Prevalence, Spread, and Effects of the Microsporidium *Thelohania solenopsae* Released into Populations with Different Social Forms of the Red Imported Fire Ant (Hymenoptera: Formicidae)

JAMES R. FUXA,[1, 2] YULIYA Y. SOKOLOVA,[1] MAYNARD L. MILKS,[1] ARTHUR R. RICHTER,[1] DAVID F. WILLIAMS,[3] AND DAVID H. OI[4]

Environ. Entomol. 34(5): 1139–1149 (2005)

ABSTRACT The microsporidium *Thelohania solenopsae* Knell, Allen, and Hazard was released into colonies of red imported fire ants, *Solenopsis invicta* Buren, at four field sites in Louisiana. Social form of the ant affected the establishment of the microsporidium; long-term epizootics developed at two sites with predominantly polygyne populations, whereas the pathogen produced a low disease prevalence before it disappeared or did not infect ants at all at two monogyne sites. This study is the first report of artificial infection of monogyne *S. invicta* field colonies. When the microsporidium became established in mixed monogyne/polygyne ant populations, prevalence rates peaked at >75% in both social forms. In mixed ant populations, the social form suffering higher prevalence of the disease decreased proportionally to the other form, possibly indicating a competitive disadvantage. Host population density or site characteristics may have influenced spread of the disease; the rate of spread was 0.9 m/mo at one epizootic site and reached 9.4 m/mo at the other. There was little seasonal variation in prevalence, which averaged 47% in February, 51% in April/May, and 57% in October/November at the two epizootic sites. The strongest evidence of microsporidian impact on *S. invicta* populations was a negative correlation between colony numbers versus percentage infection and a sporadic decrease in the number of foragers. There was some evidence of a decrease in the size and number of colonies at one epizootic site and a decrease in brood at the other. This sporadic weakening of the *S. invicta* populations did not lead to significant immigration of other ant species.

KEY WORDS *Solenopsis invicta*, biological control, entomopathogen, epizootiology, ants

THE RED IMPORTED FIRE ant, *Solenopsis invicta* Buren, is one of the few insects that is a significant medical, urban, agricultural, and ecological pest (Vinson 1997, Taber 2000, Williams et al. 2001, Wojcik et al. 2001). Chemical controls for this insect can be toxic to humans and animals, are not persistent, and at times have caused more ecological damage than the ants (Taber 2000, Williams et al. 2001). This invasive insect was introduced accidentally into the United States in the 1930s without its natural biological control agents (Jouvenaz et al. 1977, Porter et al. 1992, Briano et al. 1995). The absence of natural enemies may have allowed *S. invicta* populations to increase to greater densities in the United States than in its native regions (Porter et al. 1992).

Thelohania solenopsae Knell, Allen, and Hazard is a microsporidium that infects *S. invicta* (Knell et al. 1977). Jouvenaz et al. (1977) sampled 1,007 *S. invicta* colonies to survey for pathogens of this insect in the southeastern United States, including Louisiana. Pathogens were exceedingly rare, as the survey detected only a microsporidium (one infected colony, apparently not *Thelohania* sp.) and relatively harmless yeast and gregarines. Interestingly, *T. solenopsae* was discovered ≈20 yr later in Florida, Texas, and Mississippi populations of *S. invicta* (Williams et al. 1998, 2003, Cook 2002) and subsequently was detected in Louisiana (Milks et al. 2004, Sokolova et al. 2004a).

This microsporidium is a candidate for biological control of *S. invicta* in Louisiana. *T. solenopsae* can weaken and occasionally kill *S. invicta* colonies (Williams et al. 1999, Cook 2002, 2003, Oi and Williams 2003). In Florida, a field release resulted in persistence for 2 yr, prevalence of up to 93% infection, and intercolony transmission (Williams et al. 1999, Oi and Williams 2002), but little is known about longer-term persistence or spread. In Louisiana, *T. solenopsae* was detected at only 25 of 165 sites surveyed throughout the state (M.L.M., J.R.F., A.R.R., unpublished data), indicating that it may be spreading only very slowly,

[1] Department of Entomology, Louisiana State University AgCenter, Baton Rouge, LA 70803.
[2] Corresponding author: Department of Entomology, 402 Life Sciences Bldg., Louisiana State University, Baton Rouge, LA 70803 (e-mail: jfuxa@lsu.edu).
[3] Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611.
[4] USDA–ARS Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608.

0046-225X/05/1139–1149$04.00/0

leaving plentiful opportunity to speed this process by further releases.

A potential problem in biological control with this entomopathogen is that the two social forms of *S. invicta*—polygyne, or multiple-queen, and monogyne, or single-queen—may affect the success of releases. Polygyne but not monogyne colonies have been artificially infected in the field (Williams et al. 1999, Oi and Williams 2002), and nothing is known about the effect of a mixed-social-form host population on the pathogen's spread. *T. solenopsae* infected 95% of polygyne colonies and 0% of monogyne *S. invicta* in one field prevalence study (Oi et al. 2004) and 85% of polygyne and 3% of monogyne colonies in another (M.L.M., J.R.F., A.R.R., unpublished data). However, in a third study (Fuxa et al. 2005), 63% of monogyne colonies were naturally infected, although the microsporidium eventually disappeared from the host population. The reasons for such differences are unknown, but polygyne colonies freely exchange workers, food, brood, and newly mated females, whereas monogyne colonies are very territorial (Tschinkel 1998).

The purposes of this research were (1) to release and establish *T. solenopsae* in monogyne and polygyne *S. invicta* populations; (2) to monitor its long-term persistence and spread; and (3) to determine its effects on populations of *S. invicta* and native ants.

Materials and Methods

Four sites in Louisiana were selected for field studies after preliminary sampling indicated that *T. solenopsae* was not present. The site near St. Joseph consisted of pastures, in a flat terrain, with 30% (ground cover) dallisgrass (*Paspalum dilatatum* Poir.), 25% Bermuda grass (*Cynodon dactylon* L. Pers.), 17% perennial ryegrass (*Lolium perenne* L.), 10% little barley (*Hordeum pusillum* Nutt.), and 18% other species; the treated plot was 0.53 km from the control. The second site, near southern Baton Rouge, consisted of pastures, in a flat terrain, with 40% perennial ryegrass, 30% reversed clover (*Trifolium resupinatum* L.), 20% hairy buttercup (*Ranunculus sardous* Crantz), and 10% other species; the treated plot was 1.61 km from the control. The third treatment plot, near Clinton, was in a pasture in a swale surrounded by hills and trees, with 30% perennial ryegrass, 20% hairy buttercup, 15% timothy canarygrass (*Phalaris angusta* Nees ex Trin.), 15% annual blue-eyed grass (*Sisyrinchium rosulatum* Bickn.), 15% annual bluegrass (*Poa annua* L.), and 5% white clover (*Trifolium repens* L.); the control plot was on a hill 1.29 km from the treated plot. The fourth site, central Baton Rouge, was a flat, urban yard surrounded by trees, with 100% St. Augustine grass [*Stenotaphrum secundatum* (Walt.) Kuntze]; the treated plot was 150 m from the control. At St. Joseph, Clinton, and central Baton Rouge, a circular, 0.05-ha reference plot was established at each control and treatment site, with a permanently fixed metal stake marking the center of the plot. The same was done at the south Baton Rouge site, except that the plots were each 0.2 ha, because this site had fewer colonies per unit area than the other three sites.

