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Evidence of Intracolony Transmission of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in Red Imported Fire Ants (Hymenoptera: Formicidae) and the First Report of Spores From Pupae

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Red imported fire ant, *Solenopsis invicta*, colonies were infected horizontally by introducing live brood (mainly larvae and pupae) infected with *Thelohania solenopsae*. Live, infected brood introduced into uninfected colonies were adopted and raised to adulthood instead of being executed by the recipient colony. Introductions of infected larvae with uninfected pupae, which eclose into adult worker caste fire ants, resulted in an 80% infection rate of the inoculated colonies. Infections from introductions of infected pupae with uninfected larvae resulted in a 37.5% infection of inoculated colonies. Infections were also detected in 11.6 and 3.7% of the adult worker caste ants that eclosed from uninfected large larvae and pupae, respectively, that were held with infected adult workers. Microscopic examination of infected brood revealed sporoblasts and large numbers of spores of *T. solenopsae* in *S. invicta* pupae.

***Key Words: Thelohania solenopsae*; Microsporidia; *Solenopsis invicta;* fire ant; biological control.**

INTRODUCTION

Thelohania solenopsae is a microsporidium that infects several species of fire ants in the *Solenopsis saevissima* complex, including the imported fire ant species found in the United States, *S. invicta* and *S. richteri* (Allen and Knell, 1980; Williams *et al.*, 1998). In *S. invicta*, *T. solenopsae* slowly debilitates a queen, diminishing her reproductive capacity and causing premature death (Williams *et al.*, 1999). Vegetative stages of *T. solenopsae* are found in larvae, pupae, and ovaries of queens while mature spores are found in adult workers and queens (Knell *et al.*, 1977).

Two types of spores have been described in *T. solenopsae*: uninucleate meiospores (= octospores) within a sporophorous vesicle and binucleate nonmembrane-bound free spores (Knell *et al.*, 1977). The binucleate free spores may be responsible for transovarial transmission of *T. solenopsae*. Knell *et al.* (1977) reported the presence of diplokaryotic meronts of *T. solenopsae* in the ovaries and sporonts in queens of *S. invicta*. Briano *et al.* (1996) observed vegetative stages in eggs of *S. richteri*.

Intercolony transmission of *T. solenopsae* has been obtained under laboratory conditions by introducing live infected immature ants, or brood (consisting mainly of larvae and pupae), into uninfected *S. invicta* colonies (Williams *et al.*, 1999). This has resulted in infections in both the queens and the worker caste. Inoculations of *S. invicta* colonies with infected brood in the field have also resulted in infections and the natural spread of this pathogen (Williams *et al.*, 1999). The intracolonial pathway through which the introduction of *T. solenopsae*-infected brood results in infection within the colony is unknown. Possible pathways include the execution and feeding of infected brood to queens and the feeding of spore-laden secretions or substances to queens. Because mature fire ant queens do not forage for food, adult worker caste ants feed queens and may play a role in facilitating infection of the queens. The objective of this study was to elucidate the pathway(s) by which the introduction of *T. solenopsae*-infected brood into fire ant colonies results in the infection of their queens.

MATERIALS AND METHODS

Fate Of Brood Inoculum

To determine whether infected brood used to inoculate colonies are either killed or adopted, we introduced dyed, infected and uninfected brood into nondyed, uninfected *S. invicta* colonies originally reared in the laboratory from newly mated queens. Introduced brood were dyed by feeding five *T. solenopsae*-infected and five uninfected colonies a carbohydrate-based attractant (Vail *et al.*, 1999) formulated into a gel containing 1% (wt/wt) Nile blue A (88% dye content; Aldrich

