HOST SPECIFICITY TESTING OF THE *SOLENOPSIS* FIRE ANT (HYMENOPTERA: FORMICIDAE) PATHOGEN, *KNEALLHAZIA* (=*THELOHANIA*) *SOLENOPSAE* (MICROSPORIDIA: THELOHANIIDAE), IN FLORIDA

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The microsporidium *Kneallhazia solenopsae* (Knell, Allen & Hazard), formerly known as *Thelohania solenopsae* (Sokolova & Fuxa 2008), is a pathogen of *Solenopsis* fire ants. It was first reported in the red imported fire ant, *Solenopsis invicta* Buren, collected during South American surveys of fire ants in the early 1970s (Allen & Buren 1974) and initially described by Knell, Allen & Hazard (1977). Further details on its life cycle were subsequently provided by Sokolova & Fuxa (2008). Observations in the 1970s (Allen & Buren 1974; Allen & Knell 1980) and field studies in South America in the 1990s (Briano et al. 1995) led to the conclusion that *K. solenopsae* potentially could be utilized as a classical biological control agent. In 1996, *K. solenopsae* was detected in the U.S. from *S. invicta* populations in Florida and shortly thereafter identified in Mississippi and Texas (Williams et al. 1998; Williams et al. 2003).

The host range of *K. solenopsae* was reported to be specific to *Solenopsis* fire ants in the *Saevissima* species group with the following species being suitable hosts: *S. invicta*, *S. richteri* Forel, *S. quinquecuspis* Forel, *S. saevissima* Smith, *S. macdonaghi* Santschi, and *S. interrupta* Santschi (Oi & Valles 2009; references therein). Recently, Ascunce et al. (2010) reported that *Solenopsis geminata* (F.) and the *S. geminata* x *S. xyloni* McCook hybrid were capable of serving as hosts for *K. solenopsae*. These ant species are in the *geminata* species group and are native to North America. Examination of archived *S. invicta* specimens in Texas documented the presence of *K. solenopsae* in *S. invicta* in the U.S. as early as 1984 (Snowden & Vinson 2006). Ascunce et al. (2010) hypothesized that *K. solenopsae* in North America may have transferred from *S. geminata* to *S. invicta* in this region, but indicated this scenario remains uncertain.

Post-release monitoring for non-target effects from biocontrol agents is an important aspect of assessing the success of biological control projects (Louda et al. 2003; Van Driesche & Murray 2004; Vazquez & Porter 2005). While *K. solenopsae* from South America was never released purposely in the U.S., it is still important to ascertain its host specificity on ants in the U.S. to indicate possible host transitions and concomitant effects on native and non-target species. In addition, there is interest to determine the potential of *K. solenopsae* to control other pest ant species. Our objective was to examine the potential of *K. solenopsae* to infect non-*Solenopsis* ant species by 1) examination of various non-*S. invicta* ant species collected from areas in which *S. invicta* is infected with *K. solenopsae*, and 2) inoculation of non-*S. invicta* ant colonies with *K. solenopsae*.

In Jul and Aug 2008, various species of adult worker ants were collected from nests and food lures at 3 sites in Gainesville, Florida. *Kneallhazia solenopsae* has been documented to be present at the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) site since Apr 1996, and at another site, the former University of Florida (UF) poultry unit, since 2003 (D. H. O. unpublished data). Historical infection levels at the third site (near the UF Microbiology building) were unknown, but this site is within 0.7 km of CMAVE. The percent prevalence of *K. solenopsae* per site was based on the number of *S. invicta* samples that were infected out of the 14-28 nests or food lure samples per site. Infections were determined by phase-contrast microscopy examination of an aqueous extract of a macerated group of approximately 10-30 adult worker ants per sample for *K. solenopsae* spores (Williams et al. 1998). Detection of *K. solenopsae* in the other ant species was performed by polymerase chain reaction (PCR) (Valles et al. 2002) using pooled DNA samples of adult ants grouped by species and collection site with 2-25 ants per sample. All PCRs included positive controls to verify the proper function of the test.

