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SHORT COMMUNICATION

Assemblage of entomopathogenic fungi infesting immature stages of Noctuidae (Lepidoptera): High diversity but low effect on host populations

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Abstract. Populations of herbivorous insects are considered to be primarily regulated by natural enemies. However, little is known about the ecological role of entomopathogenic fungi. There is very little data on the diversity and prevalence of fungal pathogens in natural insect populations. In this study, the percentage mortality attributable to fungal pathogens for the immature stages of three noctuid moths feeding inside the stems of the herbaceous plant, *Typha latifolia,* were determined. The average percentage mortality caused by fungal pathogens was around 8%, with the value never exceeding 20% at any of the studied sites. As many as ten species of fungi were identified in the 52 infected larvae/pupae collected, this high diversity is consistent with the results of comparable studies. The prevalence of fungal infections did not correlate with host population density or performance of the host insects. This study contributes to the emerging generalisation that populations of insects commonly harbour diverse assemblages of pathogenic fungi, but with low overall prevalence. A significant contribution of these antagonists to regulating insect populations is unlikely.

INTRODUCTION

Populations of herbivorous insects are considered mostly top-down regulated, with natural enemies being key determinants of their population dynamics (Hochberg & Ives, 2000; Peterson et al., 2009). Natural enemies of insects are conventionally divided into predators, parasites (including parasitoids) and pathogens. For the first two groups, there is a massive body of literature on their effect on host population dynamics (Price et al., 2011). Ecological studies on insect pathogens are dominated by research on selected viruses and bacteria (Vega & Kaya, 2012). For the rest, there are primarily descriptive works on various proximate aspects of insect-pathogen interactions, with little information on the role of these interactions in host population dynamics.

Fungi are a notable group of pathogens of insects (Lacey et al., 2015). Entomopathogenic fungi (EPF) are present in several major fungal clades, with about a thousand species described so far (Dallas et al., 2022). Ecological studies on EPF have been primarily motivated by the search for new biocontrol agents (Hesketh et al., 2010). As a result, satisfactory ecological knowledge exists for a few EPF, mostly from agroecosystems (Vega, 2018), leaving large gaps in the basic ecological knowledge on the role of EPF in natural systems (Dallas et al., 2022; Hesketh et al., 2010). Even for the most basic parameter, the percentage of insects succumbing to fungal pathogens in natural populations, the knowledge is highly fragmentary, represented by scattered case studies on very different systems with no unified methodology (Barta & Cagáň, 2003; Elkinton et al., 2019 & Georgieva et al., 2014 for some examples).

In the present study, the prevalence and diversity of entomopathogenic fungi in immature stages of three Lepidoptera feeding inside the stems of a large herbaceous plant, the common cattail, *Typha latifolia* (Poales: Typhaceae), are evaluated. The primary advantage of this system is the ease of obtaining unbiased samples of immature insects: both live larvae and pupae, as well as carcasses of dead insects can be systematically collected by dissecting stems of the host plants (Teder et al., 2013, 1999; Teder & Tammaru, 2002). Such a situation differs from that of Lepidoptera that feed exophytically in which neither pupae nor naturally dead individuals can be easily traced. Moreover, *T. latifolia* typically forms dense stands in distinct patches, allowing for local populations of the associated insects to be unambiguously defined. Taking advantage of this situation, the potential for EPF to affect population dynamics of their host insects was evaluated by examining the ecological determinants of the prevalence of EPF. If EPF are involved in population regulation of their insect hosts, they should be more common at high population densities of the insects. On the other hand, EPF may contribute to the bottom-up regulation of the herbivores in the system studied (Teder

Table 1. The prevalence of fungal infections at the sites studied and in different years.

