

**HOST SPECIFICITY OF THE MICROSPORIDIAN PATHOGEN
VAIRIMORPHA INVICTAE AT FIVE FIELD SITES WITH INFECTED
SOLENOPSIS INVICTA FIRE ANT COLONIES IN NORTHERN ARGENTINA**

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ABSTRACT

The microsporidian pathogen *Vairimorpha invictae* is being evaluated for release in the United States as a potential self-sustaining biological control agent for imported fire ants. We examined the host range of this pathogen at 5 sites in northern Argentina where *Solenopsis invicta* Buren fire ant colonies had high levels of infection (28-83%). At 3 sites near the city of Corrientes, we examined 509 non-*Solenopsis* ants from 61 collections, 12 genera, and 19 species with a polymerase chain reaction-based screening procedure. No *V. invictae* infections were detected in any of the samples. At 2 sites near San Javier in Santa Fe Province, 350 km to the south, we screened another 438 non-*Solenopsis* ants from 44 baits, 4 genera, and 4 species, again with no infections. At the Corrientes sites, we also examined 235 non-ant arthropods from 10 orders, 43 families, and more than 80 species. None were infected with *V. invictae*. The results of this study indicate that, in its native South American range, *V. invictae* is specific to *Solenopsis* fire ants.

Key Words: biocontrol, host range, Formicidae, Microsporidia

RESUMEN

El microsporidio patógeno *Vairimorpha invictae* está siendo evaluado en los Estados Unidos como agente potencial de control biológico clásico para las hormigas de fuego (hormigas bravas). Examinamos el rango de hospederos de este patógeno en cinco sitios del norte Argentino en donde colonias de la hormiga brava *Solenopsis invicta* tenían altos niveles de infección (28-83%). En tres lugares cerca de Corrientes, examinamos 509 hormigas en 12 géneros (excluido *Solenopsis*), 19 especies y 61 cebos usando la técnica de acción en cadena de la polimerasa (PCR), pero ninguna estuvo infectada con *V. invictae*. En dos sitios cercanos a San Javier, Provincia de Santa Fe, 350 km hacia el sur, examinamos otras 438 hormigas no *Solenopsis* en 4 géneros, 4 especies y 44 cebos, otra vez sin infección. En los sitios de Corrientes, también examinamos otros 235 artrópodos en 10 órdenes, 43 familias y más de 80 especies. Otra vez, ninguno fue infectado con *V. invictae*. Los resultados de este estudio indican que, en su área nativa de América del Sur, *V. invictae* es específico de hormigas del género *Solenopsis*.

Translation provided by the authors.

Solenopsis fire ants are 5-10 times more abundant where they occur in the United States than they are in their South American homelands (Porter et al. 1992; Porter et al. 1997). Escape from numerous natural enemies left behind in South America (Williams et al. 2003) is a likely explanation for this difference. Natural enemies include almost 2 dozen species of phorid flies (Porter & Pesquero 2001; Brown et al. 2003), a parasitic ant (Calcaterra et al. 1999), several nematodes (Williams et al. 2003), a virus (Valles et al. 2004b; Valles et al. 2007), and two species of microsporidian pathogens: *Thelohania solenopsae* Knell,

Allen & Hazard and *Vairimorpha invictae* Jouve-
naz & Ellis.

The first microsporidian, *T. solenopsae*, was described by Knell et al. (1977) from Brazil. Briano et al. (1995a, 1995b, 1995c, 1996) published extensively on its distribution, abundance, and impacts at sites in Argentina. This pathogen was first detected in the United States by Williams et al. (1998) who found it widespread and apparently specific to imported fire ants.

The second microsporidian, *V. invictae*, was described by Jouve-
naz & Ellis (1986). This pathogen occurs in both monogyne and polygyne fire ant

colonies (Valles & Briano 2004) and appears to be more common in the red fire ant *S. invicta* than in the black fire ant *S. richteri* (Briano & Williams 2002). Its occurrence in the field is associated with smaller colonies and declining fire ant populations (Briano 2005). Recent laboratory studies have shown that this pathogen can be transmitted among fire ant colonies by the transfer of larvae, pupae, or even dead workers and that infected colonies grew 85% slower than uninfected control colonies (Oi et al. 2005).