For the colony inoculations, 0.5 - 1.0 g of brood from *S. invicta* colonies having a mean intra-colony infection prevalence of 74% ± 22 SD was placed into laboratory colonies of 6 ant species. Intra-colony prevalence of *K. solenopsae* was based on microscopic examination of wet mounts of 10-20 individual adult workers per colony or 10 Giemsa stained slide mounts of individual 4th instar larvae or prepupae per colony. The following ant species were tested: the Argentine ant, *Linepithema humile* Mayr; the bicolor trailing ant, *Monomorium floricola* (Jerdon); the Florida carpenter ant, *Camponotus floridanus* (Buckley); the pavement ant, *Tetramorium* sp. E (formerly *T. caespitum* [Schlick-Steiner et al. 2006]); the southern fire ant, *Solenopsis xyloni*; and the tropical fire ant, *Solenopsis geminata*. In addi-

tion, *S. invicta* colonies were inoculated to serve as positive controls. This method of inoculation is the only known procedure for infecting fire ant colonies (Oi and Valles 2009).

Colonies were each examined for the presence of vegetative stages of *K. solenopsae* in Giemsa-stained slides of individual larvae and/or prepupae (n = 10) and the presence of spores in extracts of macerated groups of pupae ($n \approx 10$) (Undeen 1997). These microscopy examinations were initiated at 6 or 8 wk after inoculation and continued every 2-4 wk until the studies were terminated usually after 19-24 wk. Infections were determined only from samples obtained after 8 wk (56 d) to avoid sampling brood used for inoculations. Eggs from infected and uninfected *S. invicta* colonies develop into adults within 29 to 47 d at daily minimum and maximum temperatures of 25.6-30.8 °C (Porter 1988; D. H. O. unpublished data). In addition, the volume of brood, numbers of workers and queens were visually estimated every 2-4 wk based on procedures used to assess laboratory colony status of fire ants and Pharaoh ants, *Monomorium pharaonis* (L.) (Banks & Lofgren 1991; Williams & Vail 1993). Laboratory inoculations were conducted from 1998 – 2002.

Kneallhazia solenopsae prevalence among *S. invicta* nests was 37.5% (6/16) at the microbiology site; 64.3% (18/28) at the poultry site, and 100% (14/14) at CMAVE. *K. solenopsae* was not detected in any of the 47 non-*S. invicta* samples, containing nine species, collected from the 3 field sites (Table 1). Although the presence of other ant species has been limited by the dominant *S. invicta* (King & Tschinkel 2008), the co-occurrence with infected fire ants provided an opportunity for the other ant species to acquire the pathogen. Indeed, *K. solenopsae* has been detected in parasitic *Pseudacteon* fire ant decapitating flies captured in similar habitats (Valles et al. 2009).

Kneallhazia solenopsae was not detected in any of the inoculated (n = 23) or control (n = 20) colonies comprising 6 non-*S. invicta* species. In contrast, 50% (n = 14) of the inoculated *S. invicta* colonies became infected (Table 2) while all non-inoculated controls remained uninfected. Brood volume increased during the testing period among the non-*S. invicta* colonies (Table 2), as did worker counts; queens remained alive for the duration of the test. This indicated that these colonies were growing and not negatively impacted by the inoculations.

Kneallhazia solenopsae was not detected in the non-*S. invicta* ants collected 12 yr after its first detection at CMAVE. This is concordant with its host range reported from South America (Oi & Valles 2009) and its absence from non-*S. invicta* ants collected soon after its discovery in Florida (Williams et al. 1998). Infections also did not occur in non-*S. invicta* laboratory colonies that were inoculated directly. Thus, there is no evidence from this study of *K. solenopsae* transmission to non-*Solenopsis* ants. However, the recent detection of *K. solenopsae* in *S. geminata*, and the *S. geminata* × *S. xyloni* hybrid (Ascunce et al. 2010) illustrates the wisdom of periodic post-introduction monitoring.

Summary

Post-entry host specificity testing was conducted on ants in Florida for the fire ant pathogen, *Kneallhazia solenopsae*. The pathogen was not detected in 47 samples that contained 9 non-*Solenopsis invicta* species and a total of 308 ants. Ants were collected from 3 field sites where *K. solenopsae* was present

Table 1. Species and numbers of non-*Solenopsis invicta* ant samples tested for the presence of *Kneallhazia solenopsae* collected at three sites in Gainesville, Florida. *K. solenopsae* prevalence at the sites were as follows: Center For Medical, Agricultural And Veterinary Entomology (CMAVE) 100%; poultry 56.6%; microbiology 37.5%.