Coordinates	Year	N[1]	Fungal prevalence	CI[2]	Field[3]		Laboratory[4]	Type[5]
					Larvae	Pupae		
58°16′17″N 26°11′6″E	2020	37	8.1%	2.1 – 22.0%	1	1	1	A
58°15′46″N 26°22′49″E	2020	101	9.9%	5.2 – 17.5%	5	3	2	DD
	2021	22	13.6%	3.9 – 34.2%	1	1	1	
58°8′23″N 26°42′39″E	2020	80	10.0%	4.9 – 18.8%	3	1	4	DD
	2021	46	10.9%	4.2 – 23.5%	4	1	0	
58°9′3″N 26°46′20″E	2020	93	12.9%	7.4 – 21.4%	5	1	6	DD
	2021	34	11.8%	4.1 – 27.2%	2	0	2	
58°7′48″N 26°43′33″E	2020	91	2.2%	0.1 – 8.1%	1	0	1	DD
58°14′26″N 26°12′19″E	2020	19	5.3%	0.01 – 26.5%	0	0	1	RD
58°13′50″N 26°22′32″E	2020	51	2.0%	0.01 – 11.3%	0	0	1	RD
58°10′25″N 26°47′11″E	2021	8	0%	0 – 29.3%	0	0	0	DD
58°9′43″N 27°12′2″E	2021	10	20.0%	4.6 – 52.1%	2	0	0	RD
58°17′42″N 26°55′17″E	2021	17	5.9%	0.01 – 28.9%	0	1	0	DD
58°17′25″N 26°56′22″E	2021	38	0%	0 – 7.9%	0	0	0	FM
58°12′29″N 26°30′30″E	2021	2	0%	0 – 63.1%	0	0	0	A
58°20′45″N 26°45′11″E	1995	238	0.4%	0.01 – 2.5%	0	0	1	FM
58°20′30″N 26°44′45″E	1995	1044	1.3%	0.7 – 2.1%	0	1	12	FM

[1] – number of insects collected; [2] – confidence interval for fungal prevalence was calculated using the Adjusted Wald method; [3] – number of dead larvae and pupae showing visible fungal infection when collected in the field; [4] – number of insects which were collected alive but died of a fungal infection in the laboratory; [5] – types of sites studied classified as follows: flooded meadow (FM), agricultural land (A), damp depression (DD) and roadside ditch (RD).

& Tammaru, 2002) if they are more likely to infect nutritionally stressed individuals.

MATERIAL AND METHODS

In 2020 and 2021, field populations of three species of moths: *Nonagria typhae*, *Globia* (= *Archanara*) *sparganii* and *G. algae* (Lepidoptera: Noctuidae), feeding on *T. latifolia* were sampled in the surroundings of Tartu, Estonia. They were collected at the end of July/ beginning of August, when most of these lepidopterans were in the pupal stage. Thirteen stands of *T. latifolia* (called 'sites' hereafter; Table 1, Fig. S1) were located by screening the rural landscape while driving along countryside roads. In both years at each site studied, three to four 3 × 3 m square plots were designated for recording site-specific variables, such as the size of the plot, height of the cattail plants and average weight of host pupae (Table S1). From each plot, all the moth larvae and pupae, both dead and alive, were collected. In particular, all plant shoots were carefully split in search of pupae and last instar larvae hiding between leaves and within stems. To increase sample sizes, the systematic sampling was complemented by inspecting *T. latifolia* plants growing next to the designated plots for 30 min. The results of these additional searches were used when percentage infection was calculated, but not when the values of site-specific predictors (Table S1) were estimated. The time periods when the collected insects were developing as larvae, i.e. July 2020 and July 2021, were characterized by average temperatures of 16.3°C and 21.2°C and rainfall of 89 mm and 50 mm, respectively.

All pupae and larvae approaching pupation were collected for further examination in the laboratory. Younger larvae were identified to the genus level based on their colour and released (we were unable to rear them in the laboratory), no young larvae were found dead with signs of fungal disease. The released young larvae were included in the population density estimates but disregarded when the prevalence of fungal infections was calculated. Live pupae were stored in sterile 50 ml plastic vials with pierced lids and kept in the laboratory at room temperature until the eclosion of the moth or emergence of a parasitoid. Pupae that died in the laboratory were inspected for visual signs of fungal infection. In addition, some information was retrieved from the data collected from the same system in 1995 (Teder et al., 1999, 2013; Teder & Tammaru, 2002) when, along with recording percentage parasitism, the cases of visible fungal infection of moth pupae was recorded, but the fungi were not identified.