The ecological setting at St. Joseph changed during the 6 yr that this site was sampled. Although the treated and control plots were left intact, the entire area between them was tilled just before the October 2000 sampling to convert it from pastures to row-crop agriculture beginning with the 2001 growing season. Approximately 1 ha was left undisturbed adjacent to the other three sides of the treated plot, but the grass subsequently was not grazed or cut as frequently as before October 2000. The control plot was completely destroyed by the time of the May 2004 sampling.

Thelohania solenopsae was introduced into each treatment plot by means of infected brood of *S. invicta* (Williams et al. 1999). Brood infected with *T. solenopsae* was obtained from field colonies in the vicinity of Gainesville, FL. The infected brood was shipped to Baton Rouge along with adult nurse ants. The brood was confirmed by microscopy to be infected with *T. solenopsae* and was separated from the nurse ants within 1–2 h before field inoculation. Five colonies of *S. invicta* in each treatment plot were selected randomly for inoculation among colonies with a colony rating index ≥ 8. The colony index system is a visual rating of colonies based on the number of ants and presence of worker-caste brood observed when the mound is disturbed (Lofgren and Williams 1982). A small shovel was used to dig into each treated mound ≈ 15 cm deep, and 4.8–5.0 g of the infected brood were placed into the hole and immediately covered with the removed soil. Treatment dates were 12 June 1998 at St. Joseph, 17 June 1999 at south Baton Rouge, 11 April 2001 at Clinton, and 12 April 2001 at central Baton Rouge.

On each sampling date, including immediately before treatment, the location of every *S. invicta* mound in each plot was recorded by linear measurement along compass readings from a permanently fixed stake marking the center of the plot. Colony size was estimated according to the colony index system; however, we present the presence of brood as a separate parameter in data tables. On certain sampling dates, colonies were sampled outside the treatment plot to monitor spread of *T. solenopsae.* In these cases, the colony index rating was estimated for every mound in 3.0-m-wide transects in the four cardinal directions outside the plot or in the additional area of a concentric circle defined by a greater radius than that of the plot. On 1 June 2003, the treatment site at Clinton was subjected to a final, more extensive sampling pattern for *T. solenopsae* spread. On this occasion, three sampling reference points were established at distances of 61, 122, and 244 m away from the treatment in each of the eight major compass directions, for a total of 22 reference points; there was insufficient room for the third point in two directions. An ant sample was collected, as below, from the 10–14 colonies closest to each reference point.

An ant sample was collected from every mound in the control and treatment plots as well as in the transects or extended plots for monitoring spread. For

each sample, a shovelful of mound soil ≈15 cm deep was turned, and an open, 20-ml screw-top vial with the top interior painted with Teflon TFE 30 (DuPont, Newark, DE) to prevent escape was placed angled upward in such a way that the disturbed worker ants would fall inside. The vial was collected, and the cap was screwed on when at least 50 ants were trapped, if that many were available. The vials with ants were chilled in a cooler and returned to the laboratory, where they were stored at −4°C until analysis.

Ant samples were examined for microsporidian spores, and their DNA was subjected to polymerase chain reaction (PCR) to determine the social form. Twenty ants from each mound were macerated in 200 μl of sterile distilled water in a 1.5-ml microtube with a disposable pellet pestle (Koates Glass Co., Vineland, NJ). A 25-μl aliquot of the homogenate was examined as a wet mount under phase microscopy at ×400. Another aliquot was smeared onto a slide, stained with Giemsa, and examined at ×400 (Milks et al. 2004). The remainder of the ants was stored at −20°C until further analyses; homogenate was similarly stored if there were insufficient additional ants. The social form of *S. invicta*, or proportion of monogyne versus polygyne colonies, was determined in the control and treated plots at each of the four field sites on the day of treatment and on selected sampling dates thereafter. DNA was extracted from groups of 30 ants as described in Valles and Porter (2003), and PCRs were run as outlined in Fuxa et al. (2005).

Additional ants were collected in bait stations or pitfall traps on the day of treatment and on certain dates thereafter to monitor the relative population density of *S. invicta* and other ant species. Thirteen pitfall traps were set at each plot, one in the middle and three at 6-m intervals in each cardinal direction. Each pitfall trap consisted of a 30-ml vial containing 18 ml of propylene glycol (Sierra Antifreeze; Old World Industries, Northbrook, IL); ants were removed from the pitfall traps after 24 h. Bait stations were arranged in a 7 by 7 square-grid pattern, with the grid's center at the middle of the plot and the other bait stations set at 6-m intervals from the center station. Each station consisted of an open 5-cm plastic petri dish containing cotton cosmetic pads soaked in 1 ml of a carbohydrate-based attractant (Vail et al. 1999). The bait stations were set up in the morning when the air temperature reached 21–27°C, and they were collected 30 min later, tightly covered, and placed in coolers to be transported to the laboratory. Ants in all pitfall and bait samples were identified to species and counted.

Data were analyzed by SAS programs (SAS Institute 2003). The fire ant colony index ratings and percentages of colonies containing brood were analyzed by the nonparametric Kruskal-Wallis test. Number of colonies, colony index rating, or percentage of colonies with brood was analyzed with Spearman rank correlation versus percentage infection. Data from the bait stations and pitfall traps were subjected to analysis of variance (ANOVA).

Results

The results of releasing *T. solenopsae* varied greatly among the four release sites, and successful establishment of the pathogen was more likely if the ant population was predominantly polygyne. At St. Joseph, the ratio of monogyne:polygyne ants in the treated plot was 19:81 on the release date and 26:74 over all dates tested, although this site had a relatively high proportion of colonies for which social form could not be determined (Table 1). At this predominantly polygyne site, *T. solenopsae* infections were first detected in December 1998, 6 mo after release; between 20 and 22 mo, February to April 2000, prevalence suddenly grew to epizootic proportions (Fig. 1); and sampling through May 2004 showed that the pathogen was permanently established. At Clinton, the monogyne: polygyne ratio was 40:60 in the treated plot on the release date and 23:77 over all dates tested (Table 2). At this predominantly polygyne site, *T. solenopsae* became established and grew to epizootic proportions in only 6 mo (Fig. 1), remaining at high prevalence rates through the last sampling date, 46 mo after release. At central Baton Rouge, the monogyne:polygyne ratio was 100:0 in the treated plot on the release date and 90:10 over all dates tested (Table 3). At this predominantly monogyne site, *T. solenopsae* usually infected only one or two colonies in the plot and never more than four, and it was never again detected after 18 mo. At south Baton Rouge, 19 of the 20 colonies in the treated plot were monogyne and 1 was of unknown social form on the microsporidium-release date, and no infected colony was ever found in seven sampling dates over the next 18 mo (251 colonies/dates sampled).

