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Field host range of the fire ant pathogens *Thelohania solenopsae* (Microsporida: Thelohaniidae) and *Vairimorpha invictae* (Microsporida: Burenellidae) in South America

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Abstract

We studied the field host specificity of the microsporidia *Thelohania solenopsae* and *Vairimorpha invictae* and their prevalence in the imported fire ants, *Solenopsis invicta* and *S. richteri*. Terrestrial ants were sampled by using bait traps and/or nest sampling at preselected sites in Argentina and Brazil. The sampling included the genera *Solenopsis*, *Pheidole*, *Camponotus*, *Crematogaster*, *Linepithema*, *Brachymyrmex*, *Paratrechina*, *Dorymyrmex*, and *Wasmannia*. The samples were examined under a phase-contrast microscope for the presence of microsporidian infections. The bait trap sampling revealed that: (1) *T. solenopsae* infected only *S. richteri*, *S. invicta*, and *Solenopsis* sp. at 6–67% of the sites and in 1.5–29% of the traps; (2) *V. invictae* infected only *S. invicta* at 6% of the sites and in 3% of the samples. The nest sampling revealed that: (1) *T. solenopsae* infected *S. invicta*, *S. richteri*, and *S. macdonaghi*, at 41–67% of the sites and in 11–58% of the colonies; (2) *V. invictae* infected the same species at 15–50% of the sites and in 2–26% of the colonies. We detected *T. solenopsae* and *V. invictae* in equal percentages of *S. invicta* sites (41%); however, the percentage of colonies infected with *V. invictae* was 20% and with *T. solenopsae* only 11%. At *S. richteri* sites, in contrast, *T. solenopsae* occurred at 46% of the sites and 15% of the colonies and *V. invictae* occurred at only 15% of the sites and 2% of the colonies. In *S. macdonaghi*, *T. solenopsae* was detected at 67% of the sites and 58% of the colonies, and *V. invictae* was detected at 50% of the sites and 26% of the colonies. This is the first report of *V. invictae* infecting *S. macdonaghi*. The proportion of *S. richteri* and *S. invicta* infected with *T. solenopsae* was similar. In contrast, the proportion of *S. invicta* infected with *V. invictae* was higher than *S. richteri*. We conclude that the microsporidia, *T. solenopsae* and *V. invictae*, show a very high specificity for *Solenopsis* ants in the field. It appears that *T. solenopsae* infects *S. invicta* and *S. richteri* equally but *V. invictae* may be more adapted to infect *S. invicta*. Published by Elsevier Science (USA).

Keywords: *Thelohania solenopsae*; *Vairimorpha invictae*; *Solenopsis invicta*; *S. richteri*; Host specificity; Imported fire ants

1. Introduction

The microsporidia, *Thelohania solenopsae* Knell, Allen, and Hazard (Microsporida: Thelohaniidae) and *Vairimorpha invictae* Jouvenaz and Ellis (Microsporida: Burenellidae), are pathogens associated with fire ants, *Solenopsis* spp., in South America. The former was also discovered recently in the southern United States infecting the red imported fire ant, *Solenopsis invicta* Buren, (Williams et al., 1998) and is being evaluated as a biological control agent for fire ants.

According to Tanada and Kaya (1993), the microsporidia, as a group, are the most promising protozoa-like microorganisms for use as microbial control agents. They are obligate, intracellular pathogens that produce acute or chronic infections in insects. Both *T. solenopsae* and *V. invictae* are dimorphic and produce meiospores and free spores that develop in adult fire ant workers, sexuals, and queens and, in the case of *V. invictae*, also in pupae and larvae (Briano et al., 1996; Jouvenaz and Ellis, 1986; Knell et al., 1977; J.A.B., unpublished information).

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Surveys of these microsporidia infecting fire ants have been conducted in South America since the 1970s and infections have been reported in *S. invicta*, *S. richteri* Forel, *S. saevissima* Smith, *S. quinquecuspis* Forel, *S. macdonaghi* Santschi, and *S. blumi* Buren (Allen and Buren, 1974; Allen and Knell, 1980; Allen and Silveira Guido, 1974; Briano et al., 1995; Jouvenaz, 1983, 1986, 1990; Jouvenaz et al., 1980; Williams and Whitcomb, 1974; Wojcik et al., 1987). *T. solenopsae* is the most common fire ant pathogen in South America. In some areas of Buenos Aires Province, Argentina, it infects from 40 to 80% of the fire ants (Briano et al., 1995). Much less is known about *V. invictae* field infection rates. Records of field hosts for *V. invictae* are restricted to *S. invicta* [Jouvenaz et al., 1980 (as "undescribed microsporidium"); Jouvenaz and Ellis, 1986; Wojcik et al., 1987] and *S. richteri* (Briano et al., 1995). In addition, the presence of these two pathogens in non-fire ant species has never been determined. The study of the field host range of *T. solenopsae* and *V. invictae* in their aboriginal area will help to predict their ecological host range in a new habitat. The objectives of this study were to determine the field host specificity (ecological host range) of *T. solenopsae* and *V. invictae* and to determine any difference in susceptibility of the imported fire ants, *S. invicta* and *S. richteri*, to the two microsporidian species.

