# Impact of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) on Polygyne Colonies of Red Imported Fire Ants (Hymenoptera: Formicidae)

DAVID H. OI[1] AND DAVID F. WILLIAMS

USDA–ARS Center for Medical, Agricultural, and Veterinary Entomology, 1600 SW 23rd Drive, Gainesville, FL 32608

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**ABSTRACT** Three studies were conducted to assess the effects of the entomopathogen *Thelohania solenopsae* on polygynous, red imported fire ant, *Solenopsis invicta* Buren, colonies. A total of 57 of 122 queens (46.7%) from nine, field-collected, polygyne, *S. invicta* colonies, was infected with *T. solenopsae.* Infection rate of queens for each colony ranged from 25 to 75%. Laboratory colonies of polygyne *S. invicta*, with three to 12 queens, were inoculated and infected with *T. solenopsae.* Brood levels in all infected colonies declined to 0 after 26–52 wk. Brood did not reappear in any of the colonies after 3–11 wk, even though in two of the eight infected colonies, five fertile queens that were uninfected were recovered. Thus, polygyne, *S. invicta* colonies infected with *T. solenopsae*, which were confined and isolated under laboratory conditions, did not recover. Field plots that contained polygynous *S. invicta* colonies, which were infected with *T. solenopsae*, were monitored over a 2-yr period. Infection rates increased during the study and reached a maximum of 93%. Fire ant nest density and colony sizes fluctuated over time, with maximum reduction of 63% per plot. In general, fire ant reductions were attributed to smaller colony sizes. *T. solenopsae* infections in polygynous *S. invicta* can result in a slow colony decline and death. Under field conditions, the prolonged colony death may mask the impact of *T. solenopsae* by allowing for concurrent reinfestations.

**KEY WORDS** *Solenopsis invicta*, fire ant, entomopathogen, pathogen, disease, biological control

INFECTIONS OF THE microsporidium *Thelohania solenopsae* Knell, Allen & Hazard have been reported predominately from polygynous (i.e., multiple queen) colonies of the red and black imported fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel, respectively (Briano et al. 1995a, Williams et al. 1998). The effects of *T. solenopsae* on laboratory colonies of *S. invicta* containing single queens (i.e., monogyne) have been documented by Williams et al. (1999). They reported that brood production was reduced significantly and queens died prematurely in infected colonies.

Field inoculations of polygynous, *S. invicta* colonies with *T. solenopsae* resulted in a 30% reduction in fire ant populations in the inoculated colonies after 11 mo (Williams et al. 1999). Population reductions were due to a decline in the number of ants per colony, rather than a reduction in the number of colonies (D.F.W., unpublished data). In field surveys conducted in Argentina, *T. solenopsae*-infected *S. richteri* colonies were smaller than uninfected colonies (Briano et al. 1995a). Because not all of the queens may be infected with *T. solenopsae* in polygynous fire ant colonies (Williams et al. 1998), these colonies may recover after a decrease in ant numbers. Objectives of the studies reported herein were to document per colony infection rates of queens from *T. solenopsae*-infected, field-collected, polygyne *S. invicta;* to document the impact of *T. solenopsae* inoculation on polygyne *S. invicta* laboratory colonies; and to monitor the prevalence and effect of *T. solenopsae* on a field population of polygynous *S. invicta.*

## Materials and Methods

**Queen Infection Rates of Polygyne Field Colonies.** Nests of polygynous *S. invicta* were collected from three locations in Alachua County, FL. Infections of *T. solenopsae* were detected by examining slide mounts for meiospores of the liquid portion of groups of adult, worker caste ants (30–50 ants) that were macerated in water (Williams et al. 1999). Infected colonies were separated from soil with dripping water (Jouvenaz et al. 1977, Banks et al. 1981) to locate adult, dealate female reproductives. Female reproductives were dissected to determine if their spermathecae were inseminated and then were examined individually for *T. solenopsae* meiospores following procedures described above for adult worker ants. The percentages of uninseminated and inseminated reproductives (queens) that were infected were determined for each colony.

[1] E-mail: doi@gainesville.usda.ufl.edu.