Monogyne colonies became infected at all three sites where the releases were initially successful, although the relative prevalence rates varied between the two social forms. Prevalence was higher in polygyne ants at St. Joseph and central Baton Rouge but higher in monogyne ants at Clinton. At. St. Joseph (Table 1), prevalence in monogyne and polygyne ants, respectively, was 17.5 ($n = 40$) and 33.3% ($n = 108$) beginning with the first detection of the pathogen (December 1998 to May 2004) and 22.2 ($n = 27$) and 57.7% ($n = 52$) while the disease was epizootic (April 2000 to May 2004). Similarly, at central Baton Rouge (Table 3), prevalence was 10.0 ($n = 60$) and 18.2% ($n = 11$) in monogyne and polygyne ants, respectively. However, at Clinton (Table 2), prevalence was 59.5% ($n = 37$) in monogyne and 52.5% ($n = 160$) in polygyne ants beginning in October 2001, when the epizootic began. At St. Joseph (Table 1), the proportion of monogyne to polygyne colonies in the treated plot was 19:81 on the treatment date, 19:81 from the time *T. solenopsae* was detected until it became epizootic (December 1998 to February 2000), and 34:66 during the epizootic (April 2000 to May 2004). At Clinton, the other epizootic site (Table 2), the monogyne:polygyne proportion in the treated plot was 40:60 on the day of treatment and 19:81 thereafter.

Table 1. Percentage infection (*n*) of two social forms of red imported fire ant colonies by *T. solenopsae* after its release near St. Joseph, LA

Date	Control plot				Treated plot			
	Monogyne	Polygyne	Unknown	Total	Monogyne	Polygyne	Unknown	Total
1998								
June[a]	0 (9)	0 (22)	0 (7)	0 (38)	0 (3)	0 (13)	0 (11)	0 (27)
Aug.				0 (17)				0 (22)
Oct.				0 (38)				0 (35)
Dec.				0 (52)	0 (4)	4.0 (25)	0 (18)	2.1 (47)
1999								
Feb.	0 (5)	0 (17)	0 (5)	0 (27)	25.0 (4)	11.1 (18)	0 (8)	10.0 (30)
April				0 (37)				7.0 (43)
June				0 (40)				2.3 (43)
Aug.				0 (20)				9.5 (21)
Oct.	(0)	0 (7)	0 (15)	0 (22)	(0)	50.0 (2)	0 (17)	5.6 (19)
Dec.				0 (7)				25.0 (4)
2000								
Feb.	0 (1)	0 (18)	0 (4)	0 (23)	0 (5)	18.2 (11)	0 (4)	10.0 (20)
April				0 (13)	23.1 (13)	55.6 (9)	50.0 (12)	41.2 (34)
June				0 (8)				6.3 (16)
Oct.				0 (25)	(0)	80.0 (10)	60.0 (5)	73.3 (15)
2001								
Feb.	0 (4)	0 (12)	0 (11)	0 (27)	0 (1)	66.7 (3)	80.0 (5)	66.7 (9)
April				0 (21)				72.2 (18)
June				0 (23)				6.7 (15)
Oct.	0 (3)	0 (31)	0 (6)	0 (41)	0 (1)	85.7 (7)	(0)	75.0 (8)
2002								
April				0 (25)	(0)	0 (1)	100 (1)	50.0 (2)
Nov.				0 (16)	50.0 (2)	41.7 (12)	(0)	42.9 (14)
2003								
Feb.				0 (10)				12.5 (8)
April	0 (1)	0 (11)	(0)	0 (12)	0 (3)	33.3 (6)	(0)	22.2 (9)
2004								
May	Control was destroyed				28.6 (7)	50.0 (4)	50.0 (2)	38.5 (13)

[a] *Thelohania*-infected brood released into five randomly selected colonies (circular plots, 0.05 ha, 12.7 m diam.) on this date.

It is unknown why the social form PCR had a high failure rate in samples from St. Joseph. A contributing factor may have been that this study was begun well before the social form PCR was available (Valles and Porter 2003); only old, frozen homogenates, not whole ants, were available for PCR from many of the samples. Oi et al. (2004) discussed the possible reasons for a high failure rate of this PCR in their study.

The pathogen spread within and away from the treated plots at all three release sites where it became established. The pathogen spread slowly at St. Joseph; in addition to the distance it moved within the release plot (Fig. 1), it was detected at distances of 4–8 m at 28 mo, 12–16 m at 32 mo, 32–36 m at 40 mo, and 40–44 m at 46 mo after release, a rate of movement of 0.9 m/mo. At Clinton, the pathogen spread throughout the plot within 6 mo (Fig. 1) and was almost always detected at the furthest sampling distance after we began to sample outside the release plot. It spread at least 8–12 m by 10 mo (0.80 m/mo); at 26 mo it had spread at least 244 m (9.38 m/mo), although prevalence decreased with distance, from 27% ($n = 90$) at 61 m to 17% ($n = 90$) at 122 m and 15% ($n = 72$) at 244 m. Even though *T. solenopsae* did not become permanently established at the central Baton Rouge site, infections were detected in 3 of 11 colonies up to 4 m outside the release plot during the February 2002 sampling, a rate of spread of up to 0.4 m/mo.

Thelohania solenopsae apparently had a weak impact on the *S. invicta* population at St. Joseph, although this is difficult to assess, because within-site treatment replication was not possible. At this site, there was an average of 12.5 colonies per sampling date in the treated plot and 20.1 in the control plot during the epizootic (11 sampling dates, April 2000 to April 2003; Table 1) compared with 28.3 colonies per date in the treated plot and 29.2 in the control ($n = 11$) before April 2000. Through April 2000, on certain dates, control and/or uninfected colonies in the treated plot had significantly lower colony index ratings or lower percentages of colonies with brood than infected colonies; after April 2000, however, infected colonies twice had lower index ratings than control colonies, but the differences in colony index or percentage with brood were otherwise nonsignificant (Table 4). The number of colonies in the treated plot was negatively correlated with percentage infection of colonies (Spearman $r = -0.640$, $n = 23$, $P = 0.001$), but the colony index rating (Spearman $r = 0.071$, $n = 23$, $P = 0.747$) and percentage of colonies containing brood (Spearman $r = -0.025$, $n = 23$, $P = 0.909$) were not correlated with percentage infection. Significantly fewer foragers were captured in bait stations in the treated plot than in the control on two of the four sampling dates (Table 5) during the epizootic (Table 1), but there were no differences in numbers captured in pitfall traps during that time (Table 6). There were no consistent indications that populations of non-*Solenopsis* species of ants increased because of the microsporidian epizootic in *S. invicta* (Tables 5 and 6).

