# Natural Occurrence and Laboratory Studies of the Fire Ant Pathogen *Vairimorpha invictae* (Microsporida: Burenellidae) in Argentina

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**ABSTRACT** We surveyed 154 sites in north-central Argentina and sampled 2,528 fire ant colonies for the presence and intracolonial prevalence of the microsporidium, *Vairimorpha invictae* Jouvenaz & Ellis, in the red imported fire ant, *Solenopsis invicta* Buren. The concentrations of meiospores and binucleate spores were quantified in workers and sexuals; and the occurrence and intracolonial prevalence of dual infections with *Thelohania solenopsae* Knell, Allen & Hazard were studied. To study the effect of *V. invictae* in infected colonies of *S. invicta*, we compared the proportion of infected living workers to the proportion of infected dead workers, and compared the survival of uninfected and infected workers. *V. invictae* occurred at 13% of the sites and 2.3% of the colonies. At times, the disease reached epizootic levels in certain areas. We found vegetative stages in 4.8–52.3% of eggs, larvae, pupae, and queens, meiospores in 4–56.3% of pupae and mature stages, and binucleate spores in 9.5–63% of all life stages, except eggs. Evidence for transovarial transmission is provided. The percentage of sexual males infected was significantly higher than that of sexual females (44.9 versus 15.9%, respectively). Dual infections (*V. invictae* + *T. solenopsae*) occurred in 0.24% of the colonies. *V. invictae* was present in 9.3% of living workers and in 56.7% of dead workers. Mortality rates of workers from *Vairimorpha*-infected colonies were higher than those of workers from uninfected colonies. Survival times of infected workers were 18.8–31.7% less than those of uninfected workers. The studies reported here contribute to the evaluation of *V. invictae* for use as a classical biological control agent against the red imported fire ant in the United States.

**KEY WORDS** *Vairimorpha invictae*, *Thelohania solenopsae*, *Solenopsis invicta*, intracolonial prevalence, dual infections, biological control

THE MICROSPORIDIUM *Vairimorpha invictae* Jouvenaz & Ellis (1986) (Microsporida: Burenellidae) was originally discovered in the red imported fire ant, *Solenopsis invicta* Buren, in Mato Grosso, Brazil. It was later recovered from other species of fire ants such as *S. richteri* Forel (Jouvenaz et al. 1980, Wojcik et al. 1987, Briano et al. 1995b) and *S. macdonaghi* Santschi (Briano et al. 2002) in Argentina. *V. invictae* is an obligate, intracellular, and dimorphic microorganism that produces distinct mononucleate meiospores and binucleate spores that develop sequentially in larvae, pupae, workers, sexuals, and queens. According to Jouvenaz and Ellis (1986), mature binucleate spores are present in late larvae and pupae as well as adult ants; mature meiospore production is delayed until late in pupal life with most sporulation occurring in adult ants. Thus, binucleate spore production always occurs first in immatures followed by meiospore production in adults. Efforts to transmit *V. invictae* per os and by introduction of infected brood into a healthy colony have been unsuccessful (Jouvenaz 1983; Jouvenaz and Ellis 1986; J.A.B., unpublished data).

*Vairimorpha invictae*, along with other fire ant pathogens, has been considered a potential candidate for biological control of imported fire ants in the United States since the mid-1980s. Very little is known, however, about its pathobiology, ecology, and epizootiology, and nothing has been published about its impact on fire ants. In a long-term study of microsporidia infecting fire ants in field plots in Argentina, the occurrence of *V. invictae* was rare (Briano et al. 1995a). Laboratory observations in 1990 found that colonies of *S. richteri* heavily infected with *V. invictae* and *Thelohania solenopsae* Knell, Allen & Hazard (1977) showed high mortality rates (J.A.B., unpublished data). The deleterious effects of *V. invictae* and dual infections in the field have never been documented mainly because of the low occurrence of *V. invictae* and the extremely low prevalence of both infections in an individual colony. The need for extensive surveys to find fire ant populations infected with *V. invictae* therefore becomes a top priority. The objectives of this work were as follows: (1) to extend the explorations and intensify surveys for *V. invictae* in native fire ant populations, (2) to select field sites where infections occur to initiate long-term ecological

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studies, and (3) to document the detrimental effects of *V. invictae* on fire ants under laboratory conditions.

## Materials and Methods

**Surveys.** We surveyed north-central Argentina, including the provinces of Buenos Aires, Santa Fe, Córdoba, Tucumán, Chaco, Corrientes, and Entre Rios from 1991 to 1999. From 1991 to 1994, the goal of the surveys was to search for natural enemies of fire ants, primarily *S. richteri* in Buenos Aires Province. The top priority after 1994 was to locate field populations of *S. invicta* and *S. richteri* infected with *V. invictae* as well as colonies infected with *T. solenopsae.* After the discovery of *V. invictae* in several sites in 1999, collections were intensified in those areas.