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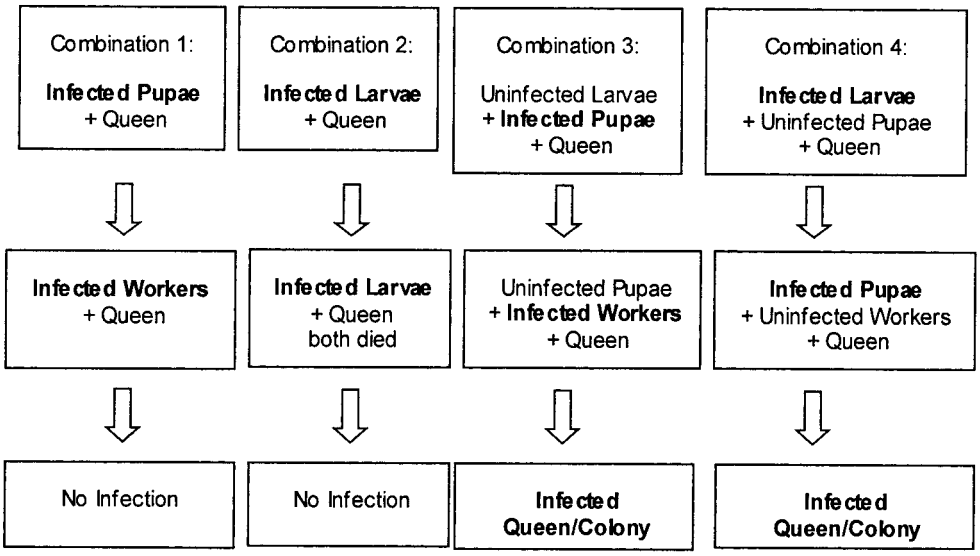

FIG. 1. Progression and outcome of combinations of live *S. invicta* larvae and pupae, obtained from *Thelohania solenopsae*-infected colonies, which were initially placed with uninfected *S. invicta* queens. Note that larvae and pupae developed into worker caste pupae and adults, respectively.

Chemical, Milwaukee, WI). The dye-stained gel was available to the colonies for 8 to 14 days without replenishment to allow the fat bodies of both larvae and pupae to be stained, while permitting the gut contents to be cleared of dye. Thus, trophallactic transfer of dye from the dye-stained introduced brood to the nondyed recipient colonies was prevented.

Uninfected recipient colonies ($n = 10$) each consisted of approximately 2000 adult workers, 7 ml of brood, and a queen. Dyed brood (0.5 g of an equal mixture of dyed larvae and pupae) was separated from adult ants and placed into a small container next to the nest cell of a recipient colony. The presence or absence of dyed brood or callow adults in the nest cell or in refuse piles of dead ants was recorded at 1- to 2-day intervals for 2 weeks. Colonies inoculated with dyed, infected brood were examined for *T. solenopsae* infection after 8 and 24 weeks by looking for vegetative stages in larvae and prepupae smeared individually onto slides and stained with a 20% Giemsa solution (Undeen, 1997). In addition, wet mounts of macerated groups of adults were microscopically examined for meiospores (Williams *et al.*, 1998).

Introduction of Infected Brood with Uninfected Queens.

S. invicta queens from uninfected polygyne colonies were exposed to different combinations of *T. solenopsae*-infected *S. invicta* larvae, pupae, and adult worker caste ants. Ants were held in plastic boxes (7.5 by 23 by 5 cm) with the inner sides of boxes coated with Fluon (ICI Flouropolymers, Downington, PA) to prevent ants from escaping. Each box contained a nest tube, which was a modification of newly mated queen cells described by Banks *et al.* (1981). Each consisted of a glass test tube half-filled with water and plugged with a layer of cotton followed by a layer of dental plaster (Castone, Dentsply Trubyte Division, York, PA). Ants were provided a diet of frozen, farm-raised crickets and sugar-water.

Four combinations of infected and uninfected *S. invicta* life stages were placed with individual uninfected queens. These combinations were selected to isolate inoculum of either infected larvae or pupae with a queen to determine which stage is needed for infection (Fig. 1). Fourth instars were used because they predigest solid foods, which are then passed to the colony by trophallaxis (Petralia and Vinson, 1978). Except for Combination 2, when larvae and/or pupae were introduced into nest tubes that contained the uninfected queen, they were allowed to complete development and eclose to provide infected or uninfected adults. This also prevented execution of queens by adult worker ants that originated from different colonies. A minimum of 25 larvae or pupae were used for each combination per uninfected queen. For each combination there were 10 replicates consisting of one queen each. All of the above combinations had corresponding controls composed of uninfected queens, larvae, and/or pupae.