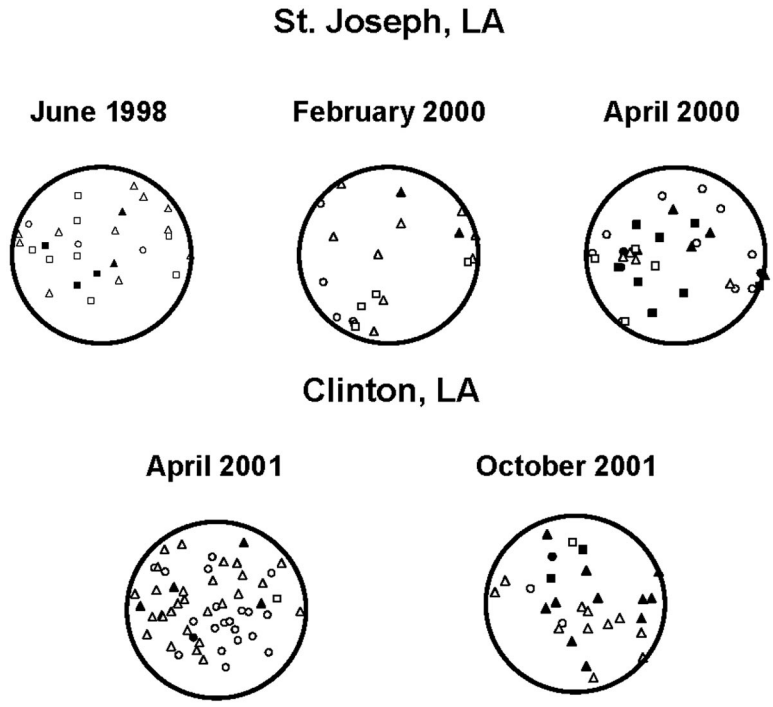

Fig. 1. Distribution of colonies of red imported fire ants on selected dates in the 0.05-ha plots treated with the microsporidium *T. solenopsae* in pastures near St. Joseph and Clinton, LA. June 1998 (St. Joseph) and April 2001 (Clinton) were the treatment dates; "infected" colonies on these dates were the ones into which *T. solenopsae* was introduced, but not all of them necessarily become infected: uninfected monogyne colony (○); infected monogyne (●); uninfected polygyne (△); infected polygyne (▲); uninfected, unknown social form (□); and infected, unknown social form (■).

Impact was even more difficult to assess at Clinton; in addition to lack of within-site treatment replication, the control plot at Clinton became predominantly monogyne shortly after the experiment began, whereas the treated site became more predominantly polygyne. At this site, there was an average of 23.0 colonies per sampling date in the treated plot and 6.3 in the control plot during the epizootic (10 sampling dates, October 2001 to February 2005), but the treated plot during that period was 19:81 monogyne:polygyne, whereas the control plot was 82:18 (Table 2). In every sampling date after 2001, infected colonies had signif-

Table 2. Percentage infection (*n*) of two social forms of red imported fire ant colonies by *T. solenopsae* after its release near Clinton, LA

Date	Control plot				Treated plot			
	Monogyne	Polygyne	Unknown	Total	Monogyne	Polygyne	Unknown	Total
2001								
April[a]	0 (10)	0 (10)	0 (2)	0 (22)	0 (21)	0 (32)	0 (1)	0 (54)
Oct.	0 (3)	0 (6)	0 (2)	0 (11)[b]	33.3 (3)	50.0 (22)	66.7 (3)	50.0 (28)
Dec.				(0)[b]				50.0 (24)
2002								
Feb.	0 (3)	0 (1)	(0)	0 (4)[b]	50.0 (2)	63.6 (11)	(0)	61.5 (13)
April	0 (4)	(0)	(0)	0 (4)[b]	44.4 (9)	71.4 (7)	(0)	56.3 (16)
Nov.			0 (6)	0 (6)[b]	(0)	41.9 (31)	(0)	41.9 (31)
2003								
Feb.	0 (4)	0 (2)	(0)	0 (6)[b]	50.0 (4)	37.9 (29)	(0)	39.4 (33)
April	0 (4)	(0)	0 (1)	0 (5)[b]	50.0 (2)	50.0 (18)	0 (1)	47.6 (21)
Oct.	0 (8)	(0)	(0)	0 (8)	77.8 (9)	63.6 (11)	20.0 (5)	60.0 (25)
2004								
April	0 (11)	(0)	(0)	0 (11)	100 (5)	76.5 (17)	(0)	81.8 (22)
2005								
Feb.	0 (8)	0 (1)	(0)	0 (9)	33.3 (3)	57.1 (14)	(0)	52.9 (17)

[a] *Thelohania*-infected brood released into five randomly selected colonies (circular plots, 0.05 ha, 12.7 m diam.) on this date.

[b] The additional area to a distance of 5.3 m outside the control plot (radius of 17.9 m from the plot's center) also was sampled. None of the sampled colonies were infected, and the additional no. colonies sampled on each date was 13 (10/2001), 11 (12/2001), 8 (2/2002), 13 (4/2002), 17 (11/2002), 2 (2/2003), and 9 (4/2003).

Table 3. Percentage infection (*n*) of two social forms of red imported fire ant colonies by *T. solenopsae* after its release at central Baton Rouge, LA

Date	Control plot				Treated plot			
	Monogyne	Polygyne	Unknown	Total	Monogyne	Polygyne	Unknown	Total
2001								
April[a]	0 (28)	(0)	(0)	0 (28)	0 (34)	(0)	(0)	0 (34)
Oct.				0 (10)[b]	33.3 (9)	100 (1)	0 (1)	36.4 (11)
Dec.				0 (9)[b]				18.2 (11)
2002								
Feb.	0 (11)	(0)	(0)	0 (11)[b]	9.1 (11)	100 (1)	0 (3)	13.3 (15)
April	0 (10)	(0)	(0)	0 (10)[b]	16.7 (12)	0 (5)	(0)	11.8 (17)
Oct.				0 (9)[b]				11.1 (9)
2003								
Feb.				0 (4)[b]				0 (15)
April	0 (6)	(0)	(0)	0 (6)[b]	0 (9)	0 (3)	(0)	0 (12)
Oct.	0 (12)	(0)	(0)	0 (12)	0 (10)	(0)	0 (1)	0 (11)
2004								
April	No sample				0 (9)	0 (1)	(0)	0 (10)

[a] *Thelohania*-infected brood released into five randomly selected colonies (circular plots, 0.05 ha, 12.7 m diam.) on this date.
[b] The additional area to a distance of 5.3 m outside the control plot (radius of 17.9 m from the plot's center) also was sampled. None of the sampled colonies were infected, and the additional no. colonies sampled on each date was 14 (10/2001), 15 (12/2001), 10 (2/2002), 12 (4/2002), 13 (10/2002), 15(2/2003), and 9 (4/2003).

icantly lower colony index ratings and/or lower percentages of colonies with brood than control colonies (Table 7); however, only the difference in brood may be meaningful because of the monogyne:polygyne ratios (Table 2). As at St. Joseph, the number of colonies in the treated plot was negatively correlated with percentage infection of colonies (Spearman $r = -0.729, n = 11, P = 0.010$), but the colony index rating (Spearman $r = -0.181$, $n = 11$, $P = 0.594$) and percentage of colonies containing brood (Spearman $r = 0.144$, $n = 11$, $P = 0.674$) were not correlated with percentage infection. Significantly fewer foragers

Table 4. Mean colony index rating[a] ± SE and percentage with brood of *S. invicta* colonies at the St. Joseph release site