2. Materials and methods

We conducted the study during 1993 (November and December, spring), 1994 (February–May, summer–fall), 1998 (October and November, spring), 1999 (April, July, August and November, fall–winter–spring), and 2000 (February, May, and October, summer–fall–spring). We sampled terrestrial ants that occurred sympatrically with fire ants infected with *T. solenopsae* and/or *V. invictae*, at 1–6 sites within each of several areas of the provinces of Buenos Aires, Santa Fe, Chaco, Corrientes, and Entre Rios, Argentina, and the state of Mato Grosso, Brazil. Ants were sampled using one or both of the following two approaches: (1) bait trap sampling and (2) nest sampling.

2.1. Bait trap sampling

Bait traps were used at 52 preselected roadside sites within the areas of Pergamino, Bragado, Saladillo, Navarro, Mercedes, Moreno, Solís, Pilar, and Escobar (Buenos Aires Province), Timbúes, Recreo, San Justo, Gobernador Crespo, Vera y Pintado, Calchaquí, Cello, Vera, Malabrigo, and Berna (Santa Fe Province). Sites that previously had *T. solenopsae* and/or *V. invictae* infecting fire ants were selected for sampling (Briano et al., 1995; J.A.B., unpublished data). Our sampling methods were similar to those reported by Porter et al. (1997). At each site, 10 bait stations consisting of a disposable 7-ml glass vial containing a small piece of canned "Vienna" sausage were established. Depending on the air temperature, the vial traps were left on the ground surface for 30–60 min. In most cases, the traps were shaded with a 15-cm plastic plate to keep traps cooler. The ants trapped, usually ranging from 50 to 400 specimens/trap, were preserved in 70% ethanol and transported to the laboratory for examination.

2.2. Nest sampling

This sampling complemented the bait traps and was primarily used to sample as many nests as possible of *S. invicta* and *S. richteri*. The purpose was to assess a differential susceptibility, if any, of the fire ant species to *T. solenopsae* and *V. invictae*. When detected, nests of other ant species were also sampled.

The sampling consisted of visually searching for ant nests and was conducted in 90 sites in Argentina and Brazil. In Argentina, we collected at the 52 sites used for bait trapping and in other areas such as Las Flores (Buenos Aires), Basail, Resistencia (Chaco), Corrientes, San Roque, Goya, Esquina (Corrientes), La Paz, Paraná, Victoria, and Gualeguay (Entre Rios). Most of these areas were selected systematically every 10–100 km along the main highways on both sides of the Paraná River during an exploratory trip to Northern Argentina. In Brazil, we sampled at four sites within the areas of Cuiaba, the type locality of both microsporidia, and Cáceres (Mato Grosso). A total of 585 ant colonies were sampled by inserting a 7-ml disposable glass vial (coated with talc) into each nest for a few minutes. The samples, containing approximately 200–3000 worker ants, were preserved in 70% ethanol and transported to the laboratory for examination.

2.3. Disease diagnosis

Alcohol-preserved samples ($n = 928$) obtained by the two methods described above were macerated individually in water with a tissue grinder (Tekmar SDT Tissumizer) and a drop of the aqueous extract was examined under a phase-contrast microscope (400×) for the presence of spores of *T. solenopsae* and/or *V. invictae*. The sensitivity of this procedure was successfully tested by Jouvenaz et al. (1977). Both meiospores and free spores of these two microsporidia are clearly distinctive in size and were easily detected when present in the samples. A few ant specimens were not macerated and were used for taxonomic identification. Most ant species were identified with available keys (Bolton, 1994, 1995; Gonçalves, 1961; Kusnezov, 1978). Gas chromatography analysis (Vander Meer and Lofgren, 1990) was used to confirm the identification of some samples of fire ants. Voucher

specimens were deposited at the USDA-ARS-South American Biological Control Laboratory in Hurlingham, Argentina and at the USDA-ARS-Center for Medical, Agricultural and Veterinary Entomology, in Gainesville, FL.