We collected samples in 154 sites, most of them selected every 10–100 km along the roadsides of the areas surveyed. In addition, some areas surveyed in Buenos Aires Province were those reported by Briano et al. (1995b) as *Vairimorpha*-infected sites. We sampled a total of 2,528 fire ant colonies, mainly *S. richteri* ($n$ = 1,996 in Buenos Aires and Entre Rios) and *S. invicta* ($n$ = 467 in Santa Fe, Chaco, and Corrientes), but also *S. macdonaghi* ($n$ = 50 in Entre Rios and Santa Fe) and *Solenopsis* spp. ($n$ = 15 in Córdoba and Tucumán). In each site, we located the fire ant colonies and sampled workers by introducing a 7-ml vial into the mounds. The vials were dusted with talc to prevent the ants from escaping. Most samples contained 200–4,000 workers and were preserved in 70% ethanol for transport to the laboratory. Once in the laboratory, we macerated the workers and examined a drop of the aqueous extract under a phase-contrast microscope (400×) for the presence of spores of *V. invictae* and *T. solenopsae.* The sensitivity of this procedure was successfully tested by Jouvenaz et al. (1977). Once located, most of the *Vairimorpha*-infected sites were repeatedly sampled until April 2001 to confirm the persistence of this microsporidium in the field.

**Intracolonial Prevalence.** We studied the intracolonial prevalence of *V. invictae* in 22 infected colonies of *S. invicta* collected at Recreo, Nelson, San Justo, M. Escalada, and Vera, Santa Fe Province, in July (winter) and November (spring) 1999, May (fall) 2000, and April (fall) 2001. After detecting the infection in the field using the methodology described above, we excavated the colonies and placed them in talc-dusted 10-liter buckets to prevent the ants from escaping. In the laboratory, we separated the colonies from the soil by flotation (Banks et al. 1981) and maintained them in plastic trays (40 by 30 by 15 cm) coated with Fluon. The presence of multiple queens (polygyny) was confirmed in three of the colonies.

We randomly selected individuals of the different castes and stages from each colony, then macerated and examined them individually with a phase-contrast microscope (400×) for the presence of spores of *V. invictae.* We detected the vegetative stages of *V. invictae* by microscopic examination (immersion oil; 1,000×) of Giemsa-stained smears of whole individuals (larvae, pupae, and queens). Eggs were examined in groups of ≈50 due to their small size.

The concentrations of meiospores and binucleate spores were quantified in minor workers ($n$ = 13) and major workers ($n$ = 19) selected at random from five infected colonies, and in sexual females ($n$ = 10) and sexual males ($n$ = 11) from four colonies. Individual ants were macerated in 1 ml of water with a tissue grinder. Meiospores and binucleate spores present in an aliquot of the suspension were counted using a hemacytometer with a phase-contrast microscope (400x).

The intracolonial prevalence of dual infections with *V. invictae* and *T. solenopsae* was studied in two polygyne colonies of *S. invicta.* We individually macerated a total of 51 worker pupae, 65 adult workers, 27 sexual females and 10 mated queens. No sexual males were found in these colonies. Additionally, the intracolonial prevalence of dual infections was determined in the worker caste in four colonies of *S. richteri* and *S. macdonaghi* by individually macerating 121 workers. Disease diagnosis was done as above. *Vairimorpha invictae* and *T. solenopsae* are morphologically very distinctive. Binucleate spores of *V. invictae* are bacilliform and measure 3.1 ± 0.3 × 11.2 ± 3.4 μm while those of *T. solenopsae* are elongate oval in shape and measure 1.8 ± 0.2 × 4.9 ± 0.6 μm. The meiospores of *V. invictae* are ovoid and measure 4.2 ± 0.7 × 6.3 ± 0.2 μm while those of *T. solenopsae* are pyriform in shape and measure 1.9 ± 0.2 × 3.3 ± 0.5 μm. In addition, the two spore types occur in very different ratios (1.2 meiospores/binucleate spore of *V. invictae* and 57 meiospores/binucleate spore of *T. solenopsae*).

**Detrimental Effect in Workers.** To study the effect of *V. invictae* in *S. invicta,* we conducted one observational and one experimental test under laboratory conditions.

*(1) Prevalence of V. invictae in Living and Dead Workers.* We compared the proportion of living workers naturally infected with *V. invictae* with the proportion of dead workers (host cadavers) with spores. One week after the excavation and 2 d after separation of the colonies from the soil by flotation, we randomly selected 146 workers from six *V. invictae*-infected *S. invicta* colonies collected in Santa Fe Province. Eighty-two workers were living, whereas 64 were dead workers collected from the trash piles of the host colonies. The ants were preserved in 70% ethanol until examination for the infection. The number of living and dead workers infected with *V. invictae* was recorded and proportions compared.

