Relationships between the fecundity of two bark beetle pests and the presence of antagonist

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Abstract

Although previous research has documented the occurrence of antagonists of bark beetles, the studies have only evaluated individual antagonists and have not assessed the overall effect of all antagonists on adult beetles. In this study, we determined which antagonists were associated with reduction in the fecundity and maternal gallery lengths of two important species of bark beetles: *Ips typographus* on spruce and *I. cembrae* on larch. We evaluated these relationships under natural conditions by collecting maternal females in galleries and dissecting and examining their internal organs. The antagonists in the *I. typographus* hemolymph had the most significant negative associations with fecundity and gallery length. These antagonists were mainly nematodes and parasitoids in the hemocoel. In contrast, a positive association was found between gregarine presence and *I. typographus* fecundity. No antagonist was found that was likely to significantly alter *I. cembrae* fecundity or maternal gallery length. Our study provides the first comprehensive assessment of antagonists that may have the potential to reduce the fecundity and thereby reduce the outbreaks of these bark beetles.

Introduction

The spruce bark beetle, *Ips typographus* (Linnaeus, 1758), is currently the most dangerous pest of spruce stands across Eurasia; the beetle is especially damaging to spruce stands that were planted on the warm side of the tree's natural climatic range and that are now experiencing higher temperatures associated with climate change¹. *I. typographus* has caused considerable damage to the entire range of spruce in recent years due to its eruptive outbreaks; it has contributed to the observed doubling in canopy mortality of spruce in Europe²⁻⁴. In contrast to *I. typographus* outbreaks, outbreaks of the large larch bark beetle, *Ips cembrae* (Heer, 1836), are rare and occur only locally on larches⁵⁻⁹.

Fecundity is the physiological maximum potential reproductive output of an individual¹⁰; for bark beetles, fecundity refers to the number of eggs deposited by one female in its maternal gallery. In general, the number of eggs laid by *Ips* bark beetles and the length of their maternal galleries are influenced by a number of factors, including the ambient temperature during oviposition^{11,12} and the population density of the infested tree¹³⁻¹⁶.

Both *I. typographus* and *I. cembrae* are polygamous species that use aggregation pheromones. Each male mates multiple times with two or three females in the case of *I. typographus*¹⁷, and with two to five females in the case of *I. cembrae*¹⁸. The adult beetles mate in a nuptial chamber, and females of both species extend the nuptial chamber into a maternal gallery system (one system per female) in the phloem of the host tree^{17,18}. Females of both species compete during the creation of gallery systems, and their larvae also compete, because the corridors of the gallery system form in a two-dimensional space. Females deposit single eggs at regular intervals (1-2 eggs per day) along both sides of the maternal gallery, preferably on the side that least interferes with other gallery systems¹⁹. Each *I. typographus* female deposits up to 80 eggs²⁰, and each *I. cembrae* female deposits up to 50 eggs¹⁸.

From the perspective of both species, the optimal density of maternal galleries is about 0.5 per dm² of bark²¹. If the density is higher, the total production of eggs and the length of maternal galleries per female decreases due to oviposition competition^{13,14}. To reduce competition, females may leave the gallery system prematurely and deposit eggs elsewhere^{13,22,23}. Another important factor reducing egg production could be the presence of some pathogens and parasites in the body cavity of the host^{24,25}.

Although antagonists of *I. typographus* is well known, their effects on beetle reproductive success have most often been studied in the laboratory, and their effects on population densities in the field are largely unknown^{26,27}. Natural enemies of *I. cembrae* have been infrequently studied^{28–30} and their effects on *I. cembrae* abundance and fecundity are largely unknown. Pathogens, nematode parasites, and parasitoids of bark beetles are taxonomically diverse, and may, in some cases, cause serious pathological and behavioral changes in their hosts^{31–33}. Both positive³⁴ and negative effects^{35–38} of antagonists have been reported in bark beetles. Although several studies suggest the effect of individual antagonists on fecundity (see above), a comprehensive analysis of the effects of all antagonists simultaneously present under natural conditions has never been presented. In the current study, we evaluated the effect of multiple antagonists on the fecundity of *I. typographus* and *I. cembrae* under natural conditions.