Terrestrial microsporidian pathogens, as a group, are normally limited to one host or a group of related hosts (Solter & Maddox 1998; Briano et al. 2002; Keeling & Slamovits 2004). Narrow host ranges also apparently apply to *Vairimorpha* (Solter et al. 2000); however, some species like *Vairimorpha necatrix* appear to have relatively broad physiological host ranges in the laboratory when lepidopteran larvae challenged with high spore doses. Nevertheless, actual ecological host ranges in the field are likely less broad (Maddox et al. 1981).

Vairimorpha invictae is a promising self-sustaining or classical biocontrol agent for fire ants because of (1) documented impacts on fire ant colonies, (2) its prevalence on red fire ants, and (3) its absence from the United States. Furthermore, *V. invictae* appears to be specific to fire ants. Briano et al. (2002) reported that none of the 9 genera of non-*Solenopsis* fire ants collected at 167 baits from >25 sites or from 50 colonies at >20 sites were infected with *V. invictae*. This absence of observed infections occurred despite the fact that *V. invictae* was found in 61 of 535 fire ant colonies collected at 18 of 90 sites.

The objective of the current study was to further examine the host range of *V. invictae* by investigating its presence in arthropods and other species of ants that co-occur in close proximity with *V. invictae* infected fire ant colonies in South America.

MATERIALS AND METHODS

We found 3 populations of mostly polygyne *S. invicta* infected with *V. invictae* in Apr 2004, 5-20 km east of Corrientes city, Argentina. The first site was a campground and boat launch area along the Paraná River (S 27°22.601', W 58°40.891'). The second site was by a bridge over a small stream near a family farm (S 27°24.283', W 58°41.127'). The third site was along Route 12 at km marker 1034 just outside the Corrientes Airport (S 27°27.414', W 58°45.743'). Two additional sites with *V. invictae* infected colonies were found near San Javier, Santa Fe Province about 350 km south of Corrientes. The first site was near Estancia Liriolay (S 30°30.951'W 60°01.052). The second site was near an intersection with a clay road marked by white tires on the roadside (S 30°31.615, W 60°00.730). The propor-

tion of polygyne colonies at these 2 sites was 33%, based on PCR tests done for a recent study (Briano et al. 2006).

In order to examine the host range of *V. invictae* at the five study sites, we used small vials (12 by 85 mm) baited with pieces of hotdog to collect other species of co-occurring ants. Sugar water baits were also used at the San Javier sites. At each site, 20-50 baits were set out at 5-m intervals in several transects. Some hand collecting was also used for ants not attracted to baits. At the Corrientes sites, we also used sweep nets to collect co-occurring arthropods. A supplemental sample of 79 non-ant Hymenoptera collected in Jan 2005. Ants and other arthropods were stored submerged in 95% ethanol to preserve the DNA (King & Porter 2004).

Infected fire ant colonies were detected by macerating about 30 workers with an electric tissue grinder. A drop of aqueous extract was then examined under a phase-contrast microscope for the presence of spores (Briano 2005). At baits, we macerated 15-20 fire ant workers for microscopic examination if that many were available. These sampling rates should have been sufficient on average to detect *V. invictae* in samples with worker infection rates as low as 3-7%.

