Akanthomyces sp.: a newly discovered fungal pathogen affecting *Solenopsis invicta* and *Pogonomyrmex badius* in Florida

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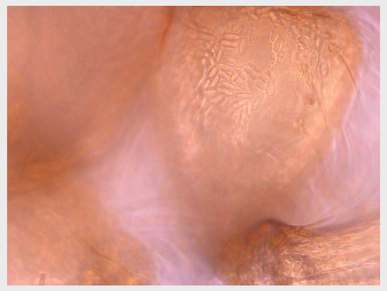
USDA
CENTER FOR MEDICAL, AGRICULTURAL & VETERINARY ENTOMOLOGY
IMPORTED FIRE ANT & HOUSEHOLD INSECTS
GAINESVILLE, FL

ABSTRACT

Workers of the red imported fire ant, *Solenopsis invicta,* and the Florida harvester ant, *Pogonomymex badius,* are naturally infected in Florida with a yeast-like fungus visible through the cuticle. Ants show no behavior change when infected by the fungus, and no signs of the disease other then the fungal cells observed under high magnification. When inoculated on *S. invicta* larvae and pupae, the pathogen causes premature mortality of the ants either in the immature or adult stages. Inoculation with lower doses allows infected ants to survive and emerge as infected adults. This fungus has been cultured *in vitro* yielding a filamentous fungus that produces thin, delicate, light beige synnemata on which conidia are formed in chains. Similar growth can be observed on infected ant cadavers placed under moist conditions. The fungus has been tentatively classified as *Akanthomyces* sp., and may be related to a fungus described as *Insecticola clavata.*

Akanthomyces cf. *clavatus* *



* Tentative identification by:
Dr. Richard A. Humber, Insect Mycologist & Curator,
USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF),
USDA-ARS Plant Protection Research Unit, Ithaca, NY.

Akanthomyces sp. may also be the agent causing this infection in *S. carolinensis,* a native thief ant.

METHODS

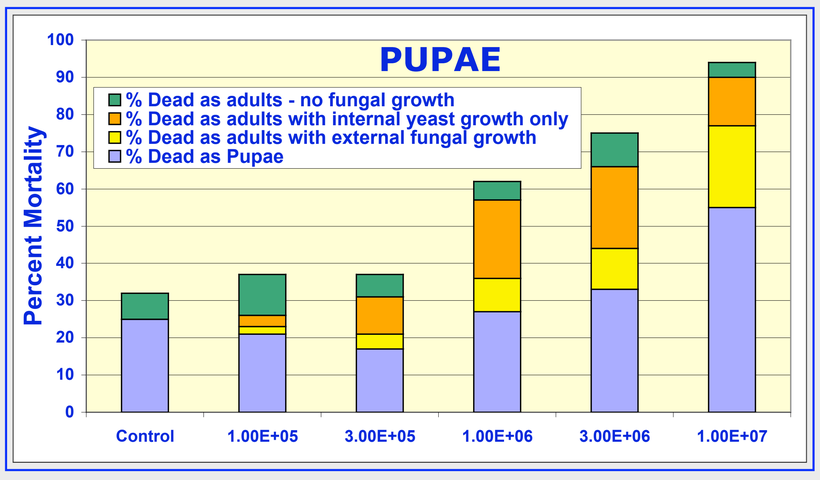
Ant Collections and Observations:

The Florida harvester ant, *Pogonomyrmex badius,* was collected from 3 colonies in Madison Co., FL (N 30.522°; W 83.289°), using a battery-operated vacuum cleaner to aspirate ants as they exited the colony galleries in the soil as the nests were excavated. *Solenopsis invicta* and *S. carolinensis* were collected from several locations in Florida, but mainly in Gainesville, FL, in and around the USDA-ARS, CMAVE. *S. invicta* was collected using plastic tubes of various sizes coated internally with Fluon® (Asahi Glass Fluoropolymers, Inc., Chadds Ford, PA) to prevent ant escape. Colonies of the red imported fire ants were collected by shoveling nest soil into Fluon®- or talc-coated 20-l plastic buckets. Ants were flooded out of the soil and maintained alive in the laboratory. *S. carolinensis* was also collected using baited traps consisting of 74-ml plastic vials containing a piece of Sandies® pecan shortbread (Keebler Co., Elmhurst, IL) and capped with a lid punctured with small (0.5-1 mm) holes. These traps were buried in soil to a depth of 10-15 cm, and collected after 1-3 days. All ants were maintained alive in the laboratory in trays, the walls of which were coated with Fluon® to prevent escape.