Date	Control plot		Treated plot			
			Infected colonies		Uninfected colonies	
	Index rating	Percent with brood	Index rating	Percent with brood	Index rating	Percent with brood
1998						
June	2.5 ± 0.1b	100 A	—	—	3.3 ± 0.2a	100 A
Aug.	1.5 ± 0.2b	0 A	—	—	2.2 ± 0.2a	0 A
Oct.	2.8 ± 0.1b	51.3 B	—	—	3.5 ± 0.2a	88.6 A
Dec.	2.5 ± 0.1a	73.1 A	3.0 a	0 AB	2.3 ± 0.1a	30.4 B
1999						
Feb.	2.8 ± 0.1a	37.0 B	2.6 ± 0.5a	60.0 A	2.5 ± 0.2a	12.0 C
April	2.5 ± 0.2a	83.8 A	2.3 ± 0.3a	100 A	2.8 ± 0.2a	87.5 A
June	2.7 ± 0.1a	65.9 A	3.0 a	100 A	2.4 ± 0.1a	68.2 A
Aug.	1.9 ± 0.2a	0 A	1.5 ± 0.5a	0 A	1.8 ± 0.2a	15.8 A
Oct.	1.2 ± 0.1b	20.0 A	3.0 a	100 A	1.7 ± 0.2a	11.1 A
Dec.	1.6 ± 0.2a	0 A	3.0 a	0 A	3.0 ± 1.2a	0 A
2000						
Feb.	1.6 ± 0.2a	4.0 B	2.5 ± 0.5a	50.0 A	1.9 ± 0.2a	0 B
April	2.3 ± 0.2b	30.8 A	2.9 ± 0.4ab	71.4 A	3.5 ± 0.2a	60.0 A
June	2.1 ± 0.4a	0 A	2.0 a	0 A	3.4 ± 0.4a	6.7 A
Oct.	2.2 ± 0.2a	60.0 A	2.4 ± 0.2a	54.5 A	2.0 ± 0.4a	25.0 A
2001						
Feb.	3.1 ± 0.2a	0 A	2.0 ± 0.3b	0 A	2.0 ± 0.6b	0 A
April	2.7 ± 0.2a	61.9 A	2.8 ± 0.1a	69.2 A	3.2 ± 0.2a	100 A
June	3.2 ± 0.2a	69.6 A	3.0a	100 A	2.4 ± 0.3a	69.2 A
Oct.	3.5 ± 0.1a	70.7 A	2.7 ± 0.2b	50.0 A	3.5 ± 0.5a	100 A
2002						
April	3.8 ± 0.2a	84.0 A	4.0 a	100 A	2.0 a	0 A
Nov.	3.9 ± 0.2a	81.3 A	3.5 ± 0.3a	66.7 A	3.5 ± 0.3a	62.5 A
2003						
Feb.	3.6 ± 0.2a	90.0 A	3.0 a	0 A	3.0 ± 0.2a	57.1 A
April	2.6 ± 0.2a	100 A	3.5 ± 0.5a	100 A	3.3 ± 0.4a	100 A
2004						
May	Control was destroyed		2.2 ± 0.2a	100 A	2.6 ± 0.2a	100 A

[a] Based on the fire ant colony index system (Lofgren and Williams 1982) after disturbing the mound surface: 1 = 1–100 ants;2 = 101–1,000; 3 = 1,001–10,000; 4 = 10,001–50,000; 5 = >50,000; observation of brood in colonies is presented as a separate parameter. Means within each row followed by the same lowercase or capital letter did not differ at $P < 0.05$, nonparametric Kruskal-Wallis test (SAS Institute 2003).

Table 5. Mean no. ± SE of *S. invicta* and otherant species captured in a grid of bait stations[a] in control andmicrosporidium-treated plots at three release sites

Site/date	*S. invicta*		*Brachymyrmex* spp.		*Cyphomyrmex* spp.		*Tetramorium* spp.	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
St. Joseph								
6/1998	216 ± 10.7a	85 ± 7.7b	0	0	0	0	0	0
5/2000	49 ± 5.7a	55 ± 5.3a	0	0	0	0	0	0
10/2001	6 ± 1.5a	2 ± 0.6b	0	0	0	0	0	0
4/2002	18 ± 3.5a	9 ± 2.6b	0	0	0	0	0	0
11/2002	7 ± 1.5a	11 ± 2.4a	0.02	0.14	0	0	0	0
Clinton								
4/2001	7 ± 2.5a	9 ± 2.0a	0	0	0	0	0	0
4/2002	6 ± 1.7a	7 ± 1.5a	0	0	0	0	0	0
6/2003	77 ± 6.1a	20 ± 1.8b	0.1	0	0	0	0	0
10/2003	11 ± 2.3a	7 ± 1.2a	0	0	0	0	0	0
Central Baton Rouge								
4/2001	13 ± 2.6a	9 ± 2.2a	0	0	0	0	0	0
4/2002	18 ± 3.2a	18 ± 3.6a	0	0	0	0	0	0
6/2003	18 ± 4.8a	2 ± 1.1b	0.7	4.4	0	0.2	0	1.0

[a] Forty-nine bait traps arranged in a 7 by 7 trap grid pattern centered on the middle of the circular plot, with 6.1 m between traps. Means within each row followed by the same letter did not differ at $P < 0.05$ (ANOVA; SAS Institute 2003).

were captured in bait stations in the treated plot than in the control on one of three sampling dates (Table 5) during the epizootic (Table 2), but significantly more foragers were captured in pitfall traps in the control on one date (Table 6). There were no consistent indications that populations of non-*Solenopsis* species of ants increased because of the microsporidian epizootic in *S. invicta* (Tables 5 and 6).

At central Baton Rouge, there was no epizootic and no impact on the *S. invicta* population. The control plot averaged 9.8 colonies and the treated plot 12.6 colonies/date over five samples taken while ants were infected (October 2001 to October 2002; Table 3). There were no differences in colony index ratings or percentages of colonies with brood, except that there was no brood in the treated plot on the last sampling date, probably an anomaly (Table 8). The number of *S. invicta* captured in bait stations (Table 5) and in pitfall traps (Table 6) in the treated versus the control plot did not differ on the only sampling date when *T. solenopsae* infections were detected (Table 3).

Discussion

This research has helped clarify the relationships between *T. solenopsae* and the *S. invicta* social forms. First, the predominant social form at a specific location affects the probability of being able to establish *T. solenopsae* in a *S. invicta* population at epizootic proportions. Years-long epizootics were induced in the predominantly polygyne populations at St. Joseph and Clinton; the microsporidium previously has been introduced into polygyne ants in the field (Williams et al. 1999). However, introduction into a monogyne population at south Baton Rouge completely failed. Additionally, the disappearance of the pathogen after 18 mo from a central Baton Rouge population that was 71–100% monogyne (Table 3) indicates that an ant population with a high proportion of the monogyne form does not seem to be able to sustain the pathogen indefinitely. This supports the results of a previous study of a predominantly monogyne population, in which polygyne ants may have been killed off by the

Table 6. Mean no. ± SE of *S. invicta* and other ant species captured in pitfall traps[a] in control and microsporidium-treated plots at three release sites

Site/date	*S. invicta* spp.		*Brachymyrmex* spp.		*Cyphomyrmex* spp.		*Hypoponera* spp.		*Trichoscapa membranifera*	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
St. Joseph										
6/1998	474 ± 39.6b	653 ± 49.4a	0	0	0	0.1	0	0	0	0
4/2002	23 ± 11.2a	3 ± 0.9a	0	0	0	0	0	0	0	0
11/2002	14 ± 7.3a	9 ± 8.1a	7.5	0.4	0	0	0	0.1	0	0
Clinton										
4/2001	3 ± 0.7a	2 ± 0.4a	0	0	0	0	0	0	0	0
4/2002	2 ± 0.8b	9 ± 1.8a	0	0	0	0	0	0	0	0
6/2003	77 ± 51.9a	17 ± 7.1a	0.1	0	0	0	0	0	0	0
10/2003	108 ± 50.8a	15 ± 2.6a	0.9	0	0	0	0	0	0	0
Central Baton Rouge										
4/2001	10 ± 5.9a	12 ± 3.0a	0	0	0	0	0	0	0	0
4/2002	1 ± 0.7a	1 ± 0.2a	0	0	0	0	0	0	0	0
6/2003	4 ± 1.4a	8 ± 7.0a	0.3	0.2	0	0	0.1	0.1	0	0.1

[a] Thirteen pitfall traps arranged with one trap at the center of the plot and three in each of the four cardinal directions at 6.1-m intervals. Means within each row followed by the same letter did not differ at $P < 0.05$ (ANOVA; SAS Institute 2003).

