MICROSPORIDIAN PATHOGENS OF THE GYPSY MOTH: RESEARCH UPDATE

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ABSTRACT

Three genera of microsporidia, *Vairimorpha*, *Nosema* and *Endoreticulatus*, infect gypsy moth larval populations in Europe and have been documented to reduce the intensity and duration of outbreaks. Manipulation of these chronic pathogens involves knowledge of taxonomic relationships, host specificity, virulence, transmission, strain variability, interspecific competition and other aspects of host-pathogen interactions. Our ongoing research has addressed these issues and continues as we now conduct inoculative introductions in the U.S. and augmentative releases in Eastern Europe. Our research objectives include the following:

- Elucidate taxonomic relationships and variability between closely related isolates
- Study the mechanisms of microsporidian transmission
- Investigate basic host-pathogen interactions
- Release and monitor microsporidia in native populations of *Lymantria dispar*

We studied interspecific competition among the microsporidia *Nosema lymantriae*, *Vairimorpha disparis*, and *Endoreticulatus schubergi*, performing simultaneous and sequential inoculations of gypsy moth larvae. *Endoreticulatus*, a gut pathogen was not influenced by competition with either *Nosema* or *Vairimorpha*, which both infect fat body tissues. Competition between *Nosema* and *Vairimorpha* altered successful establishment of infection; transmission and competition led to significant suppression of one species depending on timing and sequence of inoculation. The first inoculated species out-competed the second in sequential trials.

Nosema and *Vairimorpha* represent a closely related group with variable characteristics. One species, *Vairimorpha disparis*, was recently characterized and redescribed. Based on small subunit rDNA sequences, we consider the remaining isolates to be strains of *Nosema* lymantriae. The third genus is represented by the species

Endoreticulatus schubergi. Our studies using PCR-RAPDs, gene sequencing (rDNA, HSP70) and proteomics studies (2-D PAGE and DIGE) provide evidence of genetic variability of microsporidia in isolated *L. dispar* populations. The SSU-rDNA and HSP70 genes are nearly identical among *Nosema* isolates and differ only slightly for *V. disparis*. Soluble protein analyses suggest differences in gene expression, particularly between the octospore-producing *V. disparis* and the *N. lymantriae* isolates.

Results of field studies on the nontarget effects of ULV spray applications of *N. lymantriae* and *V. disparis* showed that *N. lymantriae* is ecologically host specific for the gypsy moth. *V. disparis* infected 10 of the 109 nontarget lepidopteran species collected (20 of 248 individuals of the susceptible species), but was not found infecting nontarget species in the same plots the following 2 years. Laboratory studies of *E. schubergi* suggest that it is a generalist pathogen and it is not being considered for release. Microsporidia recovered from nontarget species included generalists *Cystosporogenes operophterae* and *Orthosomella operophterae*. *N. lymantriae* [Bulgarian isolate] and *V. disparis* were released in Illinois in May 2008 and will be monitored over the next three seasons. Augmentative releases were also made in Bulgaria.

The three genera infecting *L. dispar* differ in virulence and in tissue specificity in the host. A series of laboratory experiments provided a good picture of the important pathways of horizontal and vertical transmission in the three genera. Laboratory experiments produced quantifiable data for input in mathematical simulation models of horizontal transmission. Horizontal transmission was also quantified in a field cage study. Acquisition of infection by test larvae increased with increasing ratio of initially infected larvae. At higher densities, percent infection in test larvae leveled off. The duration of the latent period, the time between acquiring infection and release of first spores, is important for horizontal transmission. Transmission of *N. lymantriae* began 11 d post-exposure of test larvae to inoculated larvae. We found the first infected test larvae at 20 dpi; transmission increased over time. Transmission of *V. disparis* also increased then levelled off (at 50 percent of larvae infected) with increasing ratio of larvae initially infected larvae. The correlation between probability of encounter of test larvae with dead, infected larvae and the number of test larvae developing infections is highly significant.