2.4. Statistical analysis

Chi-square tests (Minitab Statistical Software, 1991) were used to compare the prevalence of *T. solenopsae* and *V. invictae* in *S. invicta* and *S. richteri*. Other *Solenopsis* species were not considered in the analysis because of sample size limitations.

3. Results and discussion

3.1. Bait trap sampling

Nine ant genera in the subfamilies Myrmicinae, Formicinae, and Dolichoderinae, and some undetermined ants were detected in the bait traps (Table 1). *S. richteri*, *S. invicta*, *Pheidole* spp., *Camponotus* spp., and *Crematogaster* sp. were the most abundant ants collected in traps and were present at a minimum of 10 sites. Other ants trapped were *Linepithema* sp., *Pheidole radoszkowskii* Mayr, *Solenopsis* sp., *Brachymyrmex* sp., *Paratrechina pubens* (Forel), *Dorymyrmex* spp., and *Wasmannia auropunctata* (Roger) (Table 1).

Thelohania solenopsae infected only ants in the genus *Solenopsis* (Table 1). This microsporidium was found at 64% of the sites and in 29% of the traps with *S. richteri*, at 25% of the sites and in 20% of the traps with *Solenopsis* sp., and at 6% of the sites and 1.5% of the traps with *S. invicta*. However, only four sites and five traps contained *Solenopsis* sp.

Vairimorpha invictae occurred only in *S. invicta* at 6% of the sites and in 3% of the samples. The presence of this pathogen in trapped fire ants was low including those sites where it had been detected in relatively high prevalence in previous surveys (J.A.B., unpublished data). Also, it was low or even absent in sites where the presence of *V. invictae* was later confirmed through the nest sampling and examination of fire ant nests. The reason for this remains unclear. One possible explanation is that fire ant workers infected with *V. invictae* drastically reduce foraging activity and, consequently, were not trapped. This needs further investigation but some laboratory observations (J.A.B., unpublished data) revealed that colonies with *V. invictae* showed rapid mortality of infected workers which may affect the foraging capability of the colonies.

The distribution of ant species was wide. Ants were found at 51 of the 52 sites surveyed. However, the abundance of ants was low; a high proportion (177/520 = 34%) of traps were found empty. In addition, some ant species such as *Linepithema* sp., *P. radoszkowskii*, *Solenopsis* sp., *Brachymyrmex* sp., *P. pubens*, *Dorymyrmex* spp., and *W. auropunctata* occurred in insufficient numbers to allow certainty of disease presence. However, we detected infection of *T. solenopsae* in *Solenopsis* sp. that occurred at 8% (4/52) of the sites and in 1% (5/520) of the traps (Table 1).

3.2. Nest sampling

Nests of *S. richteri*, *S. invicta*, *S. macdonaghi*, *Acromyrmex* sp., *A. lundi* (Guerin–Meneville), *A. ambiguus* (Emery), *Pheidole* sp., and *Camponotus* sp. were found (Table 2). *Solenopsis invicta* and *Acromyrmex* spp. occurred at the greatest number of sites but the most abundant species were *S. richteri* and *S. invicta*.

Table 1
Field host range of *Thelohania solenopsae* and *Vairimorpha invictae*: bait trap sampling

Ants trapped	No. (%) of sampling sites per species[a]			No. (%) of traps per species[b]		
	Total	With *T. solenopsae*	With *V. invictae*	Total	With *T. solenopsae*	With *V. invictae*
Solenopsis richteri	22	14 (64)	0	75	22 (29)	0
Solenopsis invicta	16	1 (6)	1 (6)	67	1 (1.5)	2 (3)
Pheidole spp.	25	0	0	57	0	0
Camponotus spp.	19	0	0	46	0	0
Crematogaster sp.	10	0	0	28	0	0
Linepithema sp.	1	0	0	10	0	0
Pheidole radoszkowskii	4	0	0	9	0	0
Solenopsis sp.	4	1 (25)	0	5	1 (20)	0
Brachymyrmex sp.	2	0	0	2	0	0
Paratrechina pubens	2	0	0	2	0	0
Dorymyrmex spp.	2	0	0	2	0	0
Wasmannia auropunctata	1	0	0	1	0	0
Other spp.	6	0	0	10	0	0
No ants	1	–	–	177	–	–

[a] Total sites = 52.
[b] Total traps = 520.