*(2) Survival Test.* In February 2001, we collected six colonies of *S. invicta* in the area of San Justo, Santa Fe, and separated them from the soil in the laboratory by flotation. Four of the colonies were infected with *V. invictae* with a mean intracolonial prevalence in workers of 30.8% (range, 5–50%); no microsporidia infections were found in the other two colonies. Immediately after flotation, we randomly selected 30 major workers (mean head width 1.23 ± 0.08 mm; range, 1.07–1.35 mm) from each infected and uninfected colony and confined them in 160 ml-plastic Fluon-

**Table 1. Surveys for *V. invictae* in Argentina**

| Year | No. of sites infected/surveyed[a] | % of colonies infected (no. of colonies infected/checked) |
|---|---|---|
| 1991 | 0/2 | 0 (0/145) |
| 1992 | 1/2 | 2.7 (3/111) |
| 1993 | 1/2 | 4.3 (2/46) |
| 1994 | 2/3 | 2.3 (2/88) |
| 1995 | 0/8 | 0 (0/254) |
| 1996 | 0/34 | 0 (0/173) |
| 1997 | 4/14 | 1.7 (5/300) |
| 1998 | 1/61 | 0.2 (1/418) |
| 1999 | 14/63 | 4.5 (45/993) |
| Total | 20/154 | 2.3 (58/2,528) |

[a] Several sites were sampled more than once, so totals are lower than the sum of the column.

coated containers. The test was conducted in the laboratory at 28.3 ± 4.9°C (range, 19–36°C). For practical reasons (to shorten the duration of the test), the infected and uninfected workers were starved until death. A piece of moistened cotton was always present in the containers as a source of moisture.

We removed the dead workers daily, individually macerated each of them in a drop of water placed on a microscope slide, and examined as above to confirm if they were infected or uninfected. We compared the mortality rates of workers from infected and uninfected colonies. Also, we compared the survival of infected workers, uninfected workers from the same infected colonies, and uninfected workers from uninfected colonies.

**Statistical Analysis.** The concentration of *V. invictae* spores in workers and sexuals, the prevalence of *V. invictae* in living and dead workers, the survival of workers, and the prevalence of meiospores and binucleate spores in workers were analyzed with 2-sample *t*-tests. Percentages were transformed (angular transformation). The chi-square test was used to compare the proportion of males and females infected. The log rank method, an application of the Mantel-Haenszel method (Mantel and Haenszel 1959), was used to compare mortality rates of workers. Minitab Statistical Software (1991) and Systat Statistics (1996) were used to run the tests. Means are reported ± one standard deviation.

## Results and Discussion

**Surveys.** From 1991 to 1998, we found *V. invictae* at 6.6% of the sites (6/91) and in only 0.8% of the colonies surveyed (13/1,535); in 1999 we found it at 22.2% of the sites (14/63) and in 4.5% of the colonies (45/993). The overall natural prevalence of *V. invictae* infecting native fire ants in Argentina was moderately low; the pathogen was found at 13% of the sites (20/154) and in 2.3% of the colonies surveyed (58/2,528) (Table 1). The 20 *Vairimorpha*-infected sites, the proportion of colonies infected, and the primary host species are detailed in Table 2.

The *S. invicta* sites with the highest infection rates were found along 260 km of Route 11, between the towns of Recreo (km 490.8) and Malabrigo (km 750), Santa Fe province. In some of these sites and, at times, *V. invictae* reached epizootic levels [Epizootic = unusually large number of cases of disease in a host population (Steinhaus and Martignoni 1962, Tanada and Kaya 1993)]. Arbitrarily, we considered an epizootic level when at least 20% of the colonies were infected with a minimum sample size of 10 colonies. The sites along Route 11 where epizootic levels occurred were as follows: km 490.8 (April 1999), km 560 (April 1999, November 1999, February 2000, May 2000), km 578.2 (February 2001), and km 600 (April 1999) (Table 2). Some of these sites were selected for long-term ecological studies that are currently in progress. Periodic surveys of these sites revealed that 5.3–50% of the colonies was infected. Particularly high infection rates were found near San Justo (Rt. 11, km 560) where, during 2 yr of sampling, the proportion of infected colonies ranged from 6.7 to 45.5% (Table 2).

*Vairimorpha invictae* has shown a wide variation in prevalence in many sites surveyed. It is interesting that repeated samplings in places such as Saladillo, Isla Talavera, San Eladio, and Navarro detected no *V. invictae* (Table 2). For instance, Briano et al. (1995b) had reported the presence of 60% of fire ant colonies infected with *V. invictae* in Isla Talavera in April 1988. However, samplings conducted in the same area on several occasions since 1993 showed a continuous decline in the infection rates. *V. invictae* has not been detected in that area since September 1997. The reason for this behavior is unclear; however, it might indicate the following: (1) a high mortality of infected workers or entire fire ant colonies before the infection can be transmitted to neighboring host colonies, (2) a low ability of *V. invictae* to be transmitted and disseminated within a fire ant population (despite the high numbers of spores produced), and (3) a low ability of *V. invictae* to propagate itself indefinitely in the host colony. Maddox et al. (1998) reported that, for *Vairimorpha*-type infections in lepidopteran hosts, death of individuals occurred before the pathogen could produce the infectious spores necessary to infect other hosts. Also, they reported that the integument of dead host larvae infected does not rupture and, consequently, broad dissemination of infective spores does not usually occur. Since *Vairimorpha* infections from lepidopterans and fire ants are not related (Moser 1995), the effectiveness of the horizontal transmission of *V. invictae* should be investigated and the natural stability, environmental sensitivity, and persistence of the spores need to be determined. The long-term field studies that are in progress in Santa Fe province will provide, among other things, information regarding the persistence of the spores in the field.