This study had two aims:

- i) to determine whether maternal gallery length is related to the number of laid eggs,
- ii) and to determine which antagonists are associated with reduced fecundity and shortened maternal galleries for *I. typographus* on spruce and *I. cembrae* on larch.

We tested the hypotheses that some of the antagonists in the hemolymph of would be significantly associated with reduced fecundity and gallery length^{24,37}, while antagonists present in the intestine would not be significantly associated with reduced fecundity and gallery length³³.

Materials And Methods

Sampling sites

I. typographus females were collected at six sampling sites between 2009 and 2016 (Table 1), and *I. cembrae* females were collected at five study sites between 2014 and 2015 (Table 2). All sampling sites were in the Czech Republic (Fig. 1). Differences in the numbers of beetles at the sampling sites are due to differences in the presence of parental beetles in the gallery systems and to the exclusion of males from the analyses.

Proportional random-stratified sampling³⁹ was used when the number of plots (trap trees) randomly chosen at sampling sites was proportional to the overall bark beetle-infested wood in the previous year.

I. typographus, beetles were sampled from *Picea abies* (L.) H. Karst., and *I. cembrae* beetles were sampled from *Larix decidua* Mill.

Table 1
Sampling sites for *Ips typographus* and *I. cembrae*.

Species	Sampling site	Year	Altitude	Number of females
<i>I. typographus</i>	Horní Planá	2014, 2015	798	307
<i>I. typographus</i>	Kozlov	2010	620	85
<i>I. typographus</i>	Pec pod Sněžkou	2014, 2015, 2016	796	565
<i>I. typographus</i>	Potštát	2009, 2011	502	232
<i>I. typographus</i>	Prášily	2014	883	215
<i>I. typographus</i>	Zálesí	2016	739	60
<i>I. cembrae</i>	Hradec nad Moravicí	2014	264	221
<i>I. cembrae</i>	Kostelec nad Černými Lesy	2015	375	105
<i>I. cembrae</i>	Křivoklát	2015	425	53
<i>I. cembrae</i>	Město Albrechtice	2014	350	219
<i>I. cembrae</i>	Rakovník	2014	412	218

Sampling

The following sampling procedure was used at all sampling sites:

(1) In February, a series of trap trees were prepared at each study site. Healthy average trees were selected from the forest stand and felled. Branches removed from the felled spruces were placed on the trunks of the felled trees to reduce the drying of the bast. Branches were also removed from larches but were not placed on the trunks.

(2) The trap trees were monitored for the presence of bark beetle entry holes every week during May and June according to the phenology of these two bark beetles at the sampling sites. Detail monitoring included debarking of small areas (10 x 10 cm) for detecting the numbers and the life stages of bark beetles in the gallery systems.

(3) When the population density of bark beetles on the trap trees ranged from 0.5 to 1.0 per 1 dm², beetles were collected. The collection of the beetles always began when 2nd-instar larvae or later stages were present in order to ensure that the females had already completed egg deposition.

(5) For beetle collection, knives were used to carefully remove the bark and expose the maternal galleries. If there was a female in the gallery, the length of the corridor was measured with a ruler, and the eggs were counted. Only one maternal gallery from each gallery system was analysed.

(5) Each female from measured galleries was placed in a 2-ml Eppendorf tube that was marked with the sampling site, egg number, and gallery length.

Laboratory analysis

In the laboratory, the elytrae and wings were removed from each beetle and the contents of its abdomen were removed by gentle squeezing, placed in a drop of distilled water, and examined with a stereomicroscope.

All internal organs were examined, especially the intestine, adipose tissue, gonads, and hemolymph, with a Nikon Eclipse-Ci light microscope at 100-400 × magnification. If a male was found in the sample (based on the presence of male gonads), the beetle was excluded from the analysis.

The presence of nematodes, microsporidia, gregarines, viruses, and endoparasitoids was evaluated. Spores or cysts of pathogens and various developmental stages of parasites and parasitoids were mainly observed.