Combination 1: infected pupae. This combination assessed whether an uninfected queen held with infected pupae and subsequent infected adults that

eclosed from the pupae would result in her infection. Eggs that were laid by four queens were collected weekly. Because infections in previous studies with whole colonies were initially detected at 8 weeks, only eggs collected 8 and 10 weeks after pupae were introduced were stained with Giemsa (Undeen, 1997) and examined for infection. Queens also were stained and examined for vegetative stages and meiospores after 13 weeks. Because it was difficult to detect infections in eggs, larvae were examined for infections from five additional uninfected queens that were held with infected pupae. All eggs from these queens were collected weekly and reared to the 4th instar by placing them with uninfected adult worker ants. Larvae or prepupae reared from eggs collected at 7 and 12 weeks were smeared individually onto slides, stained with Giemsa, and examined for vegetative stages of *T. solenopsae*. The latter five queens were not examined for infection because of queen death, and larval examinations reflected queen infections due to vertical transmission.

Combination 2: infected 4th-instar larvae. This test was conducted to determine whether the tending, by an uninfected queen, of infected 4th instars would result in her infection. *T. solenopsae*-infected 4th instars were removed from the nest tubes when they became pupae and replaced with new infected 4th instars. Infected larvae were placed with a total of 10 uninfected queens. Five queens were obtained from field-collected polygynous colonies and 5 queens were field-collected newly mated queens that could produce eggs. Newly mated queens initially do not require tending by adult worker caste ants for survival. Evidence of *T. solenopsae* infections were to be determined from stained smears of eggs or larvae that were collected weekly.

Combination 3: uninfected 4th-instar larvae + infected pupae. This combination examined the possibility of having infected pupae and/or infected adult workers that eclosed from the pupae as the source of inoculum and uninfected 4th instars facilitating transfer of inoculum to the queen. Predigested food and secretions from 4th instars are provided to the queen by adult workers via trophallaxis (Petralia and Vinson, 1978; Tschinkel, 1995). Fourth instars or prepupae were collected and examined for *T. solenopsae* infection 8 to 20 weeks after brood introduction. Collected larvae and prepupae were assumed to originate from the queens because the 8-week sampling interval allowed for the introduced larvae to develop into pupae and adults. Larva to adult development time for infected and uninfected *S. invicta* is less than 8 weeks (Porter, 1988; D.H.O. unpublished data). For each sampling date and replicate, 10 larvae or prepupae were examined. The combination was replicated 10 times, with each replicate containing a queen, brood, and workers.

Combination 4: infected 4th-instar larvae + uninfected pupae. This combination examined the possibility of inoculum originating from infected 4th instars being trophallactically passed to the queen by uninfected adult workers that eclosed from the introduced pupae. Colony infections were determined from larval and prepupal samples using methods described in Combination 3. Combination 4 had 10 replications.

Worker to Brood Transmission

Infected ants were obtained from field-collected colonies that had 30+ meiospores per viewing field (400×) when a wet mount was prepared from a group of 15–20 ants. Uninfected ants were obtained from laboratory colonies reared from newly mated queens. Infected groups of approximately 250 worker caste adults, from the aforementioned infected colonies, were held in boxes and maintained as described in the preceding experiment. Infection rates for each group of adults were determined by examining 10 wet mounts of macerated individual ants per group for *T. solenopsae* meiospores under a phase-contrast microscope. After allowing the groups of adults to settle for 1 day in the boxes, uninfected brood consisting of approximately 50 small larvae (mainly 2nd and 3rd instars, 1.02 mm ± 0.13 SD in length), 25 large larvae (mainly 4th instars, 1.72 mm ± 0.26 SD in length), or 25 nonmelanized (white) pupae were placed separately into each box. Brood were observed daily, and when melanized pupae developed they were removed and placed into a plastic vial with a slightly dampened laboratory tissue. One to three uninfected adult workers, which tended the pupae, were added to the vials. When callow workers emerged, they were transferred to another vial and allowed to sclerotize for a minimum of 1 day before being frozen and examined individually for infection. Boxes and vials containing ants were held at 27.7–30°C. Controls followed the same procedure as above except that adult worker groups were uninfected. There were 10 replicates of infected and control groups, with replications based on colonies from which the groups of infected workers were obtained.

Detection of Spores in Immature Stages

The ability to infect healthy queens of *S. invicta* by the addition of infected larvae and/or pupae led us to examine these stages for the presence of spores that might play a role in intracolonial horizontal transmission. Larvae and pupae of *S. invicta* from *T. solenopsae*-infected colonies were separated and examined individually with a phase-contrast microscope for the presence of spores. Spores were measured using a split-image micrometer. The number of spores per individual worker caste pupa and adult ($n = 10$ for each stage) were determined with a hemocytometer.