Table 7. Mean colony index rating[a] ± SE and percentage with brood of *S. invicta* colonies at the Clinton release site

Date	Control plot		Treated plot			
			Infected colonies		Uninfected colonies	
	Index rating	Percent with brood	Index rating	Percent with brood	Index rating	Percent with brood
2001						
April	2.2 ± 0.2a	30.4 A	—	—	2.1 ± 0.1a	22.6 A
Oct.	2.6 ± 0.4a	90.9 A	2.7 ± 0.2a	92.9 A	3.1 ± 0.1a	78.6 A
Dec.	—	—	2.8 ± 0.2a	0 A	2.8 ± 0.2a	0 A
2002						
Feb.	3.3 ± 0.5a	25.0 A	1.9 ± 0.1b	0 A	2.6 ± 0.2a	0 A
April	4.8 ± 0.3a	100 A	3.2 ± 0.2b	100 A	2.7 ± 0.3b	71.4 A
Nov.	4.5 ± 0.3a	83.3 A	3.2 ± 0.2b	30.8 B	3.2 ± 0.2b	27.8 B
2003						
Feb.	4.2 ± 0.5a	83.3 A	3.9 ± 0.3a	38.5 B	3.3 ± 0.1a	5.0 C
April	4.2 ± 0.2a	100 A	3.3 ± 0.2b	100 A	3.7 ± 0.2ab	100 A
Oct.	4.0 ± 0.3a	87.5 A	2.9 ± 0.2b	46.7 B	2.9 ± 0.7b	100 A
2004						
April	4.6 ± 0.2a	100 A	2.9 ± 0.2b	100 A	3.3 ± 0.9b	100 A
2005						
Feb.	3.3 ± 0.4a	66.7 A	2.6 ± 0.2a	0 B	2.6 ± 0.3a	12.5 B

[a] Based on the fire ant colony index system (Lofgren and Williams 1982) after disturbing the mound surface: 1 = 1–100 ants;2 = 101–1,000; 3 = 1,001–10,000; 4 = 10,001–50,000; 5 = >50,000; observation of brood in colonies is presented as a separate parameter. Means within each row followed by the same lowercase or capital letter did not differ at $P < 0.05$, nonparametric Kruskal-Wallis test (SAS Institute 2003).

microsporidium that could not be sustained in a 100% monogyne population (Fuxa et al. 2005).

Second, despite this difference in probability of establishment, this research was the first to show that monogyne colonies can be directly, artificially infected in the field. Introduction of the microsporidium into a 100% monogyne population resulted in three infected monogyne colonies within 6 mo at the central Baton Rouge site (Table 3). Previously, infection of monogyne ants has been achieved only in the laboratory (Williams et al. 1999).

Third, when the microsporidium becomes established in a mixed population with both social forms, its prevalence can reach epizootic proportions, up to 78–100% of colonies infected in monogyne ants (Table 2) and 77–86% in polygyne ants (Tables 1 and 2). This is in contrast to two field surveys in which prevalence in monogyne *S. invicta* was low (M.L.M., J.R.F., A.R.R., unpublished data) or zero (Oi et al. 2004). However, these data support a third study, that of a natural epizootic in a mixed monogyne/polygyne population, in which prevalence reached 63% in monogyne ants and 89–100% in polygyne *S. invicta* (Fuxa et al. 2005).

This study adds data to previous results in support of an interesting hypothesis. The earlier research indicated that, if the microsporidium reaches higher prevalence rates in one social form than the other in a mixed population, the more infected form may be at a competitive disadvantage. In that study of a natural epizootic, polygyne ants suffering 89–100% disease prevalence disappeared from a plot where only 26% of monogyne ants were infected (Fuxa et al. 2005). During the epizootic at St. Joseph in this research, prev-

Table 8. Mean colony index rating[a] ± SE and percentage with brood of *S. invicta* colonies at the central Baton Rouge release site

Date	Control plot		Treated plot			
			Infected colonies		Uninfected colonies	
	Index rating	Percent with brood	Index rating	Percent with brood	Index rating	Percent with brood
2001						
April	2.3 ± 0.2a	48.1 A	—	—	2.3 ± 0.2a	45.5 A
Oct.	3.0 ± 0.4a	70.0 A	2.8 ± 0.6a	75.0 A	2.6 ± 0.4a	57.1 A
Dec.	3.3 ± 0.3a	55.6 A	2.5 ± 0.5a	0 A	3.2 ± 0.4a	44.4 A
2002						
Feb.	3.4 ± 0.2a	45.5 A	2.5 ± 0.5a	100 A	2.8 ± 0.2a	30.8 A
April	3.9 ± 0.4a	70.0 A	2.5 ± 0.5a	50.0 A	2.9 ± 0.3a	60.0 A
Oct.	3.6 ± 0.4a	100 A	4.0 a	100 A	3.6 ± 0.5a	62.5 A
2003						
Feb.	4.0 ± 0.7a	50.0 A	—	—	2.9 ± 0.3a	26.7 A
April	4.0 ± 0.4a	83.3 A	—	—	3.3 ± 0.4a	66.7 A
Oct.	3.3 ± 0.2a	64.3 A	—	—	3.2 ± 0.4a	81.8 A
2004						
April	3.3 ± 0.2b	80.0 A	—	—	4.3 ± 0.3a	0 B

[a] Based on the fire ant colony index system (Lofgren and Williams 1982) after disturbing the mound surface: 1 = 1–100 ants; 2 = 101–1,000; 3 = 1,001–10,000; 4 = 10,001–50,000; 5 = >50,000; observation of brood in colonies is presented as a separate parameter. Means within each row followed by the same lowercase or capital letter did not differ at $P < 0.05$, nonparametric Kruskal-Wallis test (SAS Institute 2003).

alence averaged 22% in the monogyne ants and 58% in the polygyne, and the monogyne:polygyne ratio of colonies nearly doubled from 19:81 before the epizootic to 34:66 during it. Weaker support for this hypothesis was found during the epizootic at Clinton, where prevalence averaged 60% in monogyne and 53% in polygyne ants during the epizootic, whereas the monogyne:polygyne ratio of colonies decreased from 40:60–19:81. Additional data are necessary to further test this hypothesis.

