Evaluating Spore Count and Sporophorous Vesicle Size in Ovavesicula popilliae (Microsporidia: Ovavesiculidae) in Adult Japanese Beetles (Coleoptera: Scarabaeidae)

Authors: Petty, B. M., Tripodi, A. D., Szalanski, A. L., Johnson, D. T., and Steinkraus, D. C.

Source: Florida Entomologist, 96(4) : 1626-1627

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.096.0454

EVALUATING SPORE COUNT AND SPOROPHOROUS VESICLE SIZE IN *OVAVESICULA POPILLIAE* (MICROSPORIDIA: OVAVESICULIDAE) IN ADULT JAPANESE BEETLES (COLEOPTERA: SCARABAEIDAE)

B. M. Petty, A. D. Tripodi, A. L. Szalanski, D. T. Johnson and D. C. Steinkraus
Department of Entomology, 319 Agriculture Building, University of Arkansas, Fayetteville, Arkansas 72701, USA

*Corresponding author; E-mail: bmpetty@uark.edu

Ovavesicula popilliae (Andreadis & Hanula) (Microsporida: Ovavesiculidae) is a microsporidian pathogen of the Japanese beetle, *Popillia japonica* Newman (Coleoptera: Scarabaeidae), used as a biological control agent (Cappaert & Smitley 2002). This pathogen primarily infects the Malpighian tubules but often spreads systemically (Andreadis & Hanula 1987). *Ovavesicula popilliae* was originally described from Japanese beetles in Connecticut, USA (Andreadis & Hanula 1987) and has since been found infecting *P. japonica* in Michigan (Cappaert & Smitley 2002), Kentucky (Redmond & Potter 2010), and Arkansas (Petty et al. 2012). *Ovavesicula popillae* reportedly produces 32 ovoid spores within a persistent sporophorous vesicle with a mean size of 20.0-21.0 µm × 15.0-15.5 µm (Andreadis & Hanula 1987). Our objectives were to evaluate reported sizes of *O. popilliae* sporophorous vesicles and the number of spores contained within these vesicles.

Adult Japanese beetles were collected in Battle Creek, Michigan from the Charles Binder Park Golf Course from 12-17-VII-2012 with Jumbo Jug funnel mouth traps (1.9 L) (Trécé Inc., Adair, Oklahoma) baited with compound floral and sex pheromone lures. *O. popilliae* infections were diagnosed by removing a small portion of Malpighian tubule from each beetle and examining them microscopically for sporophorous vesicles (Andreadis & Hanula 1987).

Beetle abdomens were macerated, and free sporophorous vesicles were examined under phase contrast microscopy at 400× magnification and measured with a calibrated ocular micrometer (n = 125). Spores within the vesicles were also counted at 1000× magnification. Due to overlapping of spores within a vesicle, vesicles were lightly crushed under their slide covers. Only vesicles that were not ruptured during this process had their spores counted (n = 100).

DNA extractions from the entire abdominal contents of an infected specimen was conducted (Sambrook & Russell 2001) and subjected to PCR using 2 µL DNA template with the primers 18f and 1492r (Vossbrinck & Andreadis 2007). Amplicons were purified and sequenced in both directions (UAMS, Little Rock, Arkansas). Sequences were aligned visually and a BLAST (NCBI) search was conducted with Geneious 6.0.3 (Auckland, New Zealand).

Prevalence of *O. popilliae*-infected Japanese beetle adults collected from Michigan in 2012 was 25% (n = 100). Mean sporophorous vesicle size was 13.3 µm (SE ± 0.1) × 9.3 µm (SE ± 0.1) (Fig. 1). The size of sporophorous vesicles ranged from 9.6-16.8 µm long and 7.2-13.2 µm wide. Mean numbers of spores within each sporophorous vesicle was 24.7 (SE ± 0.8), ranging from 11-64 (Fig. 2), while vesicles containing 32 spores, reported as the typical number (Andreadis & Hanula 1987), occurred 27% of the time. DNA sequencing produced a 1,266 bp amplicon, the entire length of which was unambiguously aligned to the single *O. popilliae* SSU sequence available (GenBank, EF564602).

Our results of 24.7 spores/vesicle and vesicle size of 13.3 µm × 9.3 µm greatly differ from the original measurements of 32 spores/vesicle and a vesicle size of 20.0-21.0 µm × 15.0-15.5 µm reported by Andreadis & Hanula (1987). The variation in vesicle size and the number of spores is noteworthy for two reasons. First-

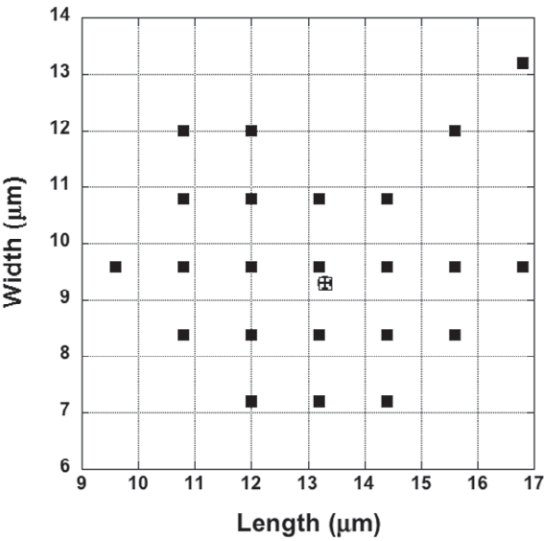

Fig. 1. Aggregated size distribution of *Ovavesicula popilliae* sporophorous vesicles, with the mean size (box and cross) of 13.3 µm × 9.3 µm (SE ± 0.1).