Table 2
Field host range of *Thelohania solenopsae* and *Vairimorpha invictae*: nest sampling

Ants sampled	No. (%) of sampling sites per species[a]			No. (%) of samples per species[b]		
	Total	With *T. solenopsae*	With *V. invictae*	Total	With *T. solenopsae*	With *V. invictae*
Solenopsis richteri	13	6 (46)	1 (15)	261	38 (15)	6 (2)
Solenopsis invicta	34	14 (41)	14 (41)	255	28 (11)	50 (20)
Acromyrmex sp.	19	0	0	21	0	0
Acromyrmex lundi	11	0	0	20	0	0
Solenopsis macdonaghi	6	4 (67)	3 (50)	19	11 (58)	5 (26)
Acromyrmex ambiguus	4	0	0	4	0	0
Pheidole sp.	4	0	0	4	0	0
Camponotus sp.	1	0	0	1	0	0

[a] Total sites = 90.
[b] Total samples (colonies) = 585.

T. solenopsae only infected *S. invicta*, *S. richteri*, and *S. macdonaghi*, at 41–67% of the sites and in 11–58% of the colonies examined. In addition, *V. invictae* infected the same hosts at 15–50% of the sites and in 2–26% of the colonies examined (Table 2).

At *S. invicta* sites, *T. solenopsae* and *V. invictae* occurred in the same percentage (41%). However, the percentage of *S. invicta* colonies infected with *V. invictae* was 20% and with *T. solenopsae* only 11% (Table 2). In contrast, at *S. richteri* sites, *T. solenopsae* occurred at 46% of the sites and *V. invictae* only at 15% of the sites. *T. solenopsae* infected 15% of the *S. richteri* colonies and *V. invictae* only 2%. In regards to *S. macdonaghi*, *T. solenopsae* occurred at 67% of the sites and infected 58% of the colonies, and *V. invictae* occurred at 50% of the sites and infected 26% of the colonies, but the sample size was limited to six sites and 19 colonies. The percentages are not comparable with those found in *S. invicta* and *S. richteri*. That is, the proportion of *S. richteri* and *S. invicta* sites with *T. solenopsae* was similar (46% and 41%, respectively; $\chi^2 = 0.095$; $df = 1$; $P > 0.1$). The proportion of *S. richteri* and *S. invicta* colonies infected with *T. solenopsae* was similar (15 and 11%, respectively; $\chi^2 = 1.481$; $df = 1$; $P > 0.1$). In contrast, the proportion of *S. invicta* sites with *V. invictae* was higher than *S. richteri* sites (41 versus 15%; $\chi^2 = 4.852$; $df = 1$; $P < 0.05$). Also, the proportion *S. invicta* colonies infected with *V. invictae* was higher than *S. richteri* colonies (20 versus 2%; $\chi^2 = 39.942$; $df = 1$; $P < 0.005$). These data suggest a possible differential susceptibility of *S. invicta* to *V. invictae* and should be confirmed with appropriate laboratory tests. These tests should be conducted when the mechanisms of transmission of *V. invictae* are known.

The information reported here regarding *T. solenopsae* is consistent with the surveys conducted in South America by Allen and Buren (1974), Allen and Silveira Guido (1974), Allen and Knell (1980), Jouvenaz (1983), Jouvenaz et al. (1980), Wojcik et al. (1987), and Briano et al. (1995), where they found *T. solenopsae* infecting colonies of *S. invicta*, *S. richteri*, *S. saevissima*, *S. quinquecuspis*, *S. macdonaghi*, and *S. blumi*. In the United States, infections of *T. solenopsae* have not been detected in field-collected *S. geminata* (Fabricius) (Williams et al., 1998) and *S. xyloni* (MacCook) (D.H.O., unpublished data). In addition, this microsporidium was not detected in other ants species in the genera *Dorymyrmex*, *Pheidole*, *Camponotus*, *Trachymyrmex*, and *Brachymyrmex* (Williams et al., 1998). Records of field hosts for *V. invictae* are restricted to *S. invicta* (Jouvenaz and Ellis, 1986; Jouvenaz et al., 1980; Wojcik et al., 1987) and *S. richteri* (Briano et al., 1995). This is the first report of *V. invictae* infecting field colonies of *S. macdonaghi*.

These results strongly suggest that the host ranges of *T. solenopsae* and *V. invictae* are restricted to closely related species within the genus *Solenopsis*. It appears that *T. solenopsae* infects *S. invicta* and *S. richteri* equally but *V. invictae* may be more adapted to infect *S. invicta*.

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