Microsporidian pathogens of the oak processionary moth, *Thaumetopoea processionea* (L.) (Lep., Thaumetopoeidae), in eastern Austria's oak forests

Gernot Hoch, Sabrina Verucchi & Axel Schopf

Department of Forest and Soil Sciences, BOKU – Universität für Bodenkultur Wien

Abstract: Mikrosporidien des Eichenprozessionsspinners, *Thaumetopoea processionea* (L.) (Lep., Thaumetopoeidae) in den Eichenwäldern Ostösterreichs

In einem zweijährigen Screening untersuchten wir das Auftreten von Mikrosporidien bei *Thaumetopoea processionea* an verschiedenen Standorten in Ostösterreich. In neun von 18 Populationen wurden Mikrosporidiosen nachgewiesen, die Prävalenzen lagen zwischen 1,9 % und 15,4 %. Basierend auf lichtmikroskopischen Befunden waren die gefundenen Pathogene den Gattungen *Endoreticulatus*, *Nosema*, *Cystosporogenes* und *Vairimorpha* zuzuordnen. *Endoreticulatus* sp. vermochte im Labor Raupen von *Lymantria dispar* zu infizieren. Das erlaubte die einfache Produktion von Inokulum sowie Untersuchungen mit einem ungefährlichen Wirtsinsekt. Laborversuche mit *L. dispar* zeigten einen langsamen Krankheitsverlauf, der aber in signifikant erhöhter Mortalität resultierte (nur 26 % der oral inokulierten Tiere entwickelten sich zu Imagines), sowie eine effiziente horizontale Übertragung. Eine inokulative Freilassung wurde versucht: dazu wurden *Endoreticulatus*-Sporen in wässriger Suspension auf Blätter isoliert stehender, von *T. processionea* befallenen Eichen ausgebracht. Die Inokulation war erfolgreich, allerdings auf niedrigem Niveau – die maximale Infektionsrate lag bei 9,5 %.

Key words: *Thaumetopoea processionea*, microsporidia, *Endoreticulatus*, inoculative release

G. Hoch, S. Verucchi, A. Schopf, Department of Forest and Soil Sciences, BOKU – Universität für Bodenkultur Wien, Hasenauerstraße 38, 1190 Wien, Austria; E-mail: gernot.hoch@boku.ac.at

Since the late 1990s, the oak processionary moth, *Thaumetopoea processionea* (L.), has been occurring at high population densities in eastern Austria. Particularly, infestations in areas of human settlement have created increasing interest in this insect due to health problems caused by the urticating hairs of the larvae. New methods for biological control are desirable. Like essentially all forest Lepidoptera, *T. processionea* is host for entomopathogenic microsporidia. These obligatory parasitic protists have been evaluated as biocontrol agents against an other oak pest, *Lymantria dispar* (Weiser & Novotny, 1987; Jeffords & al., 1988). Life history traits of *T. processionea* make this insect an even more promising target for the use of microsporidia. The larvae are highly gregarious and stay together in nests made of larval silk for resting periods and molting. Microsporidia utilize several pathways for horizontal transmission that would be aided by these features: spores can be released after host death from cadavers as well as from living larvae via silk or feces. Additionally, many microsporidia are vertically transmitted (summarized in Maddox & al., 1998). In this project, *T. processionea* larvae from various regions in eastern Austria were screened for the natural occurrence of microsporidia. One isolate, *Endoreticulatus* sp., was further studied and mass produced in a laboratory host, *L. dispar*, that is easy to rear and does not pose a health hazard for people working with the insects. An inoculative release was attempted on isolated trees infested with *T. processionea*.

Material and Methods

Microsporidia screening. Oak processionary larvae were collected at 18 locations in the federal states of Vienna, Lower Austria, Styria, and Burgenland in May and June 2004 and 2005 (Fig. 1). They were dissected and examined for the presence of microsporidia under phase contrast microscopy. Microsporidia were documented by photography and methanol-fixed, Giemsa stained smears, isolated, and stored in liquid nitrogen. A tentative determination of the microsporidian genus was based on light microscopic features.