Statistical analysis

All analyses were done in R 4.1.2.

First, we analysed the relationship between the number of laid eggs and maternal gallery length. Because these variables did not have a Gaussian distribution, we used the Spearman rank-order correlation coefficient (i.e., a non-parametric procedure).

Next, we assessed the relationship between egg number and gallery length with the generalized linear mixed model (GLMM) using *mgcv* and *nlme* packages and with study site as a random factor.

The possible collinearity of selected explanatory variables was verified using the *HH* package with $VIF = 2$ as a threshold. Both independent variables had a negative binomial distribution with a corresponding lambda value.

Results

Comparison of parameters reflecting the fecundity of *I. typographus* females

A total of 1,464 *I. typographus* maternal galleries were analysed during this research. The average length of the maternal galleries was 83.2 ± 0.8 mm (mean $\pm$ SE; min = 6, max = 220). The average number of eggs per maternal gallery was 47.0 ± 0.6 (min = 4, max = 171).

For *I. typographus*, egg number per gallery was positively correlated with maternal gallery length ($r_s = 0.72$; $p < 0.05$; Fig. 2).

In total, seven groups of antagonists were found in *I. typographus* females. Nematodes were found in the hemocoel of 21.2% of the females, which was the highest percentage of parasites detected. This was followed by intestinal nematodes, which were found in 9.7% of the females. The only microsporidian found during the study was *Chytridiopsis typographi* (Weiser 1954) Weiser 1970, which was found in 4.8% of the intestines of the females. A total of two apicomplexan species were found in the samples: the neogregarine *Mattesia schwenkei* (Purrini, 1970) was found in 2.0% of the females, and the eugregarine *Gregarina typographi* (Fuchs, 1915) was found in 6.2% of the females. Viral infection by *Entomopoxvirus typographi* (ItEPV) (Weiser & Wegensteiner, 1994) was detected in 1.9% of the females. The only endoparasitoid species observed was *Tomicobia seitneri* (Ruschka, 1924), which was detected in 2.3% of the females (Table 2).

Table 2
Number of eggs per gallery, length (mm) per maternal gallery, and number of females in which antagonists of *I. typographus* females were absent or present. Values are means $\pm$ SE.

	Number of eggs		Length of maternal gallery		Number of females	
Antagonist	Antagonist absent	Antagonist present	Antagonist absent	Antagonist present	Antagonist absent	Antagonist present
intestinal nematodes	47.1 $\pm$ 0.6	45.6 $\pm$ 1.7	83.2 $\pm$ 0.8	83.5 $\pm$ 2.2	1322	142
hemocoel nematodes	47.7 $\pm$ 0.7	44.4 $\pm$ 1.1	83.8 $\pm$ 0.9	81.2 $\pm$ 1.6	1154	310
<i>Gregarina typographi</i>	46.3 $\pm$ 0.6	56.7 $\pm$ 2.1	82.4 $\pm$ 0.8	95.9 $\pm$ 2.9	1373	91
<i>Mattesia schwenkei</i>	47.0 $\pm$ 0.6	44.4 $\pm$ 5.7	83.4 $\pm$ 0.8	73.1 $\pm$ 6.7	1435	29
<i>Chytridiopsis typographi</i>	47.0 $\pm$ 0.6	46.0 $\pm$ 2.0	83.2 $\pm$ 0.8	83.4 $\pm$ 2.5	1393	71
ItEPV	47.2 $\pm$ 0.6	36.1 $\pm$ 3.7	83.5 $\pm$ 0.8	71.2 $\pm$ 4.7	1436	28
<i>Tomicobia seitneri</i>	47.3 $\pm$ 0.6	33.5 $\pm$ 2.9	83.6 $\pm$ 0.8	68.6 $\pm$ 5.5	1431	33

All identified antagonists were tested for multicollinearity. In all cases, the VIF (the variance inflation factor) was < 2 , indicating that, in terms of presence, no antagonist of *I. typographus* was correlated (positively or negatively) with any other antagonist.