Ant Species	Site	No. Nest Samples or Collections	No. of Ants Tested	No. Samples with *K. solenopsae*
Brachymyrmex patagonicus	CMAVE	1	12	0
Camponotus floridanus	poultry	1	5	0
Cyphomyrmex rimosus	poultry	1	1	0
Cyphomyrmex rimosus	CMAVE	1	3	0
Dorymyrmex bureni	poultry	9	63	0
Dorymyrmex bureni	CMAVE	9	63	0
Dorymyrmex medeis	microbiology	11	70	0
Odontomachus brunneus	microbiology	1	3	0
Pheidole moerens	CMAVE	2	25	0
Pheidole moerens	poultry	6	48	0
Pseudomyrmex gracilis	CMAVE	2	10	0
Pseudomyrmex gracilis	microbiology	1	2	0
Pseudomyrmex gracilis	poultry	1	1	0
Solenopsis pergandei	microbiology	1	2	0
Totals:		47	308	0

TABLE 2. SPECIES AND NUMBERS OF NON-*SOLENOPSIS INVICTA* ANT COLONIES INOCULATED WITH BROOD FROM *KNEALLHAZIA SOLENOPSAE*-INFECTED *S. INVICTA* COLONIES AND TESTED FOR THE PRESENCE OF *K. SOLENOPSAE*. ASSOCIATED WITH EACH TESTED SPECIES, AND PRESENTED IN THE LAST COLUMN, ARE THE NUMBERS OF INOCULATED *S. INVICTA* COLONIES THAT BECAME INFECTED (POSITIVE CONTROLS).

Ant Species	Study Duration (weeks)	No. Colonies Infected / No. Inoculated	Avg. ± *SD* % Change in Brood Volume[1]	No. *K. solenopsae* Infected / # Inoculated *S. invicta* colonies
Linepithema humile	24	0/2	+375 (±318)	1/3
Monomorium floricola	24	0/3	+342 (±56)	—[2]
Camponotus floridanus	24	0/1	+446	—[2]
Tetramorium sp. E[3]	20-26	0/4	+60 (±136)	2/4
Solenopsis xyloni[4]	19-24	0/8	+8 (±50)	4/7
Solenopsis geminata	20-24	0/5	+64 (±59)	—[5]

[1]Average ±SD increase (+) or decrease (-) in ml of brood per colony between initial and final colony assessment.
[2]*S. invicta* colonies used as positive controls were the same colonies used for *L. humile*.
[3]*Formerly T. caespitum*. *Tetramorium*. sp. E is not in Florida.
[4]*S. xyloni* is possibly extirpated from Florida.
[5]Positive control tests were not conducted.

at the time of sampling. These sites were either at or within 0.7 km of an area where *K. solenopsae* was observed 12 yr earlier. Infections also were not detected in 23 laboratory colonies consisting of 6 non-*S. invicta* ant species that were inoculated with *K. solenopsae*, i.e., *Linepithema humile* Mayr, *Monomorium floricola* (Jerdon), *Camponotus floridanus* (Buckley), *Tetramorium* sp., *Solenopsis xyloni* McCook, and *Solenopsis geminata* (F.). Thus, transmission of *K. solenopsae* to non-*S. invicta* ants was not evident in our field and laboratory testing.

REFERENCES CITED

ALLEN, G. E., AND BUREN, W. F. 1974. Microsporidian and fungal diseases of *Solenopsis invicta* Buren in Brazil. J. New York Entomol. Soc. 82: 125-130.

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.

ASCUNCE, M. S., VALLES, S. M., OI, D. H., SHOEMAKER, D., PLOWES, R., GILBERT, L., LEBRUN, E. G., SÁNCHEZ-ARROYO, H., AND SANCHEZ-PEÑA, S. 2010. Molecular diversity of the microsporidium *Kneallhazia solenopsae* reveals an expanded host range among fire ants in North America. J. Invertebr. Pathol. 105: 279-288.