Pathology of *Endoreticulatus* sp. in the laboratory host *L. dispar*. *L. dispar* larvae were inoculated with dosages of $1.2x10^5$ spores of *Endoreticulatus* sp. (isolated from *T. processionea* in Klingenbach in 2004) on the first day in the 3rd instar. Larvae were reared individually on meridic diet at 20±1°C, 16L:8D photoperiod and checked daily. Horizontal transmission was studied by exposing 3rd instar *L. dispar* larvae (= test larvae) to infected larvae in 250-ml cups containing meridic diet. Numbers of infected larvae and test larvae were 1:9, 2:8, 4:6. Each ratio was repeated four times. After 9 days, the larvae were separated and test larvae were reared individually for another 10 days before examination for infection.

Inoculative release of *Endoreticulatus* sp. against *T. processionea*. Spores were isolated from midguts of a dozen of infected larvae. Five liters of spore suspension in water at a concentration of $1.2x10^6$ spores/ml were prepared right before the application. The suspension was sprayed on the leaves of oak trees up to a height of ca. 2 m with a manual pressure sprayer. We selected 3 or 4 isolated groups of *T. processionea*-infested oak trees (3-4 m high, max. 4 trees with connected crowns per group). In 2005, spraying was done on May 25; larvae were in the 3rd and 4th instar at the time of application. In 2006, spraying was done on May 10; larvae were 2nd or 3rd instars. *T. processionea* larvae were collected from the treated trees in (bi-)weekly intervals and microscopically examined for the presence of microsporidia.

Results and Discussion

Microsporidia screening. Microsporidia were found in 9 of the 18 screened populations (Fig. 1). The most prevalent pathogen was *Endoreticulatus* sp. It was found in 6 localities in all studied areas. The pathogen infected the midgut epithelium of the host. Spores were elliptic and measured ca. 2.5 x 1.4 µm in fixed preparations. They were enclosed in envelopes containing variable numbers of spores (typically 8, 16, or 32); many spores could be found individually on the smears. *Nosema* sp. occurred in 4 populations south of Graz, Styria. Infections affected the silk glands, fat body and potentially also Malpighian tubules and gonads. Less frequently, we found *Cystosporogenes* sp. and *Vairimorpha* sp. (in 2 and 1 populations, respectively). Generally, the prevalence of infections was low. Three high density populations that were sampled in oak forests in southern Styria in 2004 had exceptionally high prevalence (up to 15.4%). Also the microsporidian diversity was highest in these populations: all four genera occurred in the area; in one population we found larvae infected with *Endoreticulatus*, *Nosema*, and *Vairimorpha* – however, never causing mixed infections. Larvae sampled from the more isolated nests in the suburban and periurban areas of Vienna were free of infection. We can conclude that microsporidia are a typical component of the natural enemy complex of the oak processionary moth. Outbreak conditions of the host in continuous oak forests may favor the occurrence of microsporidia.

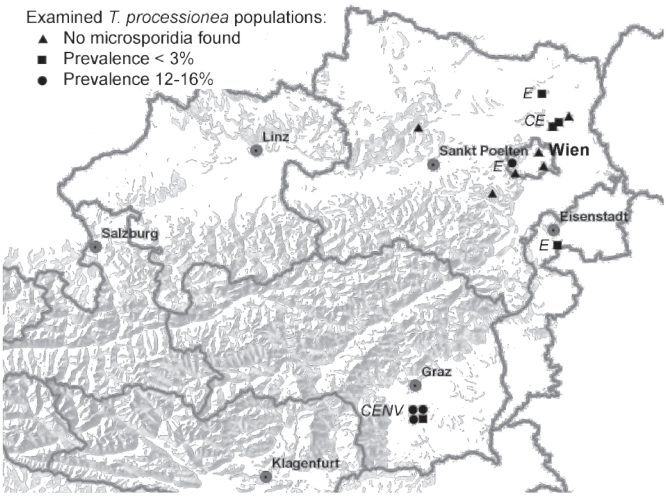

Figure 1: Prevalence of microsporidian infections in oak processionary moth populations screened in 2004 and 2005. We examined a total of 1204 larvae from 18 localities in oak forests as well as in sub- and periurban areas. The following microsporidia were found:
E = Endoreticulatus sp., *C = Cystosporogenes* sp., *N = Nosema* sp., *V = Vairimorpha* sp.

