

RESEARCH ON MICROSPORIDIA AS POTENTIAL CLASSICAL AND AUGMENTATIVE BIOLOGICAL CONTROL AGENTS OF THE GYPSY MOTH

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Three genera of microsporidia infect European gypsy moth larval populations and have been documented to reduce the intensity and duration of outbreaks. In order to introduce isolates of these pathogens into North American gypsy moth populations as classical biological control agents, the taxonomy and biology of the pathogens must be elucidated. Taxonomic relationships have been particularly difficult to evaluate and historical reports are inadequate to satisfy taxonomic guidelines for introduction. In addition, host specificity has become an increasingly important issue for introduction. The following completed and ongoing projects were designed to elucidate taxonomic relationships and variability between closely related isolates; evaluate physiological and ecological host specificity; and investigate basic host-pathogen interactions with a goal of introducing microsporidia into North American gypsy moth populations.

Host Specificity: Field studies of the non-target effects of applying ULV sprays (10^9 spores/500 m²), a method for inoculating gypsy moth populations with microsporidia, are indicating that the *Nosema* sp. microsporidia are host specific for the gypsy moth. One species, *Vairimorpha* sp., infected 7 of 80 non-target species (18/640 total individuals), but was not found infecting non-target species in the same plots the following year (0/708). *Nosema* sp. [Levishte, Bulgaria isolate] was not infective to nontarget species (0/591) when sprayed. *Endoreticulatus* species appear to be generalist pathogens and are not being considered for release.

Competition between Microsporidia: When microsporidian infections were mixed in individual host

larvae, certain species dominated in laboratory tests based on sequence of infection, but some combinations of the pathogens allowed maturation of both species. Horizontal transmission, however, may be a factor in competition among microsporidian species infecting the gypsy moth.

Taxonomy and Isolate Variability: The *Nosema* and *Vairimorpha* microsporidian pathogens of the gypsy moth represent a closely related group with variable characteristics. The redescription of one species, *Vairimorpha* (*Thelohania*) *disparis* (Timofeeva) will set the stage for taxonomic evaluation of the entire group and will serve as a basis for either accepting or redescribing the named species within the group. A clearly defined taxonomy is important in order to pursue permission to release these naturally occurring pathogens in North American gypsy moth populations. PCR-RAPDs and proteomics studies using 2-D PAGE analysis are being used to fingerprint and differentiate closely related gypsy moth microsporidia. In addition, relationships and genetic plasticity between isolated populations are being evaluated.

Transmission: Most terrestrial microsporidia are horizontally transmitted between conspecific hosts during the larval stages via feces, cadavers or contaminated silk from infected silk glands. Laboratory tests in diet cup bioassays show that the gypsy moth microsporidia are no exception, but field studies will show the extent to which horizontal transmission occurs in a more ecologically complex arena.

Immune Responses: Gypsy moth larvae fed spores of microsporidia that are natural pathogens in other forest

Lepidoptera (*Hyphantria cunea*, *Malacosoma americanum*) or spores of a virulent noctuid microsporidium, *Vairimorpha necatrix*, mounted some cellular immune response but not significantly stronger than responses elicited by *L. dispar* microsporidia. Nor was the phenoloxidase cascade that produces melanin elicited more strongly than for naturally occurring microsporidia. The strongest responses were elicited by microsporidia, both naturally occurring species and those from other hosts that heavily infected the fat body tissues.

Effects of Parasitism: Parasitism and, thus, release of immune suppressant polydnviruses (PDV) by *Glyptapanteles liparidis* promoted microsporidian infections in the gypsy moth. Parasitism, as well as PDV

+ venom, led to increased spore load in infected hosts. However, PDV + venom did not increase permissiveness of *L. dispar* larvae to microsporidia that were otherwise unable to cause patent infections.

Effects of Dimilin on Microsporidia: The chitinsynthetase inhibitor Dimilin, used extensively in Europe for gypsy moth control, does not affect environmental spores of *Nosema* sp. [Schweinfurt, Germany] directly, but appears to interfere with the formation of both the autoinfective primary spores and the environmentally resistant spores in the infected gypsy moth host. Microsporidian spores produced in larvae fed sublethal concentrations of Dimilin were not infective.