PCR was used to detect *V. invictae* in non-*Solenopsis* ants and other kinds of arthropods (Valles et al. 2004a). Briefly, PCR was carried out with primer pairs specific for the 16S rRNA gene of *V. invictae* (p90, 5'CACGAAGGAGGATAACCACGGT and p93, CGCAATCAGTCTGTGAATCTCTCA) by using the hot start method in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime: 1 cycle at 94°C for 2 min, then 35 cycles at 94°C for 15 s, 55°C for 15 s, and 68°C for 45 s, followed by a final elongation step of 5 min at 68°C. The reaction was conducted in a 25 µL volume containing 2 mM MgCl₂, 200 µM dNTP mix, 1 unit of Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA), 0.4 µM of each primer, and 0.5 µL of the genomic DNA preparation (10 to 50 ng). PCR products were separated on 1.2% agarose gel and visualized by ethidium bromide staining. For all experiments, positive and negative controls were run alongside treatments. In situations in which verification of *V. invictae* positive non-*Solenopsis* insects was required, the amplicon was cloned and sequenced. Amplicons were gel-purified, ligated into the pCR-4 vector and transformed into TOP10 competent cells (Invitrogen, Carlsbad, CA). Insert-positive clones were identified and DNA sequences of inserts were elucidated by the University of Florida, Interdisciplinary Center for Biotechnology Research (Gainesville, FL).

At the Corrientes sites, samples of ants from several baits were pooled, generally by genus and site, into 18 samples. Most pooled samples consisted of ants from 2-5 baits and contained 20-50 in-

dividuals depending on their size and availability. The arthropod samples were similarly pooled, mostly by order or family, into 33 samples. At the San Javier sites, ant samples from non-fire ant genera were similarly pooled into 16 samples with each sample containing 20-40 workers from 2-3 baits.

PCR was also conducted to verify that the test could detect the *V. invictae* target gene in other ant genera (*Linepithema*, *Dorymyrmex*, *Crematogaster*, *Paratrechina*). This experiment was done by spiking 30-70 non-*Solenopsis* workers with 5 *Solenopsis* workers from a *V. invictae*-positive colony prior to maceration for DNA extraction. Similar tests were not done with non-ant arthropods, because DNA extraction techniques are quite reliable, sample sizes were limited, and it would have considerably increased the number of samples needing testing. We also used PCR to test 3 samples of infected fire ant workers from San Javier that were initially detected by microscopic inspection. Based on the experience from a previous study conducted in Argentina (Valles & Briano 2004), *V. invictae* is always detected with PCR tests if it was previously detected by microscopic examination.

RESULTS

As expected (Valles & Briano 2004), the PCR technique identified *V. invictae* in fire ant workers in which spores had been detected by light microscopy ($n = 3$). Similarly the PCR technique detected *V. invictae* in samples of *Paratrechina*, *Crematogaster*, and *Linepithema* ants spiked with 5 infected fire ants, thereby showing that ants in these genera do not appear to have chemistry that would mask detection of *V. invictae* DNA. *Vairimorpha invictae* was not detected in the first sample with *Dorymyrmex* ants, perhaps because none of the 5 fire ant workers used to spike the sample

were infected (Briano & Williams 2002) or perhaps because of a faulty extraction. A retest with these ants, however, produced a positive result.

Infected fire ants were detected in both fire ant mounds and among foraging workers collected at baits (Table 1). At the Corrientes sites, the percentage of positive baits was lower than the percentage of positive mounds when we included baits with ≤ 4 workers. However, when we only compared baits with ≥ 5 workers, the percentages were similar. This comparison is more appropriate because only 10-60% of workers in a mound are infected (Briano & Williams 2002) and mound samples always contained many workers (15-30) while bait samples, with only a few workers, may have produced false negatives relative to their colony of origin. However, at the clay road site near San Javier (Table 1), the percent of infected baits was still about half that of the infected mounds.

All of the non-ant arthropod samples from the Corrientes area (Table 2) were negative for *V. invictae* (Table 1). One sample from the campground area containing 2 solitary bees and 2 sphecid wasps produced a faint positive band, but sequencing confirmed that the amplicon was not from the *V. invictae* 16S rRNA gene.

All of at least 19 species of ants from 12 non-*Solenopsis* genera including 947 individuals from 107 samples collected near Corrientes and San Javier (Table 3) were also negative for *V. invictae* (Table 1).