The overall occurrence of *V. invictae* reported here for *S. invicta* and *S. richteri* (13% of the sites and 2.3% of the colonies) is higher than the one reported by Briano et al. (1995b) for *S. richteri* (5% of the sites and 1% of the colonies) but much lower than the occurrence of *T. solenopsae* in *S. richteri* (25% of the sites and 8% of the colonies). The occurrence of *V. invictae* is

**Table 2. Twenty sites in Argentina where fire ants were found infected with *V. invictae* (1991–2001)**

| Province | Site | Date | % of colonies infected (no. infected/checked) | Main host |
|---|---|---|---|---|
| Buenos Aires | Rt. 205 km 180, Saladillo (35° 38′ S, 59° 46′ W) | Oct 1992 | 0 (0/21) | *S. richteri* |
| | | Nov 1992 | 6.0 (3/50) | *S. richteri* |
| | | Sep 1994 | 10.0 (1/10) | *S. richteri* |
| | | Mar 1995 | 0 (0/7) | *S. richteri* |
| | | Feb 1996 | 0 (0/7) | *S. richteri* |
| | | Sep 1997 | 0 (0/10) | *S. richteri* |
| | | Nov 1997 | 0 (0/11) | *S. richteri* |
| | | Apr 1998 | 0 (0/18) | *S. richteri* |
| | | Jun 1998 | 0 (0/6) | *S. richteri* |
| | Rt. 12 km 91, Isla Talavera (34° 04′ S, 58° 59′ W) | Apr 1993 | 10.0 (2/20) | *S. richteri* |
| | | Jun 1994 | 6.3 (1/16) | *S. richteri* |
| | | Dec 1995 | 0 (0/10) | *S. richteri* |
| | | May 1997 | 0 (0/12) | *S. richteri* |
| | | Sep 1997 | 4.8 (1/21) | *S. richteri* |
| | | Aug 1998 | 0 (0/10) | *S. richteri* |
| | | Apr 1999 | 0 (0/83) | *S. richteri* |
| | Rt. 47 km 25, San Eladio (34° 45′ S, 59° 10′ W) | May 1997 | 4.5 (1/22) | *S. richteri* |
| | | May 1997 | 0 (0/26) | *S. richteri* |
| | | Aug 1997 | 0 (0/21) | *S. richteri* |
| | | Sep 1998 | 0 (0/28) | *S. richteri* |
| | Rt. 41, 3 km N of Navarro (34° 59′ S, 59° 18′ W) | May 1997 | 25.0 (2/8) | *S. richteri* |
| | | Jul 1997 | 0 (0/6) | *S. richteri* |
| | | Jul 1998 | 0 (0/9) | *S. richteri* |
| | El Toro Ranch, Las Flores (36° 06′ S, 58° 60′ W) | Dec 1991 | 0 (0/145) | *S. richteri* |
| | | May 1994 | 0 (0/62) | *S. richteri* |
| | | Jul 1995 | 0 (0/88) | *S. richteri* |
| | | Oct 1995 | 0 (0/99) | *S. richteri* |
| | | Jul 1996 | 0 (0/30) | *S. richteri* |
| | | Oct 1997 | 0 (0/67) | *S. richteri* |
| | | Mar 1999 | 1.2 (1/82) | *S. richteri* |
| | | Aug 1999 | 3.0 (4/135) | *S. richteri* |
| Santa Fe | Rt. Rosario-S. Fe junction. Rt. to Carrizales (32° 29′ S, 60° 54′ W) | Apr 1999 | 100.0 (1/1) | *S. invicta* |
| | Rt. 11 km 490.8 (31° 26′ S, 60° 45′ W) | Apr 1999 | 29.2 (7/24) | *S. invicta* |
| | | Jul 1999 | 8.6 (3/35) | *S. invicta* |
| | | Feb 2000 | 18.8 (3/16) | *S. invicta* |
| | Rt. 11 km 537.5 (31° 03′ S, 60° 43′ W) | Apr 1999 | 6.3 (1/16) | *S. invicta* |
| | | Feb 2001 | 11.1 (1/9) | *S. invicta* |
| | Rt. 11 km 560, San Justo[a] (30° 51′ S, 60° 36′ W) | Apr 1999 | 34.5 (10/29) | *S. invicta* |
| | | Nov 1999 | 20.0 (3/15) | *S. invicta* |
| | | Feb 2000 | 28.9 (11/38) | *S. invicta* |
| | | May 2000 | 45.5 (10/22) | *S. invicta* |
| | | Oct 2000 | 6.7 (1/15) | *S. invicta* |
| | | Apr 2001 | 18.7 (14/75) | *S. invicta* |
| | Rt. 11 km 578.2 (30° 42′ S, 60° 31′ W) | Apr 1999 | 5.9 (1/17) | *S. invicta* |
| | | Feb 2001 | 27.3 (3/11) | *S. invicta* |
| | Rt. 11 km 600 (30° 26′ S, 60° 25′ W) | Apr 1999 | 22.9 (8/35) | *S. invicta* |
| | Rt. 11 km 728 (29° 26′ S, 60° 09′ W) | Feb 2001 | 20.0 (1/5) | *S. invicta* |
| | Rt. 11 km 728.5 (29° 26′ S, 60° 08′ W) | Feb 2001 | 14.3 (1/7) | *S. invicta* |
| | Rt. 11 km 729 (29° 26′ S, 60° 07′ W) | Apr 1999 | 8.7 (2/23) | *S. invicta* |
| | | Feb 2001 | 50.0 (2/4) | *S. invicta* |
| | Rt. 11 km 750 (29° 21′ S, 59° 57′ W) | Apr 1999 | 5.3 (1/19) | *S. invicta* |
| Chaco | Rt. 11 km 997 (27° 27′ S, 59° 02′ W) | Apr 1999 | 9.1 (1/11) | *S. invicta* |
| Corrientes | Rt. 12 km 919.5 (28° 17′ S, 58° 51′ W) | Apr 1999 | 7.7 (2/26) | *S. invicta* |
| | Rt. 12 km 820 (28° 55′ S, 59° 05′ W) | Apr 1999 | 7.7 (2/26) | *S. invicta* |
| Entre Rios | Rt. 12 km 135 (33° 00′ S, 59° 29′ W) | May 1997 | 4.0 (1/25) | *S. richteri* |
| | Rt. 12 km 189[a] (33° 19′ S, 59° 11′ W) | Apr 1999 | 18.2 (4/22) | *S. richteri* *S. macdonaghi* |