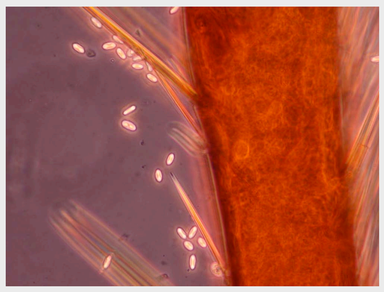
Ants, either alive or dead, were observed under microscope for the presence of yeast cells in the different body parts. Yeast infection was easier to observed in the legs and other thin body sections. Insects were also macerated on microscope slides and the body contents were observed for the presence of yeast-like structures.

Bioassays:

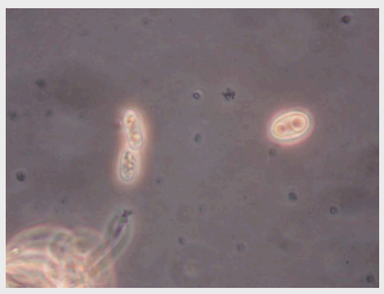
Bioassays were conducted with healthy *S. invicta* ants from colonies maintained in the laboratory. All assays were conducted with five 20-insect replicates per treatment. For assays with larvae and pupae, 10 uninoculated adult ants were added to tend the immature stages. Fourth-instar larvae and white (young) pupae were used. Insects were inoculated by shaking all insects in a replicate in a plastic container containing a small aliquot (20 µl for larvae and pupae; 60 µl for adults) of conidial suspensions. Suspensions were prepared from fungal colonies grown in SDAY plates. After inoculation, the insects were transferred to dental-plaster nest cells (35 mm plastic petri dishes) placed in arenas consisting of a square plastic petri dish (90 mm side) with a cotton-plugged tube of water and one of 10% sucrose solution. Mortality was observed until all insects were dead or had emerged as adults. All insects, whether they died during the experiment or were freeze-killed at the end of the experiment, were observed under microscope for signs of yeast infection. The insects were also surface-sterilized and placed in wells in microtiter plates, which were incubated under moist conditions. These insects were then observed for the presence of any *Akanthomyces* sp. growth.



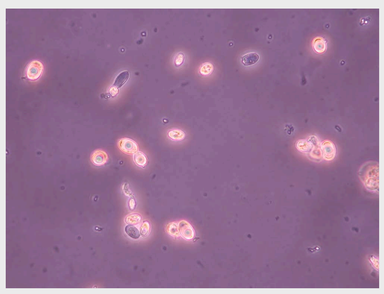
Dose-response was observed in the mortality of pupae inoculated with increasing conidial concentrations of *Akanthomyces* sp. When fungal growth was observed, most cadavers showed only internal yeast growth, with only few cadavers showing external growth and sporulation of the fungus. Adult bioassays showed lower mortality than pupa assays at equivalent doses and few adults showed *Akanthomyces sp.* growth, either as yeast or as external mycelia and sporulation. Larval assays showed greater mortality than pupae assays at equivalent doses. Larval cadavers were quickly decomposed by contaminants.



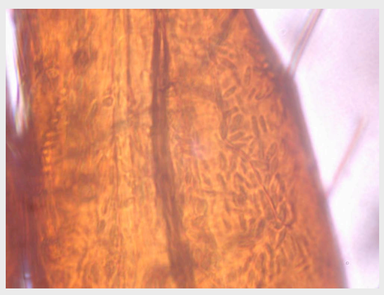
Akanthomyces sp. can be detected as yeast cells growing throughout the body of ants, such as the red imported fire ant, *S. invicta*, shown in this picture. Fungal cells very in size but are typically elongated ovals.



As the fungus matures, and especially after the death of the ant host, fungal cells tend to become round. At the same time, the cell wall thickens. This process seems to occur very quickly after death of the host, apparently in just 1 or 2 days.

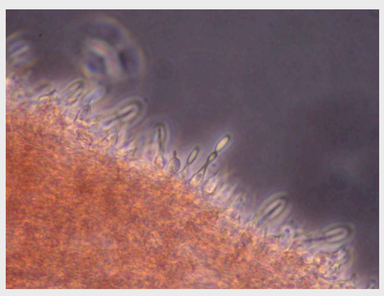


Observations have not been completed on the full cycle of this *Akanthomyces* sp. in the ant hosts. However, the fungal infections seems to follow similar developmental path in the 3 ant species that have been observed with this fungal infection.