The GLMMs indicated that the presence of *G. typographi* (Table 3) was positively related to the length of maternal galleries and the number of eggs laid by *I. typographus* females but that the presence of nematodes in the hemocoel was negatively related to the length of maternal galleries and the number of eggs laid by *I. typographus* females. Infestation with the endoparasitoid *T. seitneri* was negatively related with the number of eggs laid by females but was not related with the length of the maternal galleries.

Table 3

Relationships between the number of eggs per *I. typographus* female or maternal gallery length and the presence of different groups of antagonists as indicated by GLMMs.

Independent variable	Variable	Value	SE	t	p
Number of eggs per female	Intercept	3.8274	0.1066	35.8934	<0.0001
	intestinal nematodes	-0.0358	0.0394	-0.9063	0.3649
	hemocoel nematodes	-0.1093	0.0306	-3.5807	0.0004
	Gregarina typographi	0.1618	0.0485	3.3334	0.0009
	<i>Mattesia schwenkei</i>	0.0340	0.0816	0.4163	0.6773
	<i>Chytridiopsis typographi</i>	-0.0276	0.0575	-0.4806	0.6309
	<i>ItEPV</i>	-0.1081	0.0830	-1.3038	0.1925
	<i>Tomicobia seitneri</i>	-0.1815	0.0770	-2.3567	0.0186
Maternal gallery length	Intercept	4.3967	0.0730	60.2280	<0.0001
	intestinal nematodes	-0.0112	0.0306	-0.3678	0.7131
	hemocoel nematodes	-0.0721	0.0237	-3.0469	0.0024
	Gregarina typographi	0.1060	0.0376	2.8191	0.0049
	<i>Mattesia schwenkei</i>	-0.0911	0.0634	-1.4364	0.1511
	<i>Chytridiopsis typographi</i>	0.0065	0.0446	0.1466	0.8835
	<i>ItEPV</i>	-0.0692	0.0642	-1.0780	0.2812
	<i>Tomicobia seitneri</i>	-0.0938	0.0595	-1.5758	0.1153

Comparison of parameters reflecting the fecundity of *I. cembrae* females

A total of 820 *I. cembrae* maternal galleries were evaluated. The average length of the galleries was 76.0 ± 1.2 mm (min = 7, max = 240), and the average number of eggs per female was 23.5 ± 0.4 (min = 7, max = 91). As was the case with *I. typographus*, gallery length was positively correlated with the number of eggs deposited by *I. cembrae* females ($r_s = 0.71$; $p < 0.05$; Fig. 3).

Four groups of antagonists were identified in *I. cembrae* females: intestinal nematodes (detected in 99.9% of the females), hemocoel nematodes (detected in 19.1% of the females), adult female nematodes of the genus *Contortylenchus* (detected in 1.6% of the females), and the microsporidian *Chytridiopsis typographi* (detected in 10.5% of the females) (Table 4).

All identified antagonists were also tested for multicollinearity. In all cases VIF was < 2, indicating that, in terms of presence, no antagonist of *I. typographus* was correlated (positively or negatively) with any other antagonist.

The GLMM model indicated that the presence of any of the identified parasites and pathogens was not related to the length of *I. cembrae* galleries or with the number of eggs laid by *I. cembrae* females (Table 5).

Table 4
Number of eggs per gallery, length (mm) per maternal gallery, and number of females in which antagonists were absent or present. Values are means $\pm$ SE.

Antagonist	Number of eggs		Length of maternal gallery		Number of females	
	Antagonist absent	Antagonist present	Antagonist absent	Antagonist present	Antagonist absent	Antagonist present
intestinal nematodes	13.0 $\pm$ 0.0	23.5 $\pm$ 0.4	50.0 $\pm$ 0.0	76.1 $\pm$ 1.2	1	819
hemocoel nematodes	24.1 $\pm$ 0.5	20.8 $\pm$ 0.9	77.7 $\pm$ 1.4	68.9 $\pm$ 2.2	663	157
<i>Contortylenchus</i> sp.	23.5 $\pm$ 0.4	22.2 $\pm$ 2.2	76.1 $\pm$ 1.3	69.4 $\pm$ 5.8	807	13
<i>Chytridiopsis typographi</i>	24.0 $\pm$ 0.5	19.4 $\pm$ 1.1	76.5 $\pm$ 1.3	71.8 $\pm$ 3.7	734	86

Table 5

Relationships between the number of eggs per *I. cembrae* female or maternal gallery length and the presence of different groups of antagonists as indicated by GLMMs.