BANKS, W. A., AND LOFGREN, C. S. 1991. Effectiveness of the insect growth regulator pyriproxyfen against the red imported fire ant (Hymenoptera: Formicidae). J. Entomol. Sci. 26: 331-338.

BRIANO, J. A., PATTERSON, R. S., AND CORDO, H. A. 1995. Long-term studies of the black imported fire ant (Hymenoptera: Formicidae) infected with a microsporidium. Environ. Entomol. 24: 1328-1332.

KING, J. R., AND TSCHINKEL, W. R. 2008. Experimental evidence that human impacts drive fire ant invasions and ecological change. Proc. Nat. Acad. Sci. 105: 20339-20343.

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.

LOUDA, S. M., PEMBERTON, R. W., JOHNSON, M. T., AND FOLLETT, P. A. 2003. Nontarget effects - the Achilles' heel of biological control? Retrospective analyses to reduce risk associated with biocontrol introductions. Annu. Rev. Entomol. 48: 365-396.

OI, D. H., AND VALLES, S. M. 2009. Fire ant control with entomopathogens in the USA, pp. 237-257 *In*: A. E. Hajek, T. R. Glare and M. O'Callaghan [eds.], Use of microbes for control and eradication of invasive arthropods. Springer, New York, NY.

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

SCHLICK-STEINER, B. C., STEINER, F. M., MODER, K., SEIFERT, B., SANETRA, M., DYRESON, E., STAUFFER, C., AND CHRISTIAN, E. 2006. A multidisciplinary approach reveals cryptic diversity in Western Palearctic *Tetramorium* ants (Hymenoptera: Formicidae). Mol. Phylogenet. Evol. 40: 259-273.

SNOWDEN, K., AND VINSON, S. B. 2006. Development of *Thelohania solenopsae* as an effective biological control agent for the red imported fire ant, *Solenopsis invicta*. Texas Imported Fire Ant Research and Management Project Progress Report Year 1 September 2006, http://fireant.tamu.edu/research/projects/pdf/06snowdenvinson.pdf).

SOKOLOVA, Y. Y., AND FUXA, J. R. 2008. Biology and lifecycle of the microsporidium *Kneallhazia solenopsae* Knell Allan Hazard 1977 gen. n., comb. n, from the fire ant *Solenopsis invicta*. Parasitology 135: 903-929.

UNDEEN, A. H. 1997. Microsporidia (Protozoa): a handbook of biology and research techniques. Southern Assoc. Agric. Exp. Sta. Directors, Southern Coop. Series Bull. 387. http://www.okstate.edu/OSU_Ag/aged-cm4h/ag_news.

VALLES, S. M., OI, D. H., PERERA, O. P., AND WILLIAMS, D. F. 2002. Detection of *Thelohania solenopsae* (Microsporidia: Thelohaniidae) in *Solenopsis invicta* (Hymenoptera: Formicidae) by multiplex PCR. J. Invertebr. Pathol. 81: 196-201.

VALLES, S. M., OI, D. H., AND PORTER, S. D. 2009. *Kneallhazia (=Thelohania) solenopsae* infection rate of *Pseudacteon curvatus* flies determined by multiplex PCR. Florida Entomol. 92: 344-349.

VAN DRIESCHE, R. G., AND MURRAY, T. J. 2004. Overview of testing schemes and designs used to estimate

host ranges, pp. 68-89 *In* R. G. Van Driesche and R. Reardon [eds.], Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practice. Forest Health Technology Enterprise Team, USDA, Forest Service, Morgantown, West Virginia. FHTET-2004-03.

Vazquez, R. J., and Porter, S. D. 2005. Re-confirming host specificity of the fire ant decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) after field release in Florida. Florida Entomol. 88: 107-110.

Williams, D. F., and Vail, K. M. 1993. Pharaoh ant (Hymenoptera: Formicidae): fenoxycarb baits affect colony development. J. Econ. Entomol. 86: 1136-1143.

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., Porter, S. D., Pereira, R. M., and Briano, J. A. 2003. Biological control of imported fire ants (Hymenoptera: Formicidae). American Entomol. 49: 150-163.