For the identification of the fungi, we inoculated visible fungal material from the dead insects into Petri dishes with 2% malt extract agar (Oxoid, Cambridge, UK), a week before collecting material for DNA extraction. Fungal strains and dried specimens were deposited at the Natural History Museum, University of Tartu (collection acronyms TFC and TUF). The procedures for DNA extraction, PCR and sequencing followed the protocols described by Põldmaa et al. (2019). For all isolates, the ITS region of ribosomal DNA was sequenced at Macrogen Europe BV (Amsterdam), using the Standard-Seq service. The sequences were edited in Sequencher® version 5.4.6 (Gene Codes Co., Ann Arbor, MI, USA), uploaded via PlutoF (Abarenkov et al., 2010)

Table 2. Fungi isolated from Lepidoptera feeding on *T. latifolia*.

Fungal taxa			UNITE SH[1]	N	Field[2]		Laboratory[3]	Host insect[4]
Order	Family	Species			Larvae	Pupae		
Eurotiales	Aspergillaceae	*Aspergillus* sp.	SH3214790.09FU	2	–	–	2	*G. sparganii* *N. typhae*
		Penicillium bialowiezense	SH2071955.09FU	2	–	–	2	*N. typhae*
Hypocreales	Cordycipitaceae	*Akanthomyces muscarius*	SH2006624.09FU	3	2	1	–	*N. typhae*
		Beauveria bassiana	SH2006785.09FU	17	12	3	2	*G. sparganii* *N. typhae*
		Beauveria pseudobassiana	SH4059696.09FU	6	4	–	2	*N. typhae*
	Nectriaceae	*Fusarium langsethiae* sc[5]	SH1699024.09FU	1	1	–	–	*N. typhae*
		Fusarium sporotrichioides	SH3187143.09FU	1	1	–	–	*N. typhae*
		Fusarium sporotrichioides sc[5]	SH3194426.09FU	10	1	4	5	*G. sparganii* *N. typhae*
		Fusarium trincinctum sc[5]	SH1698094.09FU	2	–	1	1	*G. sparganii* *N. typhae*
Microascales	Microascaceae	*Microascus brevicaulis*	SH1681802.09FU	2	–	–	2	*N. typhae*
Unidentified fungi from 1995				19	3	1	15	*G. sparganii* *N. typhae*

[1] – species hypotheses according to UNITE (a database and sequence management environment, Kõljalg et al., 2020), assigned based on 1.0 or 1.5% distance threshold; [2] – number of dead larvae and pupae with visible fungal infection when collected in the field; [3] – number of insects which were collected alive but died of a fungal infection in the laboratory; [4] – no fungi were recorded from the pupae of *Globia algae*; dead larvae of *Globia* spp. could only be identified to the genus level; [5] – species complex.

and made available in the UNITE database (Kõljalg et al., 2020), which also served as the basis for species identification (Table 2).

The incidence of fungal infection (as a binary trait: yes/no) was analysed as dependent on different parameters of the sites studied (see Tables S1 and S2) using generalized linear models, package lme4 (Bates et al., 2015) in R version 4.2.3 (R Core Team, 2021).

RESULTS AND DISCUSSION

In total, 651 immature noctuid moths were collected from the stems of *T. latifolia:* 34 *G. algae*, 196 *G. sparganii* and 367 *N. typhae*, whereas 54 pupae could not be identified to species level. Fungi were found on 52 (8.0%) insects (last instar larvae and pupae), with very similar percentage infections in 2020 and 2021 (7.8% and 8.5%, respectively). In 1995, fungal infections were recorded on 1.1% (14 out of 1280) of pupae. Such low values are similar to those recorded in other studies on Lepidoptera (e.g. Gielen et al., 2022a, b; Poitevin et al., 2018). For comparison, hymenopteran parasitoids (four species, Table S3) account for 21.5% and 19.8% of pupal mortality in samples from 2020 and 2021, and 25.2% in 1995 (Teder et al., 1999).

