-infected ants were capable of harboring this virus and potentially vectoring it to other fire ant colonies. Laboratory and field experiments were conducted with *P. obtusus*, *P. litoralis*, and *P. curvatus* to determine whether these flies harbored detectable levels of SINV.

Experiments were conducted to evaluate whether SINV was detectable in *P. obtusus* or *P. litoralis* that either completed their development or oviposited in SINV-infected *S. invicta*. Nests containing SINV-infected fire ants were identified from around Gainesville, Florida, by plunging 20 mL glass scintillation vials into fire ant nests to collect a sample of workers. Total RNA was extracted from 30 to 40 worker ants by the TRIZOL method (Invitrogen, Carlsbad, CA). cDNA was synthesized and subsequently amplified with the One-Step RT-PCR kit (Invitrogen) with oligonucleotide primers p114 and p116 (SINV-1-specific) and p117 and p118 (SINV-1A-specific) (Valles & Strong 2005). Samples were positive for each virus when a visible amplicon of anticipated size (646 nt for SINV-1 and 153 nt for SINV-1A) was present after separation on a 1.2% agarose gel stained with ethidium bromide. Every RT-PCR reaction included a positive and negative control. For simplicity, positive infection by either virus genotype was labeled as SINV-infected. 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.

Three SINV-positive nests were excavated from the field and the ants were removed from the soil by the floating method (Jouvenaz et al. 1977). Ants from a SINV-infected nest were sieved to separate the worker ants by size corresponding to the oviposition preferences of *P. littoralis* and *P. obtusus* (Porter 1998). Samples of the *obtusus*-sized and *litoralis*-sized ants were evaluated for the presence of SINV-1 and SINV-1A. RNA was extracted from pooled groups of 3 worker ants of *obtusus*- (n = 14 groups) and *litoralis*-sized (n = 14 groups) workers and RT-PCR was conducted as described on each of these groups to determine the approximate rate of SINV infection. After a suitable nest of ants was identified and sieved, the appropriate sized SINV-infected workers were presented to newly-eclosed *P. obtusus* and *P. litoralis* for oviposition. Approximately 1.5 g of *P. obtusus*-sized or *P. litoralis*-sized worker ants were placed separately into a fly attack box (Vogt et al. 2003) containing newly-eclosed *P. obtusus* or *P. litoralis* phorid flies, respectively. Flies were allowed to oviposit in the ants for 1 d. A sample of flies from each species was collected about 2 h after being exposed to the ants as they were ovipositing on the ants. Parasitized worker ants were removed from the attack boxes and maintained as described by Vogt et al. (2003). Approximately 2 weeks after oviposition, parasitized ants (n = 39 from *P. litoralis* exposure; n = 17 from *P. obtusus* exposure) were removed from the holding group and evaluated for the presence of the SINV by RT-PCR. The remainder exhibited symptoms consistent with parasitization, including twitch-

TABLE 1. EVALUATION OF *PSEUDACTEON OBTUSUS* AND *PSEUDACTEON LITORALIS* FLIES OVIPOSITING IN, OR EMERGING FROM, SINV-INFECTED *SOLENOPSIS INVICTA* WORKERS.

Fly species	Days after oviposition	Development	*n* (flies)	*n* (groups)*	SINV (+/-)
P. litoralis	0	Ovipositing	10	2	—
P. litoralis	34-46	Emerging	68	9	—
P. obtusus	0	Ovipositing	67	4	—
P. obtusus	26-32	Emerging	70	8	—

*Refers to the number of individual flies pooled for analysis (5-24 worker ants comprised a group).

ing/uncoordination, morbidity, and decapitation. Approximately half of these ants were confirmed to contain a parasite. Fly pupae were observed daily for the next month. All flies emerging from these pupae were evaluated for the presence SINV-1 or SINV-1A by RT-PCR.

Field-established *Pseudacteon* fly populations were sampled and analyzed for SINV infection to determine whether the virus was present in phorid flies in their natural setting. *S invicta* mounds (10 nests at each site) were opened with a shovel in 3 areas around Gainesville, Florida, exhibiting a high incidence of SINV infection. SINV infection in these areas was determined by RT-PCR within 2 weeks of sampling for flies. *Pseudacteon curvatus* flies that were attracted to the exposed fire ant nests were collected by aspiration while they attacked worker ants. The flies were immediately returned to the laboratory and assayed for the presence of SINV. Flies were pooled in groups of 5 for analysis without regard for sex. RT-PCR was conducted on all of the collections for the presence of SINV-1 and SINV-1A.

Our data demonstrated that *Pseudacteon* flies feeding upon SINV-infected ants do not acquire the virus. SINV was not detectable by RT-PCR in flies (*P. obtusus* and *P. litoralis*) that completed their larval development in SINV-infected fire ant workers (Table 1). SINV infection of host ants did not appear to inhibit the ability of flies to complete their development (Table 2). The rate of SINV infection among parasitized groups of ants ranged from 71 to 93%. SINV was not detected in *P. curvatus* ($n \approx 90$) field-collected from areas with high a incidence of SINV infection. The lack of virus detection in flies completing development in infected ants suggests that the virus does not display a broad host range.

Although SINV does not appear to infect *Pseudacteon* decapitating flies, horizontal transmission of SINV could occur mechanically from contamination of the ovipositor. This type of virus vectoring has been reported previously and is the primary route of transmission among some ascoviruses in Lepidoptera (Stasiak et al. 2000). However, SINV infection of *S. invicta* appears to be limited to cells of the digestive tract, especially the midgut (Valles unpublished data). Further, we have not detected SINV in the hemolymph of infected fire ants. Thus, it is unlikely that oviposition into the hemocoel by *Pseudacteon* decapitating flies would result in SINV contamination of the ovipositor. Indeed, flies allowed to oviposit in SINV-infected fire ants for several hours were all negative for SINV ($n = 77$; Table 1). However, mechanical transmission still could have occurred, but was not detectable by the RT-PCR method employed; the limit of detection of SINV by RT-PCR is estimated to be between 50 and 100 copies (Valles unpublished data).

Our results indicate that SINV does not replicate within *Pseudacteon* decapitating flies that parasitize *S. invicta*. Flies appeared to develop normally from SINV-infected *S. invicta* workers. Mechanical transmission of SINV to uninfected ants by oviposition appears unlikely.

TABLE 2. RATE OF SINV INFECTION AMONG *SOLENOPSIS INVICTA* WORKERS PARASITIZED BY *P. OBTUSUS* OR *P. LITORALIS*.

Ant workers parasitized by	Ant condition	*n* (worker ants)	*n* (groups)*	% SINV infection among groups (mean ± SD)
P. litoralis	Before oviposition	21	7	100
P. litoralis	Parasitized	39	14	93 ±27
P. obtusus	Before oviposition	21	7	71 ±49
P. obtusus	Parasitized	17	7	71 ±49

*Refers to the number of individual worker ants pooled for analysis (1-3 flies comprised a group).

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