**Laboratory Inoculation of Polygyne Colonies.** Uninfected, polygyne *S. invicta* colonies were collected from a pasture in Taylor County, FL, checked for *T. solenopsae* infection, and separated from soil as described previously and maintained in the laboratory (Banks et al. 1981) at room temperature for the duration of the study. A total of 16 colonies was paired according to collection date and the number of queens per colony. Colonies in this study contained an average of 50,375 (range, 30,000–70,000) adults (workers), 40.6 ml of brood (range, 20–55 ml), and 3–12 queens. One colony in each pair was inoculated by placing 1 g of brood from a *T. solenopsae*-infected colony next to the colony's nest cell (Williams et al. 1999). Brood used for inoculum was obtained from a single *S. invicta* colony collected in Alachua County, FL. The infection level of the inoculum averaged 66.7 ± 5.8% (mean ± SD), and was determined by examining slide mounts of 10, fourth instars and/or prepupae for vegetative stages of *T. solenopsae* (Undeen 1997, Williams et al. 1998).

Brood from the inoculated colonies was examined for *T. solenopsae* at 4-wk intervals beginning with the eighth week after inoculation. Brood from control colonies was examined for *T. solenopsae* once per colony between week 26–40. At the end of the study, remaining queens from all colonies were dissected to determine if they were inseminated and examined for *T. solenopsae* meiospores. Brood volume and the number of adult worker ants per colony were visually estimated using procedures modified from Banks and Lofgren (1991). Brood and worker estimates were obtained at 2-wk intervals for 54 wk. These estimates were averaged over all sampling dates and compared between the inoculated and control colonies using a two-way analysis of variance (ANOVA) (SAS Institute 1996) with replicates comprised of the paired colonies. The association between the initial number of queens and the time required to obtain a 90% reduction in brood in infected colonies was assessed by Pearson's correlation (SAS Institute 1996). Time to 90% brood reduction was determined from each colony by regressing percentage brood reduction on either time or logarithmically transformed ($\log_{10}$) time.

***T. solenopsae* in a Field Population of Polygyne *S. invicta*.** In a pasture that contained *T. solenopsae*-infected, polygynous, *S. invicta* colonies, nest sites (mounds) within two circular plots (0.116 ha, 19.2 m radius each) were monitored for changes in *S. invicta* populations and prevalence of *T. solenopsae* infection. These plots were originally established by Williams et al. (1999), where five colonies in one plot (plot A) were inoculated with brood from *T. solenopsae*-infected colonies and a second plot (plot B) was used as a control. The control plot was still uninfected at the start of this study. For all mounds within each plot, adult worker ants were collected and examined for *T. solenopsae* meiospores, and colony population size and reproductive status of queens were assessed using the USDA population index ratings (Lofgren and Williams 1982). The population index ratings are values on a scale of 1–5 and 5, 10, 15, 20, and 25, which corresponded to visual estimates of adult ant populations that were grouped into categories shown in Table 1. Population index ratings of 1–5 were assigned to colonies of an appropriate size category when brood of the worker caste was not observed. Lack of worker brood is indicative of abnormal reproduction. When worker caste brood was observed, which indicated the presence of a reproductively viable queen(s), population index ratings of 5, 10, 15, 20, or 25 were assigned. Plots were monitored at 2–6 mo intervals, and the study was terminated after 2 yr because the landowner decided to plow the pasture.

**Table 1. USDA population index values for imported fire ant colonies with or without worker caste brood**

| No. of worker ants per colony | Population index | |
|---|---|---|
| | Without worker brood | With worker brood |
| 1 to 100 | 1 | 5 |
| 101 to 1,000 | 2 | 10 |
| 1,001 to 10,000 | 3 | 15 |
| 10,001 to 50,000 | 4 | 20 |
| > 50,000 | 5 | 25 |

Colony populations also were designated as being small, medium, or large, based on the number of adult workers estimated from the USDA population indices. Small colonies had population index ratings of 1, 2, 5, and 10 (1–1,000 workers), medium colonies had population index ratings of 3 and 15 (1,001–10,000 workers), and large colonies had population index ratings of 4, 5, 20 and 25 (10,001–50,000+ workers). Summing the population index ratings per plot gave a value that represented the number and size of colonies within the plot. Pearson's correlation (SAS Institute 1996) was used to assess the association of percentage infection with population index rating sums. A *t*-test (SAS Institute 1996) was used to compare the average population index between sample dates with high (83% infection, July and September 1999) and low infection rates (0–4% infection, March and September 1998). This comparison excluded plot A because infections were well established in the initial sampling.