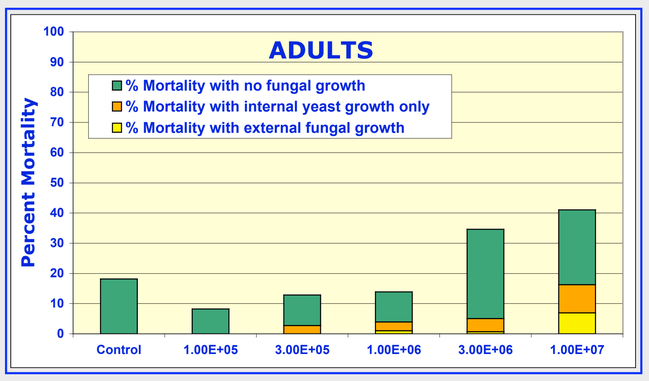


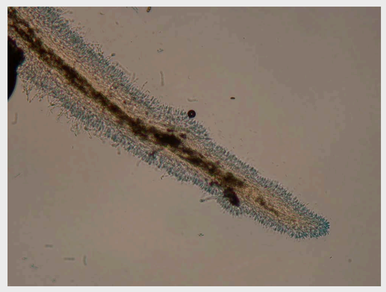
Akanthomyces sp. was isolated originally from a Florida harvester ant (*Pogonomyrmex badius*) from a colony infected with another fungal pathogen of ants, *Myrmicinosporidium durum.* The yeast infection was initially thought to represent a early stage of *M. durum* development. Similar fungus has now been isolated from *P. badius*, *S. invicta,* and *S. carolinensis.* However, the colony from this last host is too young to allow identification.

Akanthomyces sp. initially grows on artificial medium as a chlamydospore-rich hard culture seen at the center of the plate shown in this picture. The fungus grows very slowly under the condition tested so far. More than 4-6 weeks may pass before synnemata start to form, and conidia are produced.

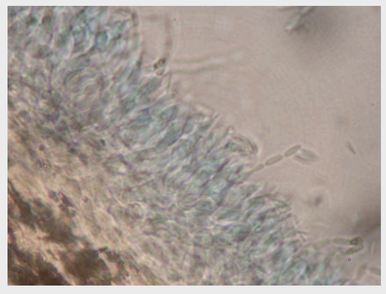


Conidia are formed at the tip pf flask-shaped conidiogenous cells over the surface of the synnemata. The conidia are cylindrical, with a slightly wider midsection.





Akanthomyces sp. produces synnemata that are approximately cylindrical, except for the terminal portion. Occasionally the hymenium many not cover the total length of some synnemata but this character seems to vary depending on conditions in the culture plate.



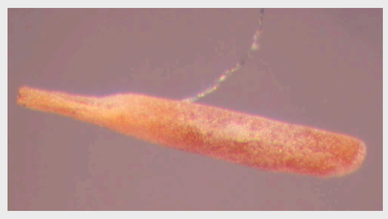
Akanthomyces sp. produces conidia in chains, with no mucous coating. Conidia production is not abundant under the conditions tested for this fungus, and may vary greatly over the surface of the same culture medium. Areas of the plate may have abundant synnemata production while others may lack synnemata completely.

Unlike other deuteromycetes such as *Metarhizium* spp. and *Beauveria* spp. that will readily grow and sporulate on host cadavers, *Akanthomyces* sp. fails to grow mycelia and produce synnemata and conidia on most of the infected hosts.

Optimum conditions for such growth have not been investigated, but adequate conidia production can be obtained occasionally on cadavers placed under 25-27°C and humid conditions.

External growth of this fungus is similar both on *S. invicta* and *P. badius.* Large numbers of *S. carolinensis* have not been inoculated to allow observation of growth on this host species. Despite efforts to surface sterilize cadavers, many cadavers infected with *Akanthomyces* sp. will also have other contaminant fungus growing by the time the pathogenic fungus has grown sufficiently for observation and isolation.

Akanthomyces sp. which infects ants in Florida, has characteristics similar to a fungus described by Mains (Mycologia 42: 566-589, 1950) as *Insecticola clavata* from a cricket collected in Gainesville, FL in 1935. The fire ant-infecting fungus has also been isolated from ants collected in Gainesville, although the original isolation was from harvester ant collected in northern Florida.



A 70-year old synnema from the cricket shown in the previous picture was obtained from the type specimen for *I. clavata* from Univ. of Michigan Herbarium. Attempts to grow this fungus were unsuccessful. Lending of the material was done under understanding that no authorization was granted to attempt isolation of genetic information. Such data would allow comparison of the museum specimen to the fungus isolated from ants.