TABLE 1

Detection of *T. solenopsae* Infections after Combinations of Infected and Uninfected Larvae or Pupae Were Held with Uninfected *S. invicta* Queens

Combination of brood stage with uninfected queen	Inoculated colonies				Control colonies		
	% Colonies infected[a] (infected/ colonies)	Total brood infected/ examined[b]	Week infection 1st detected	No. queens infected/ examined[c]	% Colonies infected[a] (infected/ colonies)	Total brood infected/ examined[b]	No. queens infected/ examined[c]
(1) Infected pupae (infected workers)	0 (0/9)	0/42	—	0/4	0 (0/5)	0/50[d]	0/5
(2) Infected larvae	0 (0/0[e])	0/0	—	0/2	0 (0/1[e])	0/0	0/1
(3) Infected pupae (infected workers) + uninfected larvae	37.5 (3/8)	10/150	16	0/3	0 (0/8)	0/110	0/0
(4) Infected larvae + uninfected pupae (uninfected workers)	80 (8/10)	34/180	8	3/4	0 (0/9)	0/140	0/2

[a] Infections determined from larvae, prepupae, and/or queens.
[b] Total number of larvae or prepupae examined among several sample dates.
[c] All queens could not be recovered for examination.
[d] Eggs examined instead of larvae or prepupae.
[e] All or most queens died in inoculated and control colonies before producing appreciable egg or larval samples.

RESULTS

Fate of Brood Inoculum

Live, infected, and uninfected dyed brood were present in all of the colonies during the 2-week observation period. Dead, dyed brood were recovered in refuse piles from three of five colonies given infected brood and three of five colonies given uninfected brood. Recoveries of the dead, dyed brood were intermittent in the inoculated colonies and did not represent a total execution of introduced brood. Recoveries of dead, uninfected introduced brood were more prevalent than in the inoculated colonies for unknown reasons. Live dyed pupae or dyed callow adults were found in all colonies after 2 weeks, suggesting that some introduced larvae and/or pupae survived to adulthood. *T. solenopsae* infection was detected in four of the five inoculated colonies and in none of the control colonies. Meiospores and vegetative stages were observed in adult and brood samples from the infected colonies, respectively, at 8 to 24 weeks after brood was introduced. The presence of infected adults further confirmed that the brood inoculum was raised to adulthood and/or that *T. solenopsae* infections occurred.

Introduction of Infected Brood with Uninfected Queens

Combination 1: infected pupae. None of the nine colonies examined was infected with *T. solenopsae* (Table 1). Eggs, four queens, and a total of 42 larvae (5 to 15 larvae per queen) had no evidence of infection. Because pupae eclosed into infected adults, this combination showed that queens did not become infected in the sole presence of infected adults. All control colonies were uninfected.

Combination 2: infected 4th instar larvae. Queens combined with either infected or uninfected (control) larvae died within 10 weeks (Table 1). Appreciable egg or larval samples could not be obtained from all queens. Because ants are eusocial insects, queens and larvae generally need adult workers to survive, and once workers are present, queens generally do not tend larvae. Thus, these results suggested that this infection pathway is unlikely.

Combination 3: uninfected 4th instar larvae + infected pupae. Infections were detected in three of eight colonies (two queens died before sampling). Infections were detected only in larval or prepupal samples obtained at 16 weeks (Table 1), and infection rates from these three samples were 10, 30, and 60% ($n = 10$ each). Infections were not detected in queen samples. *T. solenopsae* infection was not found in any of the controls. This combination suggested that colony infection occurred when larvae were present with infected adults.

Combination 4: infected 4th instar larvae + uninfected pupae. *T. solenopsae* infections were detected in larvae/prepupae or queens in 8 of 10 colonies (Table 1). Infection rates of larvae/prepupae among infected colonies averaged 45.7% (±36.4 SD) in 7 colonies. In 1 of the 8 infected colonies, an infected queen was found instead of infected larva/prepupa. A total of three of four dissected queens were infected with spores. Larval/prepupal infections initially were detected 8 weeks after infected larvae were added. *T. solenopsae* infection was not found in any of the controls. This combination indicated that infected larvae with adult workers facilitated rapid and consistent queen infections.

