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Note

# Yellow-head disease caused by a newly discovered *Mattesia* sp. in populations of the red imported fire ant, *Solenopsis invicta*

Imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae: Myrmicinae), are invasive species inadvertently introduced into the United States from South America in the early 1900s (Williams et al., 2001). The annual economic impact of imported fire ants has been estimated as 1.2 billion dollars for Texas alone (Lard et al., 2001). If this figure is extrapolated to all infested areas in the United States, the economic impact would be >6.5 billion dollars.

Surveys of pathogens conducted both in the imported fire ant homeland in South America (Jouvenaz et al., 1980) and in the United States (Beckham et al., 1982; Jouvenaz et al., 1977) have revealed few pathogens that can be used in biological control programs. Microsporidia are common pathogens but few other protozoa have been observed in fire ant populations. The Neogregarine *Mattesia geminata* infects larvae of *Solenopsis geminata* and causes mortality during the pupal stage (Jouvenaz and Anthony, 1979). Infected *S. geminata* pupae turn black before dying and infection does not occur in the adult ant. *M. geminata* also infects several other Myrmicinae ants and dying adult insects have been observed to harbor oocysts of the pathogen (Buschinger and Kleespies, 1999).

Recent surveys undertaken in Florida for the presence of *Thelohania solenopsae* revealed a new protozoan in *Solenopsis invicta* populations. This pathogen was first observed in workers collected in southwest Alachua Co., Florida. The first signs of this pathogen were observed in large workers and female alates that showed an atypical yellow-orange color head and sometimes thorax (Fig. 1a). These workers can be mistaken as callow (young) ants, whose cuticle is lighter in color due to incomplete melanization of the cuticle. However, the abdomen of an infected ant is normally as dark as that of older ants. We have designated this disease as yellow-head disease (YHD) due to this distinctive characteristic observed in adult ants. Examination of the body contents showed many spindle-shaped oocysts inside the different body parts (Fig. 1b). Oocysts can be seen through the cuticle in all body regions but are easily recognized in the head and the appendages. Oocysts occur in pairs (Fig. 1c) typical of *Mattesia* species (Weiser, 1955). Some ants with the typical yellow-head coloration have no oocysts, but several bilobed structures (Fig. 1d) can be observed in fresh mounts and Giemsa-stained preparations of body contents. These structures are similar to developing gametocysts with presporal stages described by Kleespies et al. (1997). Presence of these structures has led to the tentative placement of this pathogen in the genus *Mattesia*. A protozoan pathogen of *Myrmecia pilosula* (Hymenoptera: Formicidae: Myrmicinae), described from adult ants from Australia (Crosland, 1988), causes a discoloration of adult ants similar to that caused by YHD. Crosland did not identify the pathogenic agent, but characteristics of the gametocyst, which contains >70 spores, are not *Mattesia*-like.

To compare this new pathogen with previously described species, we measured 10 mature oocysts from each of five infected ants. Oocyst size did not differ significantly among ants; oocysts were $18.7 \pm 0.80\,\mu m$ (mean $\pm$ SEM; $n = 50$) in length and $10.3 \pm 0.80\,\mu m$ in width. These oocyst dimensions are larger than those described for *M. geminata* ($13–14 \times 8–9\,\mu m$) (Buschinger et al., 1995; Kleespies et al., 1997). Oocysts were also longer than spores of a pathogen that caused similar discoloration of adult ants in Australia (13 μm) (Crosland, 1988), *Mattesia trogodermae* from dermestid beetles (11–13 μm) (Hall et al., 1971), *Mattesia dispora* from locusts (15.4 μm) (Zizka, 1978), *Mattesia povolnyi* from sunflower moths (11 μm) (Weiser, 1952), *Mattesia grandis* from bollweevils (11.8 μm) (McLaughlin, 1965), and *Mattesia* sp. from pasture beetles (11.2 μm) (Wright, 1993). Oocysts were shorter and wider than those of *Mattesia bombi* ($21.6–27 \times 5.4\,\mu m$) (Liu et al., 1974). The oocysts of the YHD-causing agent are narrower than the published photographs of *M. geminata* oocysts with the length-to-width ratio at $1.83 \pm 0.155$ ($n = 50$) compared to ~1.44–1.48 for *M. geminata* (Buschinger et al., 1995; Kleespies et al., 1997).