[a] Sites with colonies with dual infections (*V. invictae* + *T. solenopsae*).

slightly lower than that reported by Jouvenaz et al. (1980) for *S. invicta* in Brazil (as "undescribed microsporidium" in 2.8–5.4% of the colonies). The natural occurrence of *V. invictae* is similar to the prevalence of other microsporidia in very different host-pathogens ecosystems in the United States, such as *Vairimorpha necatrix* Kramer infecting lepidopteran larvae (Maddox et al. 1988), *Nosema locustae* Canning infecting grasshoppers and crickets (Henry and Oma 1981) and *Vavraia culicis* Weiser infecting mosquitoes (Brooks 1988).

**Intracolonial Prevalence.** The presence of *V. invictae* was widely distributed among the individuals of infected colonies of *S. invicta.* Vegetative stages and/or spores were frequently present in almost every caste or stage of the host (Table 3).

**Table 3. Intracolonial prevalence of *V. invictae* in *S. invicta***

| Caste or stage | No. of individuals/colonies examined | % of individuals infected/colony mean ± SD (range) | | |
|---|---|---|---|---|
| | | Vegetative stages | Meiospores | Binucleate spores |
| Eggs | 515/3 | Yes | 0 | 0 |
| Larvae I-II-III | 87/3 | 16.7 ± 28.9 (0–50) | 0 | 13.3 ± 23.1 (0–40) |
| Larvae IV | 30/3 | 20.0 ± 10.0 (10–30) | 0 | 60.0 ± 10.0 (50–70) |
| Pupae | 63/3 | 52.3 ± 45.3 (0–80) | 4.0 ± 5.3 (0–10) | 63.0 ± 28.6 (30–80) |
| Minor workers | 46/8 | — | 56.3 ± 39.3 (0–100) | 58.7 ± 37.2 (0–100) |
| Major workers | 53/9 | — | 41.2 ± 34.6 (0–100) | 44.6 ± 35.2 (10–100) |
| Sexual females | 154/9 | — | 15.9 ± 20.8 (0–60) | 15.9 ± 20.8 (0–60) |
| Sexual males | 100/6 | — | 44.9 ± 34.9 (13–100) | 44.9 ± 34.9 (13–100) |
| Queens | 12/3 | 4.8 ± 8.3 (0–14) | 4.8 ± 8.3 (0–14) | 9.5 ± 16.5 (0–29) |

We found vegetative stages of *V. invictae* in larvae, pupae, and mated queens, from 4.8 to 52.3% of the individuals examined per colony (Table 3). We also detected several vegetative stages (meronts and stages of binucleate spore sporogony) in some eggs, providing evidence for the transovarial (vertical) transmission of *V. invictae* in *S. invicta.* The importance of vertical transmission in the life cycle of *V. invictae* is uncertain because of the low prevalence of the infection found in eggs. This low prevalence would be in accordance with the low prevalence of *V. invictae* found in mated queens (2 infected/12 checked). Higher numbers of infected queens are needed to determine the actual role of vertical transmission. Transovarial transmission was also reported for *T. solenopsae* in *S. richteri* (Briano et al. 1996).