TABLE 2

Number of *T. solenopsae*-Infected *S. invicta* Adults That Eclosed from Brood Held with Groups of Adult, Worker Caste *S. invicta* Infected with *T. solenopsae* (Treated) or Held with Uninfected Workers (Control)

Small larvae[a]			Large larvae[b]			White pupae		
% Infect. group (No. reps)[c]	Treated infected/ eclosed	Control infected/ eclosed	% Infect. group (No. reps)[c]	Treated infected/ eclosed	Control infected/ eclosed	% Infect. group (No. reps)[c]	Treated infected/ eclosed	Control infected/ eclosed
100 (1)	0/26	2/17	90[d] (1)	0/17	0/0	90[d] (3)	0/27	0/12
90[d] (2)	0/14	0/16	80 (2)	10/32	0/3	70[d] (1)	0/7	0/6
70[d] (1)	0/8	0/0	70[d] (2)	1/22	0/5	60 (3)	3/21	0/23
60 (3)	0/16	0/44	60 (3)	0/22	0/5	50 (1)	0/4	0/13
50 (2)	0/24	0/29	50 (1)	0/0	0/6	40 (2)	0/22	0/15
30 (1)	0/8	0/3	40 (1)	0/2	0/10	—	—	—
Total	0/96	2/109		11/95	0/29		3/81	0/69

[a] Small larvae were mainly 2nd and 3rd instars, 1.02 mm ± 0.13 SD in length.
[b] Large larvae were mainly 4th instars, 1.72 mm ± 0.26 SD in length.
[c] Percentage infection of a sample of 10 ants obtained from the groups of adult workers held with the uninfected brood and the number of replicates with the given group percentage infection.
[d] Infection rate obtained from a single sample of adults from the source colony and/or from the adult worker groups.

Worker to Brood Transmission

T. solenopsae infection rates of 11.6 and 3.7% were detected in groups containing either large uninfected larvae or white pupae, respectively. Infections were not detected from the small larvae groups except for 2 of 17 workers from one replicate in a control group (Table 2). Because infections were not detected in any of the other control groups, it was assumed that this was an isolated occurrence of contamination. When white pupae were tended (i.e., groomed, because pupae do not feed) by infected adults, infections occurred in 3 of 81 individuals (Table 2) and probably reflected an inefficient mode of infection because of the low infection rate or possible contamination. Contamination could occur if infected workers, used in the test, also carried infected pupae from the source colony. Average infection rates of the adult worker ants that tended the introduced brood ranged from 30 to 100% with an average of 65.7%.

Detection of Spores in Immature Stages

In fresh squashes of *S. invicta* pupae infected with *T. solenopsae* we found what appeared to be binucleate sporoblasts (Fig. 2a) and large numbers of spores (Fig. 2b). The mature spores were ovate and measured 4.5 ± 0.1 × 2.3 ± 0.05 μm (range 3.6–5.6 × 2.0–2.7 μm, $n = 20$, fresh). The posterior vacuole was large and prominent in fresh spores (Fig. 2b). The ratio of these spores, found in individual pupa, to meiospores and free spores, found in adult ants, was 59.5:67.5:1.0. Spores were not observed in larvae infected with *T. solenopsae*.

DISCUSSION

T. solenopsae infection occurred when live, infected brood was adopted and reared by a colony, in contrast to all introduced brood being executed. Evidence of queen infections with *T. solenopsae* was observed most