As many as ten species of fungi belonging to four families and three orders were recorded infecting immature noctuid moths feeding on *T. latifolia* (Table 2). The most abundant species (overall infection 2.6%) was *Beauveria* (= *Cordyceps*) *bassiana*, a well-known cosmopolitan generalist entomopathogen, followed by *Fusarium* cf. *sporotrichioides* (1.8%). Most of the species (complexes) encountered had previously been reported infesting Lepidoptera (Gielen et al., 2022b, 2021) and some other insects (Dallas et al., 2022, UNITE database).

The prevalence of fungal infections varied in the 13 sites studied (Table 1, logistic regression: $\chi^2 = 23.94$, df = 11, p = 0.01), but none of the site-level predictors had an effect on the prevalence (Table S2). This was also the case when the prevalence of the most abundant fungus (*B. bassiana)* was analysed separately. Thus, the data from 2020–2021 does not support the hypothesis that the incidence of fungal infections depends on host population density or herbivore performance. Notably, however, the percentage of infected pupae was particularly low in 1995, a year with highly favourable nutritional conditions for the noctuids as indicated by their exceptionally high pupal masses (average pupal mass of *N. typhae* 1.27 times higher than the average in 2020–2021, Teder et al., 1999).

The present study is one of the surprisingly few documenting the prevalence and diversity of entomopathogenic fungi in natural populations of insects, and Lepidoptera in particular. The present study contributes to the emerging picture that indicates that natural populations of insects tend to host diverse assemblages of fungal pathogens, but the mortality attributable to the fungi is low. Combined with the failure to identify environmental factors affecting the prevalence of entomopathogens (consistent with Gielen et al., 2022a), the observations support the idea that these pathogens typically do not play a major role in regulating natural moth populations (see, however, Elkinton et al., 2019). Nevertheless, as the effects of different mortality factors are not necessarily additive, time series approaches (also suggested by Peterson et al., 2009) could add further information for evaluating the effects of different antagonists, such as the hymenopterous parasitoids in the system studied.

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CONFLICT OF INTEREST. The authors declare that they have no conflict of interest.

REFERENCES

Abarenkov K., Tedersoo L., Nilsson R.H., Vellak K., Saar I., Veldre V., Parmasto E., Prous M., Aan A., Ots M. et al. 2010: *PlutoF – a Web Based Workbench for Ecological and Taxonomic Research, with an Online Implementation for Fungal ITS Sequences: Evolutionary Bioinformatics.* URL: https://doi.org/10.4137/EBO.S6271

Barta M. & Cagáň L. 2003: Entomophthoralean fungi associated with the common nettle aphid (*Microlophium carnosum* Buckton) and the potential role of nettle patches as reservoirs for the pathogens in landscape. — *Anz. Schädlingsk.* **76**: 6–13.

Bates D., Mächler M., Bolker B. & Walker S. 2015: Fitting linear mixed-effects models using lme4. — *J. Stat. Softw.* **67**: 1–48.

Dallas T.A., Carlson C.J., Stephens P.R., Ryan S.J. & Onstad D.W. 2022: insectDisease: programmatic access to the Ecological database of the world's insect pathogens. — *Ecography* **2022**: e06152, 6 pp.

Elkinton J.S., Bittner T.D., Pasquarella V.J., Boettner G.H., Liebhold A.M., Gould J.R., Faubert H., Tewksbury L., Broadley H.J., Havill N.P. & Hajek A.E. 2019: Relating aerial deposition of *Entomophaga maimaiga* conidia (Zoopagomycota: Entomophthorales) to mortality of gypsy moth (Lepidoptera: Erebidae) larvae and nearby defoliation. — *Environ. Entomol.* **48**: 1214–1222.

Estonian Land Board 2022: *Web Maps. Geoportal of Estonian Land Board.* URL: https://geoportaal.maaamet.ee/eng (last accessed 28 Nov. 2022).