Pathology of *Endoreticulatus* sp. in the laboratory host *L. dispar*. All isolated microsporidia were tested for their infectivity for *L. dispar* larvae. Only *Endoreticulatus* sp. isolated from *T. processionea* collected in Klingenbach, Burgenland, in June 2004 was able to produce regular infections in *L. dispar*. Spores propagated in *L. dispar* were infective for *T. processionea* when administered orally. One *Nosema* sp. from Styria also caused infections in *L. dispar*, however, spore numbers were so low that they could not be isolated for further use. The *Endoreticulatus*-infection in *L. dispar* was comparable to the natural host; after ca. 20 days the larval midgut tissue was usually completely infested and the cells filled with spores. The infections caused by *Endoreticulatus* sp. in *L. dispar* were not highly virulent. 49% of treated larvae managed to pupate, 26% developed into adults. Nevertheless, survivorship was significantly lower than in controls (Fig. 2). The low virulence is comparable to an *Endoreticulatus* sp. isolated from *L. dispar* (Solter & al. 2002). We found *Endoreticulatus* spores in 100% of inoculated insects that successfully developed into adults. This is an important prerequisite for potential vertical transmission of the pathogen to the next generation. In addition to affecting survival of the host, infection also prolonged the larval development. Infected males pupated 42.5±2.0 days post inoculation, control males after 36.8±1.2 days (Student's t test: P = 0.015). In females, the difference was not significant. The fresh mass of those insects that were able to pupate successfully was not significantly reduced.

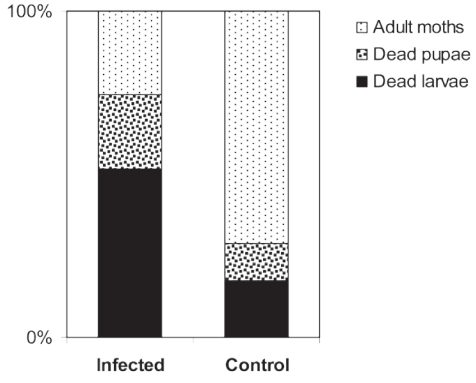

Figure 2: Mortality of *L. dispar* during the larval and pupal stage and successful development to the adult stage in *Endoreticulatus*-infected and uninfected insects (n = 35).

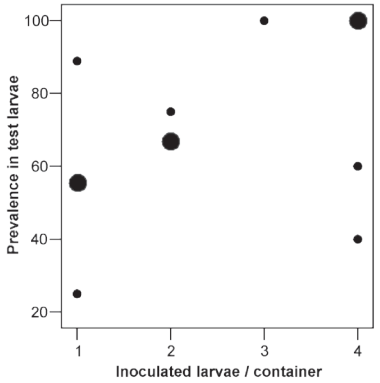

Figure 3: Prevalence of *Endoreticulatus*-infections in susceptible *L. dispar* test larvae after exposure to different numbers of infected larvae for 9 days. An increase of transmission with increasing numbers of originally infected larvae was noticeable; however, a logistic regression did not explain the observed data well (after arcsin-transformation of percent values: R^2=0.215, F=2.740, P=0.129).

The infection was efficiently transmitted horizontally. Prevalence of infection in test larvae after exposure to infected larvae was usually above 50%, in three cases 100% (Fig. 3). The high level of horizontal transmission is due to spore release from living larvae. For gastric microsporidia, this typically occurs with feces (Maddox & al. 1998).