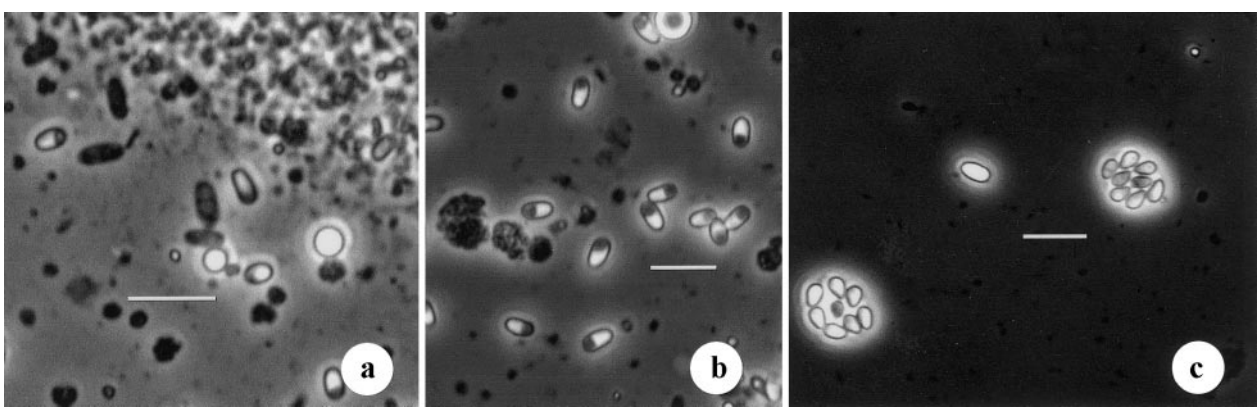

FIG. 2. Sporoblast and spores of *Thelohania solenopsae* in pupae and adults of *Solenopsis invicta*. All fresh and phase contrast; bar, 5 μm. (a) Sporoblast of *T. solenopsae* in pupae. (b) Spores of *T. solenopsae* in pupae. Note the large posterior vacuole at one end of the spore. (c) Binculeate "free" spore and octospores in adult workers of *S. invicta*.

consistently in the combination containing live infected larvae and worker caste adults that eclosed from uninfected pupae. Infections were also obtained from the combination of infected pupae and uninfected larvae. However, infections from the latter combination were detected at 16 weeks, and not at 8 and 12 weeks, which was twice as long as the former combination. The maximum duration from egg through larval stages for *S. invicta* is less than 6 weeks (Porter, 1988). This suggested that queen infection occurred later because the introduced larvae developed into pupae while introduced, infected pupae developed concurrently into infected adults. Thus, queen infection was delayed until new 4th-instar larvae developed from eggs laid by a queen. Because queens did not become infected in the sole presence of infected adult workers (Combination 1), *T. solenopsae* was most likely passed by the adults to the larvae and then to the queen. Thus, spores (type unknown) are probably being transferred from adults to larvae during trophallaxis, perhaps in oral secretions.

We were able to detect infection in adults that eclosed after large larvae and white pupae were reared by infected adult workers. However, infections rates were less than 12% and occurred in only 2 of 10 replicates for either brood stage. Transmission of *T. solenopsae* from live infected, adult workers to uninfected brood has been reported by Allen and Knell (1980). They reported a 25% infection rate when brood (stage not specified) was held with adults that were approximately 100% infected. These results suggested that infected workers could distribute spores to brood. Adult workers of the tropical fire ant *Solenopsis geminata* transmit the microsporidian *Burnella dimorpha* by mechanically passing spores collected from dead infected pupae to uninfected 4th-instar larvae (Jouvenaz *et al.*, 1981). They reported that the binucleate, nonmembrane-bound spores were infective, while the uninucleate, meiospores found in fat bodies were not. Attempts at *per os* transfer of *T. solenopsae* spores in a diet of chicken egg yolk to small groups of workers and brood of *S. invicta* did not result in infections (Allen and Knell, 1980). Presumably, these spores were primarily meiospores because they were obtained from adult ants. Meiospores are found in much greater abundance in adult ants than the free spores (Knell *et al.*, 1977). It is possible that the meiospores of *T. solenopsae* are not infective to other individuals of the same host species. *T. solenopsae* meiospores have been mixed with various food attractants, ingested by 4th-instar *S. invicta* larvae and observed in gut contents and meconium. However, all of these spores remained ungerminated (B. A. Moser, personal communication). Such spores may infect an intermediate host as exemplified in the life cycle of some microsporidia of mosquitoes (Becnel and Andreadis, 1999). However, an intermediate host for *T. solenopsae* has not been reported (Briano *et al.*, 1996; D.F.W. personal observation). Thus, it seems that intracolony transmission of *T. solenopsae* may be dependent on the transfer of a less prevalent infective spore instead of the more numerous meiospores.