Georgieva M., Takov D., Georgiev G., Pilarska D., Pilarski P., Mirchev P. & Humber R. 2014: Studies on non-target phyllophagous insects in oak forests as potential hosts of *Entomophaga maimaiga* (Entomophthorales: Entomophthoraceae) in Bulgaria. — *Acta Zool. Bulgar.* **66**: 115–120.

Gielen R., Meister H., Tammaru T. & Põldmaa K. 2021: Fungi recorded on folivorous Lepidoptera: High diversity despite moderate prevalence. — *J. Fungi* **7**: 25, 8 pp.

Gielen R., Põldmaa K. & Tammaru T. 2022a: In search of ecological determinants of fungal infections: A semi-field experiment with folivorous moths. — *Ecol. Evol.* **12**: e8926, 12 pp.

Gielen R., Robledo G., Zapata A.I., Tammaru T. & Põldmaa K. 2022b: Entomopathogenic fungi infecting lepidopteran larvae: A case from entral Argentina. — *Life* **12**: 974, 9 pp.

Hesketh H., Roy H.E., Eilenberg J., Pell J.K. & Hails R.S. 2010: Challenges in modelling complexity of fungal entomopathogens in semi-natural populations of insects. — *BioControl* **55**: 55–73.

Hochberg M.E. & Ives A.R. 2000: *Parasitoid Population Biology.* Princeton University Press, Princeton, NJ, 384 pp.

Kõljalg U., Nilsson H.R., Schigel D., Tedersoo L., Larsson K.-H., May T.W., Taylor A.F.S., Jeppesen T.S., Frøslev T.G., Lindahl B.D. et al. 2020: The taxon hypothesis paradigm – On the unambiguous detection and communication of taxa. — *Microorganisms* **8**: 1910, 25 pp.

Lacey L.A., Grzywacz D., Shapiro-Ilan D.I., Frutos R., Brownbridge M. & Goettel M.S. 2015: Insect pathogens as biological control agents: Back to the future. — *J. Invertebr. Pathol.* **132**: 1–41.

Peterson R.K.D., Davis R.S., Higley L.G. & Fernandes O.A. 2009: Mortality risk in insects. — *Environ. Entomol.* **38**: 2–10.

Poitevin C.G., Porsani M.V., Poltronieri A.S., Zawadneak M.A.C. & Pimentel I.C. 2018: Fungi isolated from insects in strawberry crops act as potential biological control agents of *Duponchelia fovealis* (Lepidoptera: Crambidae). — *Appl. Entomol. Zool.* **53**: 323–331.

Põldmaa K., Bills G., Lewis D.P. & Tamm H. 2019: Taxonomy of the *Sphaerostilbella broomeana*-group (Hypocreales, Ascomycota). — *Mycol. Progress* **18**: 77–89.

Price P.W., Denno R.F., Eubanks M.D., Finke D.L. & Kaplan I. 2011: *Insect Ecology: Behavior, Populations and Communities. 1st ed.* Cambridge University Press, Cambridge, UK, 816 pp.

R Core Team 2021: *R: A language and Environment for Statistical Computing.* R Foundation for Statistical Computing, Vienna, URL: https://www.R-project.org/

Teder T. & Tammaru T. 2002: Cascading effects of variation in plant vigour on the relative performance of insect herbivores and their parasitoids: Cascading effects and population dynamics. — *Ecol. Entomol.* **27**: 94–104.

Teder T., Tammaru T. & Pedmanson R. 1999: Patterns of host use in solitary parasitoids (Hymenoptera, Ichneumonidae): field evidence from a homogeneous habitat. — *Ecography* **22**: 79–86.

Teder T., Tammaru T. & Kaasik A. 2013: Exploitative competition and coexistence in a parasitoid assemblage. — *Popul. Ecol.* **55**: 77–86.

Vega F.E. 2018: The use of fungal entomopathogens as endophytes in biological control: a review. — *J. Forest Res.* **110**: 4–30.

Vega F.E. & Kaya H.K. 2012: *Insect Pathology. 2nd ed.* Academic Press, Cambridge, MA, 508 pp.