The rate of spread of *T. solenopsae* may have been influenced by alate production or by the number of colonies per unit area. The different development of epizootics between St. Joseph and Clinton, within and away from the release plots, apparently was a function of disease transmission. The pathogen spread throughout the Clinton treated plot in only 6 mo, whereas it required almost 2 yr to do so at St. Joseph (Fig. 1). However, when *T. solenopsae* did finally begin to spread within the St. Joseph treated plot, it did so rapidly (Fig. 1). Similarly, the rate of spread outside the plot was only 0.9 m/mo at St. Joseph but 9.4 m/mo at Clinton. This was despite the fact that mean prevalence rates during the epizootic in primary sampling months (February, April, May, October, November) were similar, 49.4% ($n = 10$) at St. Joseph (Table 1) and 54.6% ($n = 9$) at Clinton (Table 2), as were the overall ratios of monogyne:polygyne colonies (26:74 at St. Joseph and 23:77 at Clinton). However, the greater prevalence of infected monogyne colonies at Clinton may have contributed to more successful dispersal of infected alates; alate production (Vargo and Fletcher 1987) and dispersal ability (DeHeer et al. 1999) are greater in monogyne than in polygyne *S. invicta.* Also, Clinton averaged more mounds per plot than St. Joseph, both before (53/plot versus 17/plot, respectively) and during (22/plot versus 10/plot, respectively) the epizootic. High host population density is known to be conducive to spread and epizootics of many entomopathogens (Andreadis 1987).

Another possibility is that the difference in rate of spread, and therefore transmission, may have been caused by site characteristics. St. Joseph is a flat region with extensive row-crop agriculture, whereas the Clinton release plot was in a swale with pastures surrounded by hills and forests. It is possible that, during swarming, infected alates (Cook et al. 2003, Oi and Williams 2003) flew or were transported by wind away from the general area of the release plot in the flat terrain of St. Joseph but were more constrained by the topography at Clinton, remaining in the general vicinity of the plot to accelerate transmission of *T. solenopsae.*

Thelohania solenopsae is transmitted vertically and horizontally, but the specific mechanisms remain unknown. Evidence for vertical transmission is strong: vegetative stages of the microsporidium have been detected in *S. invicta* eggs (Briano et al. 1996), spores collect adjacent to female ovarioles (Sokolova et al. 2004b), and new colonies become infected if founded by an infected female alate (Oi and Williams 2003). *T. solenopsae* forms four or five types of spores (Shapiro et al. 2003, Sokolova et al. 2004b) whose specific functions in transmission remain unknown. Mechanisms for horizontal transmission are even more mysterious, although the addition of infected brood to a colony, either artificially or by brood raiding, can result in its infection (Oi et al. 2003).

Prevalence rates of the microsporidium showed little seasonal variation during the epizootics except in the heat of summer. When data from St. Joseph and Clinton are combined, the mean prevalence of *T. solenopsae* was 46.6% ($n = 5$) in February, 51.2% ($n = 8$) in April/May, and 57.2% ($n = 6$) in October/November. Samples in June, which were collected only at St. Joseph, averaged 6.5% infection ($n = 2$) during that epizootic, but this low prevalence probably reflected difficulty in sampling infected ants during high summer temperatures.

The impact of *T. solenopsae* on *S. invicta* populations at the two epizootic sites was weak and sporadic. The strongest evidence of negative effects on red imported fire ant was (1) the colony numbers decreased as percentage infection increased at the two epizootic sites, and (2) the treated plot had fewer foragers captured at bait stations than the control plot at certain times at both St. Joseph and Clinton (Table 5), although there were no differences in pitfall trap captures (Table 6). Other effects were not as consistent. Colonies in the treated plot at St. Joseph had smaller index ratings than in the control on two dates during the epizootic, although there were no differences between infected and uninfected colonies in the treated plot (Table 4); a similar finding at Clinton (Table 7) was not valid because the ants in the control plot became mostly a monogyne population. The correlations of colony index rating versus percentage infection were nonsignificant at both sites, a finding similar to that of Oi and Williams (2002). The percentage of colonies with brood was lower in the treated plot at Clinton than in the control on four of the last six sampling dates (Table 7), but there were no significant differences in this parameter during the St. Joseph epizootic (Table 4). The correlations of percentage of colonies with brood versus percentage infection were nonsignificant at both sites. Colony numbers were reduced by 38% in the treated plot versus the control plot at St. Joseph after the epizootic began (Table 1); however, within-site replication to support this observation was not possible, and colony-number data at Clinton were not valid because the control became predominantly monogyne ants (Table 2), which produce only 0.10–0.33 as many mounds per unit area as polygyne ants (Vinson 1997, Tschinkel 1998). The microsporidian epizootics in *S. invicta* did not weaken this species sufficiently to allow significant immigration of other ant species (Tables 5 and 6) to restore biodiversity, a goal of *S. invicta* pest management (Wojcik et al. 2001). It is possible that long-term epizootics would be necessary over larger areas than in this study for native ant species to become evident. In previous laboratory and field research, effects of *T. solenopsae* on *S. invicta* have included lower brood and worker numbers, lower queen weight, shorter

queen life span, lower rate of egg-laying (Williams et al. 1999), reduced weight in female alates (Cook et al. 2003), and increased susceptibility to an entomopathogenic fungus (Brinkman and Gardner 2000) and a chemical pesticide (Valles and Pereira 2003).

Thus, *T. solenopsae* has impressive features for classical biological control of *S. invicta* as well as some weaknesses. This microsporidium can be established in an ant population and spread from a release site, where it seems able to produce long-lasting epizootics and weaken the host population in various ways without further treatment or cost. However, methods for releasing this pathogen into an ant population are inefficient; its introduction is not always successful in monogyne ant populations; it may require a certain proportion of polygyne colonies in the host population to sustain an epizootic; and it is not virulent to the point where it will destroy a high proportion of ant colonies. These characteristics clearly indicate that this pathogen can succeed as a classical biological control that is intended to weaken, not destroy, ant populations. Further research could increase its usefulness by improving efficiency of release, improving epizootics in predominantly monogyne ant populations, and identifying sublethal affects that can interact with other natural and artificial agents to more greatly impact *S. invicta* populations.

Acknowledgments

We thank I. Sokolov, I. Isakova, and M. McMichael (Department of Entomology, Louisiana State University AgCenter) for help with the sampling and D. M. Ferguson (Department of Biological Sciences, Louisiana State University) for identification of plant species. This research was supported by the Louisiana Fire Ant Research and Management Legislative Grant and by Grant 99-34103–8067 from the USDA Southern Region Integrated Pest Management Program. This paper was approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript 05-26-0227.

References Cited

Andreadis, T. G. 1987. Transmission, pp. 159–176. *In* J. R. Fuxa and Y. Tanada (eds.), Epizootiology of insect diseases. Wiley, New York.

Briano, J., R. Patterson, and H. Cordo. 1995. Relationship between colony size of *Solenopsis richteri* (Hymenoptera: Formicidae) and infection with *Thelohania solenopsae* (Microsporida: Thelohaniidae) in Argentina. J. Econ. Entomol. 88: 1233–1237.