Independent variable	Variable	Value	SE	t	p
Number of eggs per female	Intercept	1.3953	1.3865	1.0064	0.3145
	intestinal nematodes	0.3767	0.5008	0.7522	0.4521
	hemocoel nematodes	-0.0573	0.0453	-1.2635	0.2067
	<i>Contortylenchus sp.</i>	0.1510	0.1413	1.0690	0.2854
	<i>Chytridiopsis typographi</i>	-0.0638	0.0595	-1.0712	0.2844
Maternal gallery length	Intercept	2.5153	1.4770	1.7029	0.0889
	intestinal nematodes	0.2918	0.4483	0.6510	0.5152
	hemocoel nematodes	-0.0566	0.0413	-1.3703	0.1709
	<i>Contortylenchus sp.</i>	0.0030	0.1289	0.0231	0.9815
	<i>Chytridiopsis typographi</i>	0.0638	0.0540	1.1803	0.2382

Discussion

Several antagonists were associated with the number of deposited eggs and maternal gallery length for *I. typographus*. In particular, the number of *I. typographus* eggs and the length *I. typographus* maternal galleries were negatively related with presence of nematodes in the hemocoel, and the number of *I. typographus* eggs was negatively related with the presence of endoparasitoids in the body cavity. Surprisingly, the presence of gregarines was positively related with the two variables, i.e., gregarines seemed to have increased *I. typographus* fecundity and maternal gallery length. This result suggests that, rather than being a bark beetle pathogen as is usually assumed, *G. typographi* may be more of a mutualist. The occurrence of other antagonists was not associated with the number of eggs laid or the gallery length per *I. typographus* female. For *I. cembrae*, the number of eggs and in the length of the galleries were not statistically associated with the presence of any of the identified antagonists, although means tended to be lower when nematodes were present in the hemocoel and when *Contortylenchus sp.* or *Chytridiopsis typographi* were detected.

The mean number of eggs deposited by one *I. typographus* female was 47, and the mean length of the galleries was 83 mm. These values are consistent with previous reports^{15,17,40}.²² found that the number of eggs laid in the absence of spatial competition for the first maternal gallery ranged from 35 to 50, which suggests that spatial competition was not a major factor affecting *I. typographus* females in the current study. For the less-studied *I. cembrae*, the mean length of the galleries was 76 mm, which is in the range according to⁴¹ but is lower than the range of 100 to 115 mm reported by⁴². The average number of eggs per *I. cembrae* female (n = 24) was in the range of previously reported values¹⁸. Because bark beetle

females gradually bore the maternal galleries and lay eggs in them continuously, it is reasonable that gallery length is positively correlated with the number of eggs deposited for both species, as documented in the current study (Fig. 2 and 3) and in previous studies^{13,15}. This close relationship between fecundity and gallery length suggests that it is a standard property for both species of bark beetles.

A total of four pathogens of *I. typographus* and one of *I. cembrae* were recorded. *Chytridiopsis typographi* microsporidia were found in the intestine of both species. It is a common pathogen reported in most bark beetles of the genus *Ips*³². The infection was found in the midgut epithelium in the form of cysts. Our results suggest that this microsporidian has little or no effect on the fecundity of the two species of bark beetles. The question, therefore, remains whether *C. typographi* is indeed a pathogen, because only two studies have assessed the effects of *C. typographi* on bark beetle populations. One laboratory study reported 100% mortality of infected bark beetles within 60 days⁴³; this long time (60 days) before death, however, could allow females to complete gallery construction and egg deposition. A second study compared *C. typographi* infection rates in *I. typographus* collected in pheromone traps over a 10-year period; the results suggested that infection might hinder flight ability and interfere with pheromone perception³⁴.