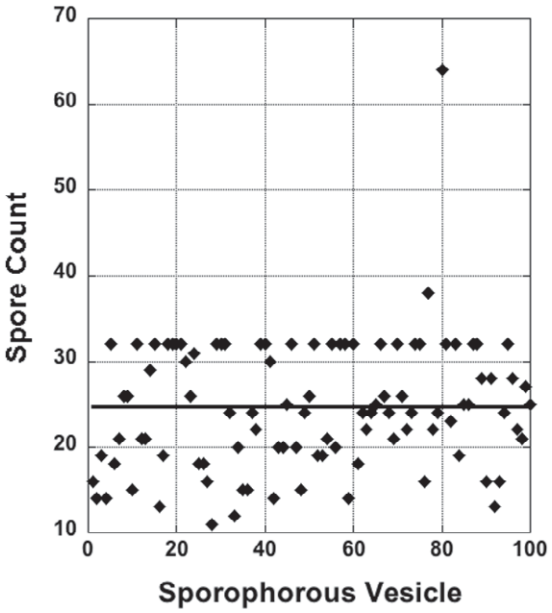

Fig. 2. Spore count distribution of *Ovavesicula popilliae* within sporophorous vesicles, with the mean spore count (bar) of 24.7 (SE ± 0.8).

ly, the vesicle size and number of spores differ from the original description, and they are used for taxonomic identification. Secondly, it is important to know the average number of spores/vesicle when planning experimental applications, such as making spore suspensions. *Ovavesicula popilliae*'s sporophorous vesicles are highly persistent and do not readily break when disturbed. The assumption that 32 spores are present may lead to erroneous estimations of spore density.

One explanation for the difference in size of our sporophorous vesicles and those originally described may be that our *O. popilliae* sporophorous vesicles were collected from adult Japanese beetles. The host life stage, by some unknown mechanism, may affect vesicle size. Alternatively, the differences in sporophorous vesicle size may be evidence of the existence strains of the pathogen in addition to the one found in Michigan and the one was originally described from Connecticut.

The variability in spores may be due to failed divisions of the vegetative stages (Andreadis & Hanula 1987), an unknown effect of the adult beetle's immune system, death of spores, or some unknown factor. Number of spores/vesicle or size of vesicle may differ by host life stage or strain of *O. popilliae* and these variations should be recognized when working with this pathogen for practical purposes, such as counting spores in suspension or identification of taxa.

SUMMARY

Ovavesicula popilliae is a microsporidian pathogen used as a biological control agent of the Japanese beetle (*Popillia japonica* Newman), and reportedly produces 32 spores within a sporophorous vesicle measuring 20.0-21.0 µm × 15.0-15.5 µm. We determined the sporophorous vesicles to have a mean size of 13.3 µm × 9.3 µm and each with a mean of 24.7 spores. Varying number of spores may be due to failed divisions of sporonts, death of spores after formation, host effects or different pathogen strains.

Key Words: amplicon, DNA, host life stage, pathogen strains, *Popillia japonica*

RESUMEN

Ovavesicula popilliae es una microsporidio patógeno utilizado como agente de control biológico del escarabajo japonés (*Popillia japonica* Newman), que según reportes produce 32 esporas dentro de vesículas con medidas de 20.0-21.0 µm × 15.0-15.5 µm. Determinamos que las vesículas tienen un tamaño de 13.3 µm × 9.3 µm y una media de 24.7 esporas. La variación en el número de esporas puede deberse a fallas en la división de los sporonts, muerte de las esporas después de su formación, efectos del hospedero, o diferencias en las cepas del patógeno.

Palabras Clave: amplicon, ADN, estadio del hospedero, cepas del patógeno, *Popillia japonica*

REFERENCES CITED

ANDREADIS, T. G., AND HANULA, J. L. 1987. Ultrastructure study and description of *Ovavesicula popilliae* n. g., n. sp. (Microsporidia: Pleistophoridae) from the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). J. Protozoology 34: 15-21.

CAPPAERT, D. L., AND SMITLEY, D. R. 2002. Parasitoids and pathogens of Japanese beetle (Coleoptera: Scarabaeidae) in southern Michigan. Environ. Entomol. 31: 573-580.

PETTY, B. M., JOHNSON, D. T., AND STEINKRAUS, D. C. 2012. Survey of pathogens and parasitoids of *Popillia japonica* (Coleoptera: Scarabaeidae) in northwest Arkansas. Invert. Pathol. 111: 56-59.

REDMOND, C. T., AND POTTER, D. A. 2010. Incidence of turf-damaging white grubs (Coleoptera: Scarabaeidae) and associated pathogens and parasitoids on Kentucky golf courses. Environ. Entomol. 39: 1838-1847.

SAMBROOK, J., AND RUSSELL, D. W. 2001. Molecular Cloning: A Laboratory Manual 3rd edition, CSH Press, NY, 2,344 pp.

VOSSBRINCK, C. R., AND ANDREADIS, T. G. 2007. The phylogenetic position of *Ovavesicula popilliae* (Microsporidia) and its relationship to *Antonospora* and *Paranosema* based on small subunit rDNA analysis. J. Invert. Pathol. 96: 270-273.