Briano, J. A., R. S. Patterson, J. J. Becnel, and H. A. Cordo. 1996. The black imported fire ant, *Solenopsis richteri*, infected with *Thelohania solenopsae:* intracolonial prevalence of infection and evidence for transovarial transmission. J. Invertebr. Pathol. 67: 178–179.

Brinkman, M. A., and W. A. Gardner. 2000. Enhanced activity of *Beauveria bassiana* to red imported fire ant workers (Hymenoptera: Formicidae) infected with *Thelohania solenopsae.* J. Agr. Urban Entomol. 17: 191–195.

Cook, T. J. 2002. Studies of naturally occurring *Thelohania solenopsae* (Microsporida: Thelohaniidae) infection in red imported fire ants, *Solenopsis invicta* (Hymenoptera: Formicidae). Environ. Entomol. 31: 1091–1096.

Cook, T. J., M. B. Lowery, T. N. Frey, K. E. Rowe, and L. R. Lynch. 2003. Effect of *Thelohania solenopsae* (Microsporida: Thelohaniidae) on weight and reproductive status of polygynous red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae), alates. J. Invertebr. Pathol. 82: 201–203.

DeHeer, C. J., M.A.D. Goodisman, and K. G. Ross. 1999. Queen dispersal strategies in the multiple-queen form of the fire ant *Solenopsis invicta.* Am. Nat. 153: 660–675.

Fuxa, J. R., M. L. Milks, Y. Y. Sokolova, and A. R. Richter. 2005. Interaction of an entomopathogen with an insect social form: an epizootic of *Thelohania solenopsae* (Microsporidia) in a population of the red imported fire ant, *Solenopsis invicta.* J. Invertebr. Pathol. 88: 79–82.

Jouvenaz, D. P., G. E. Allen, W. A. Banks, and D. P. Wojcik. 1977. A survey for pathogens of fire ants, *Solenopsis* spp., in the southeastern United States. Fla. Entomol. 60: 275–279.

Knell, J. D., G. E. Allen, and E. I. Hazard. 1977. Light and electron microscope study of *Thelohania solenopsae* n. sp. (Microsporida: Protozoa) in the red imported fire ant, *Solenopsis invicta.* J. Invertebr. Pathol. 29: 192–200.

Lofgren, C. S., and D. F. Williams. 1982. Avermectin B_1a: highly potent inhibitor of reproduction by queens of the red imported fire ant (Hymenoptera: Formicidae). J. Econ. Entomol. 75: 798–803.

Milks, M. L., Y. Y. Sokolova, I. A. Isakova, J. R. Fuxa, F. Mitchell, K. F. Snowden, and S. B. Vinson. 2004. Comparative effectiveness of light-microscopic techniques and PCR in detecting *Thelohania solenopsae* (Microsporidia) infections in red imported fire ants (*Solenopsis invicta*). J. Eukaryot. Microbiol. 51: 187–191.

Oi, D. H., and D. F. Williams. 2002. Impact of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) on polygyne colonies of red imported fire ants (Hymenoptera: Formicidae). J. Econ. Entomol. 95: 558–562.

Oi, D. H., and D. F. Williams. 2003. *Thelohania solenopsae* (Microsporidia: Thelohaniidae) infection in reproductives of red imported fire ants (Hymenoptera: Formicidae) and its implication for intercolony transmission. Environ. Entomol. 32: 1171–1176.

Oi, D. H., S. M. Valles, and R. M. Pereira. 2004. Prevalence of *Thelohania solenopsae* (Microsporida: Thelohaniidae) infection in monogyne and polygyne red imported fire ants (Hymenoptera: Formicidae). Environ. Entomol. 33: 340–345.

Porter, S. D., H. G. Fowler, and W. P. MacKay. 1992. Fire ant mound densities in the United States and Brazil (Hymenoptera: Formicidae). J. Econ. Entomol. 85: 1154–1161.

SAS Institute. 2003. SAS/STAT user's guide, version 9.1. SAS Institute, Cary, NC.

Shapiro, A. M., J. J. Becnel, D. H. Oi, and D. F. Williams. 2003. Ultrastructural characterization and further transmission studies of *Thelohania solenopsae* from *Solenopsis invicta.* J. Invertebr. Pathol. 83: 177–180.

Sokolova, Y. Y., R. L. Bossard, J. R. Fuxa, D. W. Sanson, and L. D. Foil. 2004a. The microsporidium *Thelohania solenopsae* in red imported fire ants (Hymenoptera: Formicidae) from Louisiana pastures. Southwest. Entomol. 29: 271–276.

Sokolova, Y. Y., L. R. McNally, J. R. Fuxa, and S. B. Vinson. 2004b. Spore morphotypes of *Thelohania solenopsae* (Microsporidia) described microscopically and confirmed by PCR of individual spores microdissected from smears by position ablative laser microbeam microscopy. Microbiology. 150: 1261–1270.

Taber, S. W. 2000. Fire ants. Texas A&M University Press, College Station, TX.

Tschinkel, W. R. 1998. The reproductive biology of fire ant societies. BioScience. 48: 593–605.

Vail, K. M., D. F. Williams, and D. H. Oi. 1999. Ant bait attractive to multiple species of ants. U.S. Patent No. 5,939,061. 17 August 1999.

Valles, S. M., and R. M. Pereira. 2003. Hydramethylnon potentiation in *Solenopsis invicta* by infection with the microsporidium *Thelohania solenopsae.* Biol. Control. 27: 95–99.

Valles, S. M., and S. D. Porter. 2003. Identification of polygyne and monogyne fire ant colonies (*Solenopsis invicta*) by multiplex PCR of *Gp-9* alleles. Insect Soc. 50: 1–2.

Vargo, E. L., and D.J.C. Fletcher. 1987. Effect of queen number on the production of sexuals in natural populations of the fire ant, *Solenopsis invicta.* Physiol. Entomol. 12: 109–116.

Vinson, S. B. 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae). Am. Entomol. 43: 23–39.

Williams, D. F., G. J. Knue, and J. J. Becnel. 1998. Discovery of *Thelohania solenopsae* from the red imported fire ant, *Solenopsis invicta*, in the United States. J. Invertebr. Pathol. 71: 175–176.

Williams, D. F., D. H. Oi, and G. J. Knue. 1999. Infection of red imported fire ant (Hymenoptera: Formicidae) colonies with the entomopathogen *Thelohania solenopsae* (Microsporidia: Thelohaniidae). J. Econ. Entomol. 92: 830–836.

Williams, D. F., H. L. Collins, and D. H. Oi. 2001. The red imported fire ant (Hymenoptera: Formicidae): an historical perspective of treatment programs and the development of chemical baits for control. Am. Entomol. 47: 146–159.

Williams, D. F., D. H. Oi, S. D. Porter, R. M. Pereira, and J. A. Briano. 2003. Biological control of red imported fire ant (Hymenoptera: Formicidae). Am. Entomol. 49: 150–163.

Wojcik, D. P., C. R. Allen, R. J. Brenner, E. A. Forys, D. P. Jouvenaz, and R. S. Lutz. 2001. Red imported fire ants: impact on biodiversity. Am. Entomol. 47: 16–23.

Received for publication 29 April 2005; accepted 14 July 2005.