The neogregarine *Mattesia schwenkei* is the only pathogen thus far detected in the adipose tissue of bark beetles^{29,44}. The infection level, however, is usually very low^{40,45}, which is consistent with our findings. Although this pathogen breaks down the fat in the hemocoel and apparently reduced beetle fecundity and gallery length in the current study, the reductions were not statistically significant. *M. schwenkei* is thought to possibly reduce the establishment of a sister brood of bark beetles and to reduce the survival of overwintering bark beetles⁴⁶. Similar effects have been assumed to result from infection of bark beetles by the ItEPV virus, which is usually found in the middle intestine⁴⁶. In the current study, the virus was associated with a 23% reduction in the number of *I. typographus* eggs deposited and a 13% reduction of *I. typographus* gallery length, but the reductions were not statistically significant.

The only organism that was positively associated with increases in *I. typographus* fecundity and gallery length was the gregarine *Gregarina typographi*. Gregarines are thought to have many kinds of relationships with their hosts, including symbiotic, mutualistic, and parasitic relationships⁴⁷. *G. typographi* has been found in many *Ips* species and is generally considered a pathogen of the anterior part of the intestine lumen^{33,48-50}. Horizontal transmission of *G. typographi* occurred between beetles via spore ingestion in the nuptial chambers⁴⁹. Evidence of a negative effect of gregarines on bark beetles is rather speculative. Only³⁴ suggested a positive influence of gregarines on beetles, because swarming of *I. typographus* adults was greater for infected than uninfected individuals. For the first time, our results show a statistically significant positive association between the presence of gregarines and *I. typographus* fecundity and gallery length. The life cycles of gregarines differ significantly from those of most other apicomplexans because gregarines generally utilize only one group of host organisms⁴⁷. The high degree of host specificity of gregarines⁴⁷ suggests the coevolution of gregarines and their hosts⁵¹. We therefore suspect that gregarines are not parasites of bark beetles but instead are part of the host's

non-damaging natural flora. *G. typographi* might even contribute to *I. typographus* fitness via symbiosis.⁵² reported that the mechanical and physiological damage caused by gregarines to the midgut according⁵³ can be easily repaired and that the pathological effects of gregarines are generally unimportant.

One adult bark beetle may contain a very large number of nematodes. It is not uncommon to find bark beetles with hundreds of nematode larvae in the gut, in the hemocoel, and under the elytrae⁵⁴. Nematodes associated with bark beetles can be either commensal or parasitic. In commensal relationships, the effects on hosts are minimal and host fitness is not reduced³⁵. The influence of parasitic nematodes on the fecundity of bark beetles has been documented in several studies^{55,56}. The nematodes that develop inside the beetle's body have been shown to remove nutrients from their host. This is evidenced by the reduced number of fat cells in infected bark beetles^{37,57} and the decreased level of proteins in the hemolymph^{36,58}.

Parasitorhabditis and *Cryptaphelenchus* are two dominant genera of nematodes found in the bark beetle intestines⁵⁹. Because the occurrence of intestinal nematodes in the two bark beetles of the current study species was unrelated to the number of eggs laid or gallery length, we assume that these species do not reduce fecundity or gallery length. This is inconsistent with the findings of⁶⁰, who found significant damage to gastrointestinal epithelial cells in *Ips sexdentatus* individuals parasitized by *Parasitorhabditis* spp. In a subsequent study,²⁵ reported a slight decrease in the density of oviposition incisions and in the number of eggs in *Ips sexdentatus* individuals parasitized by *Parasitorhabditis* sp. In the current study, the presence of nematodes in the intestines of *I. typographus* was associated with a decrease in fecundity of two eggs per female.

Three species of nematodes, *Contortylenchus diplogaster* (v. Linstow, 1890) Rühm 1956, *Parasitylenchus dispar* (Fuchs, 1915), and *Cryptaphelenchus* sp., were previously found in the hemocoel of *I. cembrae* and *I. typographus*^{59,61}. Previous study has revealed that the hemocoel of individual beetles can be simultaneously infected with multiple species of parasitic nematodes^{35,59,62}. Although *Parasitylenchus* spp. may kill bark beetles under particular circumstances⁶³ studies on *Contortylenchus* species have not demonstrated any lethal effects³¹. The fatal effects of nematodes on bark beetles could be easily overlooked in nature because infested dead beetles are difficult to detect but living and heavily infested beetles are easy to detect³³.