## Results and Discussion

**Queen Infection Rates of Polygyne Field Colonies.** The infection rate for all queens examined from the nine colonies was 46.7% (57/122), and the average (±SD) infection rate per colony was 49.6 ± 16.6% (Table 2). Inseminated spermathecae were found in 98% (93/95) of the dissected queens, and 44.1% of these mated queens were infected with *T. solenopsae.* Of the two unmated queens, one was infected (Table 2). Infection rates reported here were higher than the 31% reported by Williams et al. (1998), which was a composite of two colonies containing 40% (4 of 10 queens) and 17% (1 of 6 queens) infected queens per colony (D.F.W., unpublished data). In *S. richteri* from Argentina, an average of 66% (range, 25–100%; $n = 7$ colonies) of the queens were infected per colony (J. A. Briano, personal communication).

**Table 2. Percentage of *T. solenopsae*-infected *S. invicta* queens per colony that were inseminated (fertile) and uninseminated (infertile) from nests excavated in Alachua County, Florida**

| Colony | % queens infected (*n*) | | |
|---|---|---|---|
| | Totals | Fertile | Infertile |
| 1 | 75.0 (8) | — | — |
| 2 | 66.7 (6) | 60.0 (5) | 100.0 (1) |
| 3 | 66.7 (3) | 66.7 (3) | − (0) |
| 4 | 50.0 (10) | — | — |
| 5 | 44.4 (63) | 44.4 (63) | − (0) |
| 6 | 44.4 (9) | — | — |
| 7 | 37.5 (8) | 42.9 (7) | 0.0 (1) |
| 8 | 36.4 (11) | 36.4 (11) | − (0) |
| 9 | 25.0 (4) | 25.0 (4) | − (0) |
| Avg ± SD | 49.6 ± 16.6 | 45.9 ± 15.3 | 50.0 ± 70.7 |

—, Queens not dissected.

**Laboratory Inoculation of Polygyne Colonies.** *T. solenopsae*-infected queens were found in two of the seven inoculated colonies that had inseminated queens at the end of the study. In these colonies, two of five and three of five queens were infected. *T. solenopsae* was not detected in either brood smears or queens from any of the control colonies.

*Thelohania solenopsae* was detected in brood produced by queens in all inoculated colonies after 8–16 wk, with an average maximum brood infection level per colony of 46.3% (±26.7% SD). By the end of the study, there were significantly lower volumes of brood ($F = 38.1$; df = 1, 7; $P < 0.01$) and numbers of workers ($F = 47.7$; df = 1, 7; $P < 0.01$) in the inoculated colonies than in the controls. Inoculated colonies initially increased in brood volume and adult workers for ≈10 and 16 wk, respectively, and then declined (Fig. 1). There were no brood in inoculated colonies after an average of 39 wk (±9.0 wk SD), and these colonies remained without brood for an average of 6.25 wk (range, 2–11 wk) before the study was terminated (Fig. 1A). Reductions in adult worker populations were less dramatic than brood reductions, with an average reduction of 45.8% (±39.6 SEM) from initial populations (Fig. 1B).

The initial number of queens per colony was not correlated with the estimated time to a 90% reduction in brood volume ($r = 0.42$, $n = 8$, $P = 0.30$). Because queen age and other factors affect fecundity and the initial queen infection rates were unknown, associating rate of brood decline and queen numbers from infected colonies probably would require a much larger sample size. However, in monogyne laboratory colonies infected with *T. solenopsae*, Williams et al. (1999) reported a 92% reduction in brood after 22 wk from an initial brood volume of 68 ml. In this study, the average estimated time for a 90% reduction in brood from colonies with three to four queens and a smaller initial brood volume of 51.7 ml (±5.8 SD) was 37.9 wk (±4.4% SD), and colonies with six to 12 queens and 34 ml (±13.9 SD) of brood required 45.6 wk (±6.1% SD). Thus, there is evidence for slower brood decline in polygyne colonies. This is not unexpected because the collective oviposition rates of queens from uninfected,