No obvious signs of the disease have been observed in immature stages of the ant and no stages of the pathogen have yet been observed in imported fire ant larvae or pupae. Oocyst dimensions and shape, presence of oocysts in adult ants, and the yellow-head symptom are evidence that the causative agent of YHD is a newly discovered species. These characteristics are distinct from those de-


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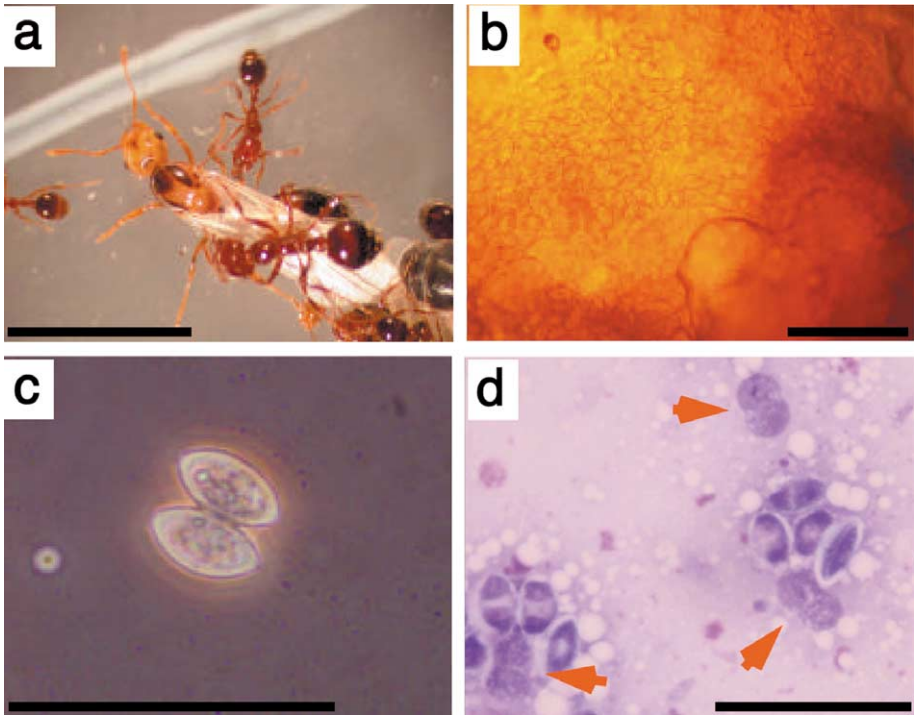


Fig. 1. Yellow-head disease (YHD) in *Solenopsis invicta*: (a) alate female showing typical "yellow-head" sign (compare yellow coloration of alate with normal workers) (bar = ~5 mm); (b) oocysts of the YHD-causing agent seen through the head cuticle of worker ant (bar = 50 μm); (c) pair of oocysts (within gametocyst) seen under phase contrast microscope (bar = 50 μm); (d) bilobed developing gametocysts (arrows) (bar = 50 μm).