When we resampled ant colonies at the 3 Corrientes sites 20 months after the initial sampling (Dec 2006) and tested them for *V. invictae* infections, we found that *V. invictae* had disappeared from the bridge site (0/8) and only one weakly positive sample was found each at the campground and airport sites (1/12; 1/19), a considerable decline from what we found in 2004 (Table 1). This drop in frequency of *V. invictae* infections was not

TABLE 1. PERCENT OF FIRE ANTS, OTHER KINDS OF ANTS, AND OTHER INSECTS INFECTED WITH THE MICROSPORIDIAN PATHOGEN *VAIRIMORPHA INVICTAE* AT 5 SITES IN ARGENTINA. NUMBERS IN PARENTHESES SHOW POSITIVES DIVIDED BY TOTAL SAMPLE.

Taxon	Sites				
	Campground ¹	Airport ¹	Bridge ¹	Liriolay ²	Clay Rd. ²
Fire Ants					
Mounds	69% (9/13)	28% (4/14)	83% (10/12)	43% (15/35)	62% (16/26)
Baits (all)	45% (5/11)	17% (2/12)	50% (7/14)	—	33% (6/18)
(≥ 5 ants)	56% (5/9)	29% (2/7)	64% (7/11)	—	33% (6/18)
Other Ants					
Baits ³	0% (0/31)	0% (0/23)	0% (0/9)	0% (0/29)	0% (0/15)
Other Arthropods					
Individuals	0% (0/184)	0% (0/39)	0% (0/12)	—	—

¹Near Corrientes, Argentina.

²Near San Javier, Santa Fe Province, Argentina.

³Seven *Acromyrmex* samples were from nests rather than baits.

TABLE 2. NON-ANT ARTHROPODS COLLECTED AT CORRIENTES SITES, APR 2004. NUMBER OF MORPHOSPECIES, FAMILIES, AND INDIVIDUALS GROUPED BY ORDER AND SITE WHICH WERE TESTED BY PCR FOR THE PRESENCE OF THE MICROSPORIDIAN PATHOGEN *VAIRIMORPHA INVICTAE*.

Order	Species-Families (individuals) by site		
	Campground	Airport	Bridge
Araneae	8-? (8)	4-? (4)	4-? (4)
Odonata ¹	—	1-1 (1)	1-1 (1)
Orthoptera ²	5-3 (17)	1-1 (3)	2-2 (2)
Homoptera ³	9-3 (10)	3-1 (12)	—
Hemiptera ⁴	8-5 (18)	6-3 (7)	2-2 (2)
Psocoptera	1-1 (1)	—	—
Coleoptera ⁵	12-5 (16)	5-3 (6)	—
Diptera ⁶	15->7 (23)	3-3 (3)	2-2 (2)
Lepidoptera ⁷	5-4 (5)	2-2 (2)	—
Hymenoptera ⁸	7-4 (7)	1-1 (1)	1-1 (1)
supplemental sample ⁹	58-15 (79)	—	—
Estimated Totals	121-44 (184)	26-16 (39)	12-9 (12)

Identified Families: ¹Libellulidae; ²Acrididae, Tettigoniidae, Gryllidae, Tetrigidae; ³Cicadellidae, Fulgoridae; ⁴Myridae, Lygaeidae, Coreidae, Pentatomidae, Berytidae; ⁵Curculionidae, Buprestidae, Chrysomelidae, Scolytidae, Dermestidae; ⁶Tephritidae, Bombyliidae, Culicidae, Dolichopodidae, Tipulidae, Bibionidae, Rhagionidae, Sarcophagidae; ⁷Lycaenidae, Nymphalidae, Pieridae, Sphingidae, Geometridae; ⁸Chrysididae, Halictidae, Sphecidae, Cynipidae, Apidae, Ichneumonidae, Braconidae, Diprionidae, Bethyidae, Vespidae, Anthophoridae; ⁹Collected 26 Jan 2005.

unexpected because similar drops in infection rates have been reported previously, often in conjunction with lower nest densities (Briano 2005; Briano et al. 2006).

TABLE 3. THE NUMBER OF ANT SPECIES, BAITS, AND INDIVIDUALS (EXCLUDING FIRE ANTS) GROUPED BY GENUS AND SITE WHICH WERE TESTED BY PCR FOR THE PRESENCE OF *VAIRIMORPHA INVICTAE*.