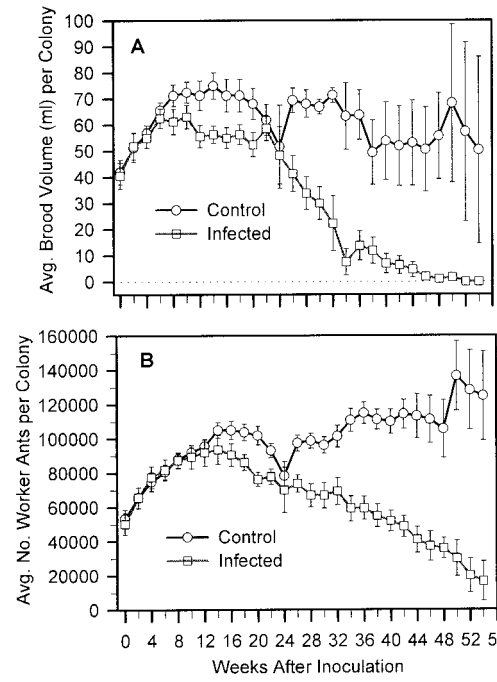


**Fig. 1.** Mean ± SEM ($n = 8$) *S. invicta* brood volume (A) and live adult workers (B) per colony from *T. solenopsae*-inoculated and control laboratory colonies.

polygyne colonies can be significantly greater than that of monogyne colonies (Vander Meer et al. 1992). In addition, initiation of infections in queens may not be uniform, and some queens may remain uninfected, which could allow for staggered declines in brood production.

***Thelohania solenopsae* in a Field Population of Polygyne *S. invicta*.** *T. solenopsae* infection rates increased and *S. invicta* populations fluctuated during the 2-yr monitoring period (Table 3; Fig. 2). Percentage infection rates were not correlated with the sums of the population index ratings ($r = -0.11$, $P = 0.69$, $n = 16$) for plots among different sample dates. This was probably due to an increase in the number of small colonies that were infected during different sampling intervals. This increase in small, infected colonies raised the percentage infection rates (Table 3), but not enough time had passed for these colonies to exhibit reductions in brood and workers. Thus, the sums of the population index ratings increased or remained the same despite the higher infection rates.

Forty-four to 52 wk after infections were initially detected, colonies from plot B had an average population index of 12.2 (±0.38 SE, $n = 144$), which was significantly ($t = 12.3$, df = 361, $P < 0.01$) smaller than the 18.2 (±0.31 SE, $n = 219$) average population index before infection was detected (March 1998). The time interval required for the significant decrease in the population index was similar to that for the 90% brood reductions and decline in worker populations in the laboratory study. Colony numbers and sizes increased in plot B during the January and March 1999, which

**Table 3. Percentages of colonies infected with *T. solenopsae* and percentage changes in population index sums from two, 0.116-ha plots containing polygynous *S. invicta***

| Sample date | Plot A | | Plot B | |
|---|---|---|---|---|
| | % infection ($n^a$) | % change PI$^b$ ($n$) | % infection ($n$) | % change PI$^c$ ($n$) |
| Mar 1998 | 26.4 (53) | — | 0.0 (19) | — |
| Sept 1998 | 59.6 (52) | −40.0 (58) | 4.3 (47) | — |
| Jan 1999 | 89.4 (104) | 29.4 (119) | 66.3 (80) | 12.6 (142) |
| Mar 1999 | 77.6 (85) | 2.7 (86) | 86.2 (138) | 39.4 (140) |
| May 1999 | 76.2 (63) | −61.5 (66) | 61.7 (94) | −12.1 (104) |
| July 1999 | 71.7 (60) | −41.4 (60) | 82.6 (69) | −50.3 (74) |
| Sept 1999 | 78.8 (66) | −41.1 (68) | 82.5 (63) | −62.5 (69) |
| Nov 1999 | 89.2 (93) | 29.4 (93) | 92.5 (120) | −4.2 (125) |
| Mar 2000 | 72.1 (68) | −17.4 (69) | 85.2 (88) | −39.1 (93) |

PI, population index rating.