As indicated previously, *T. solenopsae* currently is characterized by two spore types that occur only in adult ants. The predominant spore type is formed in packets of eight uninucleate pyriform spores (meiospores = octospores) while oval binucleate (free) spores are rare (Fig. 2c). The adult binucleate (free) spore measured ($4.93 \pm 0.58 \times 1.85 \pm 0.16$ μm), lacks a prominent vacuole, and represents less than 2% of the total spores (Knell *et al.*, 1977). The spores reported here from pupae were smaller in size than the binucleate spore in adults, produced in greater numbers, and distinguished by the prominent posterior vacuole (Fig. 2b) indicating a functional difference (Becnel and Andreadis, 1999). These two spore types also differed by their occurrence in different stages of the host (pupae versus adults). This is the first report for large numbers of spores being produced in pupae and may represent the first report of a third spore type in *T. solenopsae*. Briano *et al.* (1996) reported the presence of a single meiospore in a pupa of *S. richteri*. The role of this spore formed in pupae in the life cycle of this pathogen is unknown. In this study, after the introduction of reciprocal combinations of infected or uninfected larvae and pupae (Combinations 3 and 4) colonies were allowed to continue to grow. Thus, infected pupae, and presumably the same spore type, were present in large numbers. *S. invicta* workers have a buccal tube that filters out particle sizes >0.88 μm (Glancey *et al.*, 1981), which is smaller than the size range of both types of free spores and the meiospores (Knell *et al.*, 1977). However, limits of particle ingestion by queens have not been reported, so there may be a possibility that spores can be ingested by queens.

In summary, the most consistent intracolony infection of *S. invicta* queens by *T. solenopsae* was obtained when live infected larvae and uninfected pupae were placed with an uninfected queen. Delayed queen infection resulted from the combination of infected pupae and uninfected larvae. Large numbers of what appeared to be binucleate spores of *T. solenopsae* were observed in the pupae of *S. invicta*. Studies are underway to determine the possible role for this spore in the life cycle of *T. solenopsae* and to examine its ultrastructural features.

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REFERENCES

Allen, G. E., and Knell, J. D. 1980. Pathogens associated with the *Solenopsis saevissima* complex in South America. *Proc. Tall Timbers Conf. Ecol. Animal Control Habitat Management* **7,** 87–94.

Banks, W. A., Lofgren, C. S., Jouvenaz, D. P., Stringer, C. E., Bishop, P. M., Williams, D. F., Wojcik, D. P., and Glancey, B. M. 1981. Techniques for collecting, rearing, and handling imported fire ants. Advances in Agricultural Technology. U.S. Dept. Agriculture, Science and Education Administration, AAT-S-21/April 1981.

Becnel, J. J., and Andreadis, T. G. 1999. Microsporidia in insects, *In* "The Microsporidia and Microsporidiosis" (M. Wittner and L. M. Weiss, Eds.), pp. 447–501. Am. Soc. Microbiol. Press, Washington, DC.

Briano, J. A., Patterson, R. S., Becnel, J. J., and Cordo, H. A. 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.

Glancey, B. M., Vander Meer, R. K., Glover, A., Lofgren, C. S., and Vinson, S. B. 1981. Filtration of microparticles from liquids ingested by the red imported fire ant, *Solenopsis invicta* Buren. *Insects Soc.* **28,** 395–401.

Jouvenaz, D. P., Lofgren, C. S., and Allen, G. E. 1981. Transmission and infectivity of spores of *Burenella dimorpha* (Microsporida: Burenellidae). *J. Invertebr. Pathol.* **37,** 265–268.

Knell, J. D., Allen, G. E., and Hazard, E. I. 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.

Petralia, R. S., and Vinson, S. B. 1978. Feeding in the larvae of the imported fire ant, *Solenopsis invicta*: Behavior and morphological adaptations. *Ann. Entomol. Soc. Am.* **71,** 643–648.

Porter, S. D. 1988. Impact of temperature on colony growth and developmental rates of the ant, *Solenopsis invicta*. *J. Insect Physiol.* **34,** 1127–1133.

Tschinkel, W. R. 1995. Stimulation of fire ant queen fecundity by a highly specific brood stage. *Ann. Entomol. Soc. Am.* **88,** 876–882.

Undeen, A. H. 1997. "Microsporidia (Protozoa): A Handbook of Biology and Research Techniques." South. Assoc. Agric. Exp. Sta. Directors, South Coop. Ser. Bull. 387. [http://www.okstate.edu/OSU_Ag/agedcm4h/ag_news.]

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

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