Inoculative release of *Endoreticulatus* sp. against *T. processionea*. Oak processionary larvae collected before the spray were free of naturally occurring microsporidia. Contaminated leaves from the test trees sampled after the spray were infective when fed to *L. dispar* larvae in the laboratory. Leaves collected from the trees one hour after treatment in 2005 caused infections in 31.6% of the challenged *L. dispar*; leaves collected one day after treatment in 2006 caused infections in 11.1% and 30.8% of larvae fed with those leaves for one (n = 27) and four days (n = 39), respectively. Feeding 1 µl of the originally sprayed spore suspension to *L. dispar* larvae in our standard inoculation procedure led to 100% infection (n = 24 in both years). The inoculation of the *T. processionea* field population was successful, however, the prevalence of

infections by the released microsporidium was low. In 2005, first *Endoreticulatus* infections were detected in old larvae sampled on June 22, i.e., 4 weeks after the application. Prevalence of infections on the treated groups of oaks was 3%, 8.9%, and 0%, respectively (n = 99, 79, 77 larvae). No larvae sampled at earlier dates were positive. In 2006, the first infected larvae were found in the sample taken on June 19. Prevalence was 5.1%, 9.5%, 0%, and 1.6%, respectively (n = 59, 21, 100, 62). The highest prevalence occurred on the same group of trees in both years. This group had the smallest crown area, thus, probably a higher percentage of the leaves were treated. The late occurrence of infected larvae reflects the slow progress of the disease. It may also indicate that it takes some time before larvae encounter leaves that were contaminated.

Overall, we showed that the microsporidium *Endoreticulatus* sp. can be introduced into natural populations of *T. processionea* by spraying spore suspensions produced in a laboratory host. Our technique was very simple, improvements of application and spore suspension could increase the efficacy. Application in the evening could increase the uptake of viable spores by larvae; this was not feasible in our case because the release site was located in a recreation area. The results from the laboratory host *L. dispar* indicate that the disease will be transmitted horizontally to other larvae in the field due to spore release with feces. This can happen through contamination of leaves or inside of the larval nests that also contain fecal pellets. Although the gonads are not infected, *Endoreticulatus* has the potential for transovum transmission on the egg surface (Maddox & al. 1998). However, the progress of the disease is slow and its virulence is low. The main reason for using *Endoreticulatus* in the field experiment was that it could be cultured in *L. dispar* as an alternate host. It would be much more cumbersome with *T. processionea* since separate rearing chambers and precaution for people working with this insect are necessary to avoid contact with the urticating hairs. Our screening of *T. processionea* revealed, that the species is host for a variety of microsporidia. The ideal candidate for inoculative would have higher virulence than *Endoreticulatus* and would be similarly efficiently transmitted horizontally via spore release from living larvae or from decaying cadavers. Additionally, it would be transmitted to the next generation. *Nosema* spp. may have these features. If a suitable laboratory host can be found to produce these microsporidia in larger quantities, they should be evaluated in field conditions.

Acknowledgements
We gratefully acknowledge the support of this study by the Forestry Departments of Vienna, Styria, and Lower Austria. The help of state and district foresters in pointing out oak processionary moth infestations for our screening program is highly appreciated. The Forstverwaltung Lobau (Forestry Office and Urban Agriculture, City of Vienna) kindly permitted us to conduct our field experiment on their territory.

References

Jeffords, M.R., Maddox, J.V., McManus, M.L., Webb, R.E., Wieber, A. (1988): Egg contamination as a method for the inoculative release of exotic microsporidia of the gypsy moth. – J. Invertebr. Pathol. **51**: 190-196.

Maddox, J.M., McManus, M.L., Solter, L.F. (1998): Microsporidia affecting forest lepidoptera. – in: McManus, M. L. & A. M. Liebhold (Eds.): Proceedings: Population Dynamics, Impacts, and Integrated Management of Forest Defoliating Insects. Aug. 18-23, 1996, – Banská Štiavnica, Slovak Republic. Gen. Tech. Rep. NE-247. USDA Forest Service, Radnor, Pennsylvania. 198-205.

Solter, L.F., Siegel, J.P., Pilarska, D.K., Higgs, M.C. (2002): The impact of mixed infection of three species of microsporidia isolated from the gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae). – J. Invertebr. Pathol. **81**: 103-113.

Weiser, J., Novotny, J. (1987): Field application of *Nosema lymantriae* against the gypsy moth, *Lymantria dispar* L. – J. Appl. Ent. **104**: 58-62.