Subfamily Genus	Species-Baits (individuals) by site				
	Campground ^a	Airport ^a	Bridge ^a	Liriolay ^b	Clay Rd. ^b
Ponerinae					
<i>Ectatomma</i>	1-2 (2)	—	—	—	—
<i>Pachycondyla</i>	1-1 (1)	—	—	—	—
Myrmicinae					
<i>Acromyrmex</i>	—	—	1-3 (15) ^c	1-4 (48) ^c	—
<i>Crematogaster</i>	4-4 (21)	1-8 (90)	1-2 (22)	—	—
<i>Pheidole</i>	4-5 (32)	—	—	—	—
<i>Wasmannia</i>	1-4 (42)	—	—	—	—
<i>Zacryptocerus</i>	1-1 (1)	—	—	—	—
Dolichoderinae					
<i>Dorymyrmex</i>	—	1-3 (31)	1-4 (24)	1-5 (44)	—
<i>Linepithema</i>	1-11 (101)	—	—	—	—
Formicinae					
<i>Camponotus</i>	1-1 (3)	1-2 (7)	—	1-1 (7)	—
<i>Brachymyrmex</i>	1-1 (30)	1-1 (20)	—	—	—
<i>Paratrechina</i>	1-1 (1)	1-9 (66)	—	1-19 (191)	1-15 (148)
Total	16-31 (234)	5-23 (214)	3-9 (61)	4-29 (290)	1-15 (148)

^aNear Corrientes, Argentina.

^bNear San Javier, Santa Fe Province, Argentina.

^cCollected from nests rather than baits.

DISCUSSION

Overall, these data indicate that *V. invictae* is specific to *Solenopsis* fire ants because this pathogen was not found in any of the sympatric ants or co-occurring arthropods. Briano et al. (2002) failed to find *V. invictae* in 217 collections of non-*Solenopsis* ants from 9 genera in Argentina; although, it appears that only 20% of the sites may have had *V. invictae* present in co-occurring fire ants. While *V. invictae* has not been found in other ant genera, it has been reported from 3 species of *Solenopsis* fire ants (*S. invicta*, *S. richteri*, *S. macdonaghi*; Briano et al. 2002), all in the saevissima complex of South American fire ants (Trager 1991). It likely occurs in other South American *Solenopsis* species as well. The known geographic range of this pathogen extends from Mato Grosso, Brazil (Jouvenaz & Ellis 1986) south through Paraguay and part of Bolivia (Briano et al. 2006) to Buenos Aires Province, Argentina (Briano et al. 2002). Extensive searches in the United States for *T. solenopsae* and other pathogens (Jouvenaz et al. 1977; Williams et al. 1998) have failed to detect *V. invictae* infections.

We currently do not know whether *V. invictae* has the ability to infect native *Solenopsis* fire ants in the United States (e.g.; *S. geminata*, *S. xyloni*) which belong to a different complex of fire ants (*geminata*) or the more distantly related group of very small *Solenopsis* thief ants. Laboratory tests in quarantine are currently being conducted by D. H. Oi in an effort to answer the question of whether our native North American fire ants and other species of ants are susceptible to *V. invictae* infections.

Low levels of infection in native fire ants would probably be tolerable (Porter 2000) because the native fire ants can be pests in their own right, especially the tropical fire ant *S. geminata* which has become a world-wide exotic pest. However, infections in other ant genera or in other families of insects would be a complicating factor which would likely require further tests and impact evaluations across a range of ant genera or arthropod families.

ACKNOWLEDGMENTS

Chuck Strong is thanked for conducting the PCR tests and Euripides Mena is thanked for doing the visual microscope scans. Laura Varone and Alicia Delgado (SABCL) helped with field collections in Corrientes and Juan P. Livore, a former SABCL intern, helped with field collections at the San Javier sites. Roberto Pereira and Yoshifumi Hashimoto are thanked for helpful comments.

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