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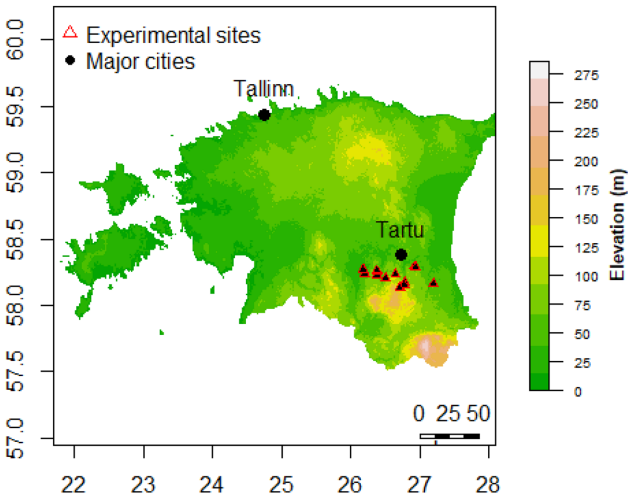

Fig. S1. Map of Estonia showing the sites sampled. Sites sampled were selected while driving along countryside roads and scanning the landscape for distinct patches with dense growth of *T. latifolia*. All patches easily accessible and large enough to incorporate the 3 × 3 m^2 square were accepted.

Table S1. Parameters of the sites studied that were used to explain the prevalence of entomopathogenic fungi in GLMM models (Table S2).

Parameter	Description	Min	Median	Max
Population density	Population density of moths (individuals per m^2)	0.4	1.9	6.2
Plant vigour	Vigour of the host plants estimated as the average lenght of the vegetative shoots of *T. latifolia* (cm)	134	162	213
Plant density	Density of *T. latifolia* shoots (per m^2)	8.5	14.9	21.0
Area	Area of the stand of *T. latifolia* (m^2)	160	6566	31 602
Age*	Age of the stand of *T. latifolia* (years)	1	10	84
Pupal weight	Live weight of non-parasitized moth pupae, three species combined.The site-specific average is calculated from the values standardized to species-specific means. In the study system, pupal weight is a correlate of moth fitness and depends on host plant vigour (Teder & Tammaru 2002).	0.142	1.097	1.939
Parasitoid prevalence	Percentage of parasitism by hymenopterous parasitoids (Table S3)	0.0	19.2	26.5
Population density	Population density of moths (individuals per m^2)	0.4	1.9	6.2

* Calculated using historical and current ortophotos obtained from Estonian Land Board Geoportal (2022).

Table S2. Results of a GLMM model of the incidence of fungal infection (as a binary variable: yes/no) in immature noctuid moths, combined for all fungi and for the most abundant fungus (*B. bassiana*) considered separately. The effects of different predictors were evaluated in a one-way model including the focal predictor only, and, alternatively, in a multi-way model including all predictors. Site studied was included as a random factor.

Parameter	One-way model		Multi-way model		*B. bassiana* One-way model	
	χ^2	p	χ^2	p	χ^2	p
Population density	0.03	0.86	0.36	0.55	0.04	0.85
Plant vigour	0.08	0.78	0.01	0.94	0.19	0.67
Plant density	0.10	0.75	0.21	0.65	1.10	0.29
Area	0.03	0.86	0.25	0.62	0.04	0.84
Age	0.38	0.54	0.51	0.48	—	—
Pupal weight*	1.89	0.17	—	—	—	—
Parasitoid prevalence	0.22	0.64	0.18	0.67	0.73	0.39
Year	0.04	0.85	0.04	0.85	0.37	0.54

* Some models including weight and age did not converge.

Table S3. Numbers of parasitoids reared from moth pupae found in *T. latifolia* stems.

Parasitoid species	Host species	Year	
		2020	2021
Chasmias paludator	*G. algae*	5	0
	G. sparganii	20	3
	N. typhae	40	16
Exephanes occupator	*N. typhae*	16	9
Spilichneumon limnophilus	*G. algae*	1	0
	G. sparganii	8	3
	N. typhae	11	4
Vulgichneumon saturatorius	*G. sparganii*	1	0