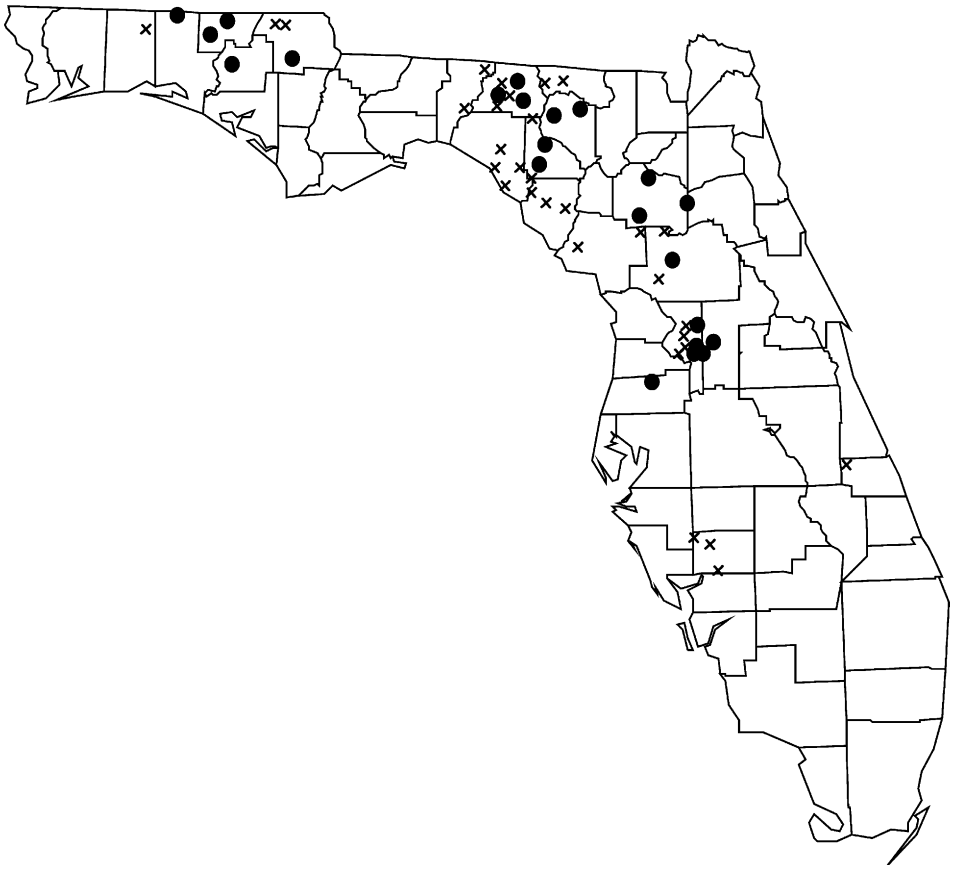

Fig. 2. Florida, USA map showing positive (●) and negative (×) sites for yellow-head disease.

scribed for any other ant disease including *M. geminata* (Jouvenaz and Anthony, 1979; Buschinger et al., 1995; Kleespies et al., 1997), *Mattesia*-like pathogen (Crosland, 1988), and other described *Mattesia* spp.

After initial observation, other sites visited during *T. solenopsae* surveys were examined for the presence of YHD. A total of 64 sites and 1017 nests were visited throughout central and northern Florida; each site consisting of a group of nests collected within a certain area or property. Samples ranged from 1 mound per site to a maximum of 81 mounds collected at the Bar-L Ranch near Marianna, FL. Samples generally consisted of ants collected in a 7-ml plastic vial (Kimble Glass, Vineland, NJ) coated internally with Fluon to prevent escape of the captured ants (Banks et al., 1981). Occasionally, whole colonies were collected by excavating the mound into a 20-liter bucket and later separated from the soil by flotation (Banks et al., 1981). In the laboratory, ants showing the yellow-head symptom were examined intact under a microscope (200×) for the presence of oocysts in any body part. Ants were also macerated in water and observed by phase-contrast microscopy (400–630×) for presence of the mature oocysts or bilobed prespore form.

The YHD seems to be widely distributed in Florida (Fig. 2), and was observed in both polygyne and monogyne *S. invicta* colonies. The disease was present in 34% of the sites and in 8% of nests. In the 22 infected sites, the pathogen was observed in an average of 19% of the nests. Most of the infected sites were in northern Florida, and there was a weak tendency ($P = 0.08$) for site infection rates to increase further northeast in the state. In contrast, *M. geminata* was observed in only 1 of 307 colonies of *S. geminata* from 74 sites in Florida, but in approximately 20% of the colonies from 1 infected site (Jouvenaz and Anthony, 1979). Several colonies have been identified in Florida in which a dual infection with YHD and *T. solenopsae* occurs. Dual infections were also observed in individual ants, but consequences of these dual infections have not yet been determined.