[a] Number of colonies sampled for *T. solenopsae* does not necessarily equal the total number colonies assigned a PI within a plot.

[b] Percentage change from initial population index sum of 1,267 ($n = 80$) from March 1998. March 1998 PIs were obtained from visual estimates of colony sizes where small colonies were given a PI of 10, medium colonies a PI of 15, and large colonies a PI of 20.

[c] Percentage change from initial population index sum of 2025 ($n = 104$) from September 1998.

was an abnormally mild winter. In plot A, where infections were established longer, a similar pattern of increase in population index ratings to that of plot B occurred (Fig. 2). Lower populations in the summer are often the result of sampling during hot dry weather, which typically makes brood detection difficult. To avoid this sampling limitation, we timed our sampling to occur after sufficient rainfall to ensure that nests and brood were readily visible. Briano et al. (1995b) reported cyclic fluctuations of *S. richteri* colony densities in a *T. solenopsae*-infected pasture in Argentina. These fluctuations were not seasonal, and the greatest reductions occurred in the fourth year, through the fall, winter, and spring.

Infection rates of field-collected, polygyne, *S. invicta* colonies indicated that only a portion of the queens were infected. Our laboratory inoculations of confined, isolated, polygyne colonies also resulted in infections of only some of the queens. However, after brood was no longer observed, brood production was not evident for several weeks and there were not any indications of recovery in these colonies. In the infected field sites, however, numerous small colonies were always present, and fire ant populations recovered in the winter. Based on our laboratory results with 3–12 queens per colony, the presence of small colonies in the field is not due to the recovery of the original infected colonies that had only some of their queens infected. However, the number of queens per colony can vary widely, with various studies reporting from 5 to 186 queens, but 6–43% of these queens were not inseminated (Lofgren and Williams 1984, Vargo and Fletcher 1989). Our results also reflected this variability (Table 2) in queen numbers. Alternatively, the presence of small colonies may be attributed to the adoption of uninfected, newly mated queens (Glancey and Lofgren 1988) or established polygyne queens (Vander Meer and Porter 2001); budding, or splitting, off of larger colonies (Vargo and Porter 1989); and/or establishment of new colonies by queens from mating flights (Porter et al. 1988). In Argentina, Briano et al. (1995b) reported an 83% decrease in nest densities from a polygynous *S. richteri* field population infected with *T. solenopsae.* This also may be indicative of the lack of recovery of *T. solenopsae*-infected, polygyne colonies. However, for polygynous *S. invicta,* which are not territorial, the extended decline of individual colonies prolongs the opportunity for movement of infected brood and workers from infected colonies to uninfected colonies (unpublished data), which can favor the perpetuation of the pathogen. The natural mechanism of transmission of *T. solenopsae* between colonies has not been documented. However, based on evidence of transovarial transmission (Knell et al. 1977, Briano et al. 1996) and artificial inoculations with infected brood (Williams et al. 1999, Oi et al. 2001), brood raiding between colonies (Tschinkel 1992) is one plausible method of transmission. To date, no evidence exists of infected queens recovering from *T. solenopsae* infections. Because the impact of *T. solenopsae* on *S. invicta* colonies is a chronic debilitation of queens (Williams et al. 1999), long-term suppression of *S. invicta* populations under field conditions may be dependent on increasing the ratio of infected to uninfected colonies

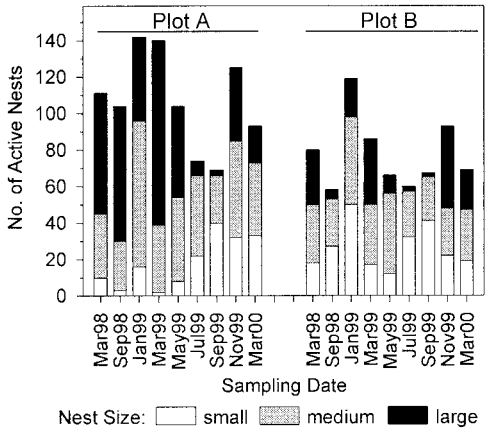


**Fig. 2.** Number and size of active, *S. invicta* nests observed on different sample dates from *T. solenopsae*-infected field plots.

and/or limiting the rate of reinfestation by uninfected colonies.

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