Vegetative stages in eggs, prefourth instars, and mated queens of *S. invicta* were scarce and difficult to observe, consequently, the percentages of infection reported here might be conservative. In contrast, vegetative stages were numerous and easily detected in fourth instars and pupae. The presence of vegetative stages of *V. invictae* in *S. invicta* is similar to the presence of vegetative stages of *T. solenopsae* reported by Briano et al. (1996) for *S. richteri.*

We found meiospores of *V. invictae* in pupae and all mature stages of *S. invicta* from 4–56.3% of the individuals examined per infected colony (Table 3). The mean number of meiospores in infected major workers was $6.4 \times 10^4 \pm 12.8 \times 10^4$ (range, $2.5 \times 10^2$-$5.4 \times 10^5$) and in infected minor workers was $1.2 \times 10^4 \pm 1.4 \times 10^4$ (range, $2.5 \times 10^2$-$4.3 \times 10^4$). This difference was not statistically significant because of the high variability in the number of meiospores as indicated by the standard deviations ($t = 1.44$, df = 30, $P < 0.16$). The concentration of meiospores of *V. invictae* in *S. invicta* workers was similar to the concentration of meiospores of *T. solenopsae* in workers of *S. richteri* (Briano et al. 1996).

On average, we found binucleate spores of *V. invictae* in 9.5–63% of the individuals in all stages and castes, except eggs (Table 3). The mean number of binucleate spores in infected major workers was much higher than in minor workers. In major workers it was $1.6 \times 10^4 \pm 1.9 \times 10^4$ (range, $2.5 \times 10^2$-$6.5 \times 10^4$) and in minor workers was $3.2 \times 10^3 \pm 3.6 \times 10^3$ (range, $2.5 \times 10^2$-$1.2 \times 10^4$) ($t = 2.4$, df = 30, $P = 0.023$).

The presence of binucleate spores in early larval stages of *S. invicta* and the presence of meiospores in pupae were not reported by Jouvenaz and Ellis (1986) when describing *V. invictae,* but the description was based on only one colony of *S. invicta.* Spores of *V. invictae* were not found by Moser (1995) in larval stages of fire ants. Binucleate spores of *V. invictae* occurred more frequently in individuals of *S. invicta* (9.5–63.0%; Table 3) than did binucleate spores of *T. solenopsae* in *S. richteri.* The latter were found only in workers (0.7–6.4%; Briano et al. 1996).

Binucleate spores might play an important role in the intracolony and intercolony transmission of *V. invictae* in *S. invicta,* as it does the related microsporidium *Burenella dimorpha* Jouvenaz & Hazard (Microsporida: Burenellidae) in the tropical fire ant, *Solenopsis geminata* (Fabricius) (Jouvenaz et al. 1981). However, several recent attempts to transmit this disease artificially (per os) were unsuccessful (J.A.B., unpublished data). Jouvenaz and Lofgren (1984) reported that the predominant spore for *B. dimorpha* was the binucleate spore and that the production of meiospores was temperature dependant. Whether or not the production of meiospores in *V. invictae* depends on temperature is unknown.

The number of *V. invictae* meiospores in workers was not significantly different from the number of binucleate spores ($t = 1.77$, df = 62, $P = 0.082$) due primarily to high variability. Additionally, meiospores and binucleate spores were present in similar proportion in minor and major workers (i.e., meiospores: 56.3 versus 41.2%, respectively; $t = -0.79$, df = 15, $P = 0.44$; binucleate spores: 58.7 versus 44.6%, respectively; $t = -0.70$, df = 15, $P = 0.5$).

In *S. invicta* sexuals infected with *V. invictae*, the mean percentage of males infected per colony was higher than that of females (44.9 versus 15.9%, respectively; $t = -2.24$, df = 13, $P = 0.043$). However, the concentration of spores in both sexes was similar. The mean number of meiospores per male was $1.1 \times 10^6 \pm 1 \times 10^6$ (range, $1.2 \times 10^4$-$3.3 \times 10^6$) and per female it was $3.6 \times 10^6 \pm 4.8 \times 10^6$ (range, $1 \times 10^3$-$14 \times 10^6$) ($t = 1.54$, df = 9, $P = 0.16$). The mean number of binucleate spores per male was $2.5 \times 10^5 \pm 1.7 \times 10^5$ (range, $5 \times 10^4$-$5.1 \times 10^5$) and per female it was $1.2 \times 10^6 \pm 2.8 \times 10^6$ (range, 0–$9.2 \times 10^6$) ($t = 1.05$, df = 9, $P = 0.32$).

It is interesting that we found only one colony (probably monogyne) of *S. invicta* infected with *V. invictae* in which both sexual females and males were abundant. The intracolonial prevalence of the infection in both sexes was consistently significantly different. The percentage of males infected was 59.5% (22/37) and in females it was 5.6% (2/36) ($\chi^2 = 24.025$, df = 1, $P < 0.001$). In workers of the same colony, the percentage of infection was 26.7% (8/30) and the only queen found was uninfected. The biological implications of the different infection rates in the sexuals are unknown. Microscopic examination of males showed the presence of sperm in both uninfected and infected individuals. The viability of the sperm was not confirmed. According to Issi (1986), microsporidia may affect the reproductive abilities of males and the sex ratio in heavily infected insect populations.