Field colonies brought into our laboratory have had high mortality of YHD-infected ants within days after arrival. For instance, a 250-mg live ant sample (196 ants from a >50,000-ant colony) from a colony collected on November 30, 2001, and sampled on December 10, 2001, had only 3.6% of ants with oocysts or bilobed gametocysts typical of the YHD-causing agent (Fig. 3a). Oocysts or bilobed gametocysts were found in 49% ($n = 100$) of cadavers accumulated over the next week (Fig. 3b). Although YHD occurs in all worker sizes, YHD infects a greater proportion of larger than of smaller workers both in live and dead ant samples. A rapid mortality of infected ants indicates that this disease may have significant impact on fire ant populations. Perhaps the rapid mortality observed in field-collected colonies was due to stresses to which ants were subject

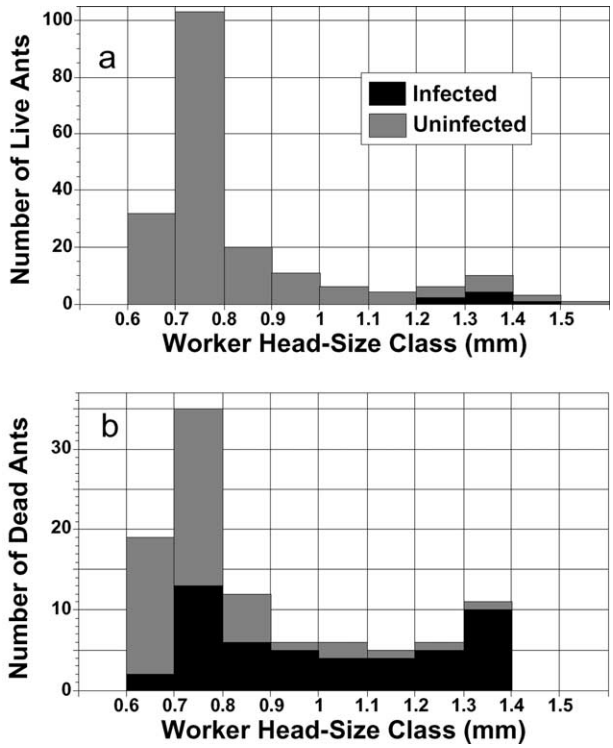


Fig. 3. Frequency of ants in different head capsule-size classes in live (a) and dead ant (b) samples from a colony infected with yellow-head disease (YHD) collected on November 30, 2001, in Walton, Florida. Live sample was taken on December 10, 2001 and the cadaver sample on December 19, 2001.

after collection. External stress factors, such as drought, food shortage, and extreme temperatures may enhance mortality of protozoan-infected insects (Weiser, 1963).

Laboratory cross transmission experiments using YHD oocysts collected from adult *S. invicta* have not produced infections in ants from the same species. Similar attempts with the pathogen *M. geminata* and its host *S. geminata* were not successful (Jouvenaz and Anthony, 1979). These authors suggested two possible explanations for their results: the production of noninfective oocysts in a possibly factitious host (*S. geminata*), or the lack of necessary conditions to allow germination of *M. geminata* oocysts. Attempts by Buschinger and Kleespies (1999) to infect *S. invicta* and *Solenopsis xyloni* with *M. geminata* were also unsuccessful, demonstrating the difficulty in transmitting *Mattesia* diseases in *Solenopsis* ants.

Self-sustaining biological controls are the only long-term area-wide solution for the fire ant problem in the United States. The disease described herein is now found widely spread in Florida, despite no previous observations in surveys and intensive fire ant field studies. Wide distribution of a previously undetected disease with obvious external sign (yellow head) is an indication that the causative agent may have dispersed through the fire ant population very rapidly. High dispersal potential is a

desirable characteristic for biological control agent. Because this disease has never been observed in the fire ants in either South or North America, its origin is unknown. Additional studies, including genetic ones, are planned or underway to determine the taxonomic identity of the causative agent, its mode of transmission, its effect on individual infected ants and fire ant populations, and its biological control potential.

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