The prevalence of *V. invictae* in mated queens (4.8–9.5%) seemed lower than in unmated females (15.9%) of *S. invicta.* Statistical tests were not run because of sample size limitations in individuals and colonies (Table 3). Further investigation is needed to determine whether there is an increased mortality rate of infected females that died before they were inseminated or before colony foundation.

The wide occurrence of various life stages of *V. invictae* in different life stages of *S. invicta* suggests that the pathogen can infect several life stages of the host (Harper 1987) and may indicate that *V. invictae* is an important disease causing mortality in *S. invicta.* According to Tanada and Kaya (1993), the transstadial prevalence is typical of those pathogens producing chronic infections in insects and could indicate that the microsporidium is appropriate for classical biological control programs.

**Dual Infections.** The microsporidium *T. solenopsae* occurred much more frequently than *V. invictae* in surveys conducted since 1991. We found the former at 42.9% of the sites (66/154) and in 11.9% of the fire ant colonies (300/2,528). The occurrence of dual infections was extremely low. We detected both *V. invictae* and *T. solenopsae* at 7.8% of the sites (12/154) but fire ant colonies with dual infections were found only at 1.3% of the sites (2/154). We found only 0.24% of the colonies (6/2,528) with dual infections: (1) Two *S. invicta* colonies at Rt. 11 km 560, San Justo, Santa Fe and (2) four *S. richteri* and *S. macdonaghi* colonies at Rt. 12 km 189, Entre Rios Province.

**Table 4. Intracolonial prevalence of *V. invictae* and *T. solenopsae* in the two dual-infected colonies of *S. invicta***

| Caste or stage (*n*) | % of individuals infected mean ± SD | | |
|---|---|---|---|
| | *V. invictae* | *T. solenopsae* | Dual |
| Pupae (51) | 85.1 ± 7.2 | 22.0 ± 31.0 | 22.0 ± 31.0 |
| Workers (65) | 33.9 ± 5.5 | 45.2 ± 13.8 | 14.1 ± 1.2 |
| Sexual females (27) | 10.8 ± 2.4 | 18.2 ± 25.7 | 4.5 ± 6.4 |
| Queens (10) | 14.3 ± 20.2 | 0 | 0 |

The occurrence of sites with both infections (7.8%) was similar to the probability of finding *V. invictae* (13%) and *T. solenopsae* (42.9%) in the same site ($0.13 \times 0.429 = 0.056 = 5.6\%$). The percentage of field colonies with dual infections (0.24%) was almost identical to the probability of finding *V. invictae* (2.3%) and *T. solenopsae* (11.9%) infecting simultaneously the same colony ($0.023 \times 0.119 = 0.0027 = 0.27\%$). This suggests the lack of an enhanced pathogenicity of the two microsporidia infecting the same fire ant colony. If a synergistic interaction existed, the actual proportion of dual infected colonies in the field would be lower than the probability predictions. However, mixed infections must be studied using appropriate laboratory tests to confirm the lack of synergistic or antagonistic effects. The presence of dual infections with the neogregarine *Mattesia geminata* Jouvenaz & Anthony and the microsporidium *Burenella dimorpha* was also observed occasionally in pupae of the tropical fire ant *Solenopsis geminata* (Jouvenaz and Anthony 1979).

The extremely low occurrence of dual infections is consistent with surveys conducted by Briano et al. (1995b). These authors found *V. invictae* and *T. solenopsae* on several occasions infesting the same sites, mostly *S. richteri* sites, but never infecting the same colonies. Dual infections appear to be more common in *S. invicta* sites because *V. invictae* was more frequent in *S. invicta* than in *S. richteri* (Briano et al. 2002).

The intracolonial prevalence of *V. invictae* and *T. solenopsae* in the two dual-infected polygyne colonies of *S. invicta* can be seen in Table 4. We found *V. invictae* in 10.8–85.1%, *T. solenopsae* in 0–45.2% and dual infections in 0–22.0% of the individuals examined. *T. solenopsae*, and, consequently dual infections, were not detected in queens. The workers and sexual females infected with only one of the two microsporidia were more abundant than the workers and sexual females with dual infections. As shown in Table 4, the proportion of dual-infected pupae (22%) was higher than the proportion of dual-infected workers (14.1%) indicating that dual-infected pupae died before eclosing. This could represent and important mortality factor within dual-infected colonies that should be further tested. In the other four colonies of *S. richteri* and *S. macdonaghi*, we found dual infections only in 2.7 ± 2.3% of the workers ($n = 121$). Heavy dual infections with *V. invictae* and *T. solenopsae* were reported by Moser (1995) in individual workers of *S. richteri* originally collected in Argentina.

**Table 5. Prevalence of *V. invictae* in living and dead workers of *S. invicta***

| Colony no. | Living workers | | Dead workers | |
|---|---|---|---|---|
| | No. of workers examined | No. (%) of workers with spores | No. of workers examined | No. (%) of workers with spores |
| 1 | 10 | 1 (10) | 10 | 2 (20) |
| 2 | 10 | 1 (10) | 10 | 8 (80) |
| 3 | 10 | 2 (20) | 10 | 0 |
| 4 | 10 | 0 | 10 | 9 (90) |
| 5 | 30 | 1 (3.3) | 12 | 8 (66.7) |
| 6 | 12 | 2 (12.5) | 12 | 10 (83.3) |
| Total (mean ± SD) | 82 | 7 (9.3 ± 7.0) | 64 | 37 (56.7 ± 37.6) |

**Detrimental Effect in Workers.** *(1) Prevalence of V. invictae in Living and Dead Workers.* In five of the six colonies observed, the infection rate with *V. invictae* in dead workers was much higher than in living workers (2- to 8-fold; Table 5). The mean percentage of infected dead workers was 56.7 ± 37.6% (range, 0–90%), whereas only 9.3 ± 7.0% (range, 0–20%) of the living workers were infected ($t = -2.67$, df = 6, $P = 0.037$). According to Kramer (1970), the survival of microsporidian spores in the host cadavers at room temperature may range from a few weeks to over a year. Thus, some spore dessication could have occurred and the actual proportion of host cadavers containing spores could have been even higher. This suggests a high virulence of *V. invictae* in the worker stage of stressed laboratory colonies and would be consistent with the apparent low persistence of infected colonies in the field. However, whether deaths occurred as a direct effect of microsporidiosis or were associated with secondary infections such as bacterial septicemia was not determined. A high degree of virulence was reported for the congeneric *Vairimorpha necatrix*, which has been one of the few protozoans considered as a microbial control agent against a wide range of lepidopteran pests in the United States (Maddox et al. 1988, Lacey and Goettel 1995).

*(2) Survival Test.* Mortality rates of workers of *S. invicta* from colonies naturally infected with *V. invictae* ($n = 4$) were higher than that of workers from uninfected colonies ($n = 2$) (Fig. 1; logrank method, $\chi^2 = 11.516$, df = 1, $P < 0.001$). In infected colonies, mortality of workers began on day 1, whereas in workers from uninfected colonies, it began on day 9. At day 30, mortality of workers from infected colonies had reached 100% while mortality of workers from uninfected colonies was 68.1%. The median lethal time, $LT_{50}$ (time required for 50% of the individuals to die), in workers from infected colonies was 17.7 ± 3.8 d while in workers from uninfected colonies it was 25.2 ± 11.0 d. This difference was not statistically significant because of the small numbers of experimental units (colonies) ($t = -1.357$, df = 4, $P = 0.246$).

The mean survival time for infected workers ($n = 36$) was 13.8 ± 8.8 d (range, 1–27), for uninfected workers ($n = 82$) from the same infected colonies was 17.0 ± 7.2 d (range, 1–30) ($t = 2.086$, df = 116, $P = 0.039$), and for all uninfected workers ($n = 124$) including those from uninfected colonies was 20.2 ± 10.0 d (range, 1–63) ($t = 3.452$, df = 158, $P = 0.001$). This represented an 18.8–31.7% reduction in the survival of infected workers compared with uninfected ones. The reduced survival of workers infected with *V. invictae* is consistent with the increased prevalence of *V. invictae* spores in the host cadavers mentioned above. Reduced longevity is a typical consequence of microsporidiosis in insects (Issi 1986, Tanada and Kaya 1993). Although the age of the workers at the beginning of our study was unknown, the large sample size guarantees meaningful comparisons. The use of field infected workers was the only way to quantify the impact of this pathogen because horizontal transmission of *V. invictae* in the laboratory has not been successful (Jouvenaz and Ellis 1986; J.A.B., unpublished data).

We conclude that the overall occurrence of *V. invictae* infecting fire ants in Argentina is low and discontinuous. However, at times and in certain areas, the infection reached epizootic levels. Field studies are in progress in *S. invicta* areas to determine the long-term ecological impact of *V. invictae* and of dual infections. *V. invictae* showed a high degree of intracolonial prevalence in *S. invicta* and high pathogenicity in stressed workers under laboratory conditions. Evidence for transovarial transmission was provided but its importance in the life cycle of *V. invictae* was not determined. Additional studies are needed to determine the actual pathogenicity of dual infections. These studies

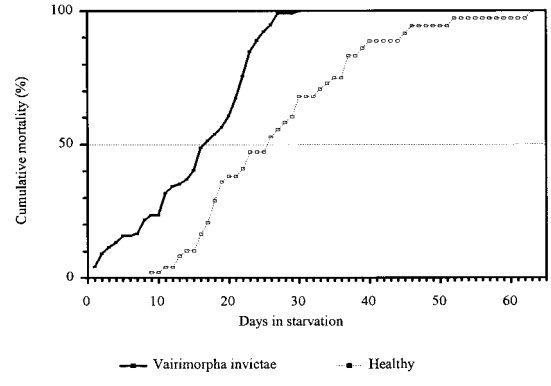


**Fig. 1.** Mortality rate under laboratory conditions of starved *S. invicta* workers infected with *V. invictae* and uninfected.

should be conducted when the mechanisms of transmission and infectivity of both *V. invictae* and *T. solenopsae* are well known. The studies reported here contribute to an evaluation of the potential use of *V. invictae* as a classical biological control agent against the imported fire ants in the United States.

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