In several studies in Europe, *Contortylenchus diplogaster* was the nematode most frequently found in the hemocoel of *I. typographus* and *I. cembrae*^{28,61}. The presence of nematodes of *C. diplogaster* in the hemocoel was associated with a significant decrease (7%) in *I. typographus* fecundity and a significant decrease (3%) in the length of *I. typographus* maternal galleries. These values are much lower than the 20 to 50% reduction in the number of eggs and the 25 to 27% reduction in gallery length reported by³⁶.³⁷ reported that individuals of *Ips sexdentatus* parasitized by *Parasitaphelenchus* sp. and *Contortylenchus* sp. had smaller fat bodies and ovaries and less developed terminal oocytes than non-parasitized individuals. Such effects on fat bodies ovaries could delay the maturation of oocytes.

In our study, intestinal nematodes were found in all *I. cembrae* adults except one. We were therefore unable to determine the association between *I. cembrae* fecundity and the presence of intestinal nematodes. On the other hand, the presence of nematodes in the hemocoel rather than in the intestine was associated with fecundity of *I. typographus* but not in *I. cembrae*. Perhaps this difference was due to differences in the proportion of beetles with parasitic nematodes in the hemocoel and intestine, the presence of nematodes in the intestines of nearly 100% of *I. cembrae* females. According to⁶¹, *C. diplogaster* is mainly found in the hemocoel of *I. cembrae*, but can also occur in the intestine and Malpighian tubules after being ingested with frass in the gallery³³. With such a level of parasitism, the individual stages of nematodes could appear not only in the hemocoel but also in the intestines, making the clear identification of these groups difficult.

Two common obligate endoparasitoids were previously identified in *I. typographus* adults in Europe: *Tomicobia seitneri* and *Ropalophorus clavicornis* (Wesmael, 1835)^{27,33,64}. In our study, we found only one pteromalid endoparasitoid, *T. seitneri*; the latter endoparasitoid locates its host via a host aggregation pheromone^{65,66}. Females oviposit directly through the thorax or elytra of bark beetle adults. In our study, we found both eggs and larvae of *T. seitneri* but in only 2% of analyzed females. The level of *T. seitneri* parasitism varied considerably among previous reports and ranged from 20 to 100 % in⁶⁶, from 0 to 19 % in⁶⁴, and from 0 to 35 % in⁶⁷; the level of parasitism apparently depends on the time of beetle collection and other factors. Our results are in line with the predictions of²⁴ because infected females continue to lay eggs and the parasitoids do not hatch until after eggs are deposited. We found that the presence of *T. seitneri* was associated with an almost 16% reduction in *I. typographus* fecundity, but reductions as high as 30% were previously reported²⁴, and even higher reductions were reported for *Tomicobia tibialis* Ashmead, 1904 on *Ips pini* females, i.e., parasitized *I. pini* females produced 50% fewer offspring than unparasitized females³⁸. On the other hand, gallery length is not affected by *Tomicobia* parasitism, because infected females still create galleries. The parasitoid apparently destroys the eggs in the host's body and thereby prevents the eggs from being laid. *Tomicobia* larvae feed on the tissue in the hemocoel. The adult wasp gnaws its way out of the bark beetle elytral declivity and leaves the gallery³³. *T. seitneri* has not been found in *I. cembrae* beetles and is not known in the literature as a parasitoid of *I. cembrae*²⁹.

In conclusion, our findings provide the first comprehensive data on pathogens, nematodes, and endoparasitoids of two bark beetles, *I. typographus* and *I. cembrae*. This information is important because pathogens, nematodes, and endoparasitoids could potentially reduce beetle fecundity, which can in turn greatly affect the severity of outbreaks by these beetles. The results of our study should be included in models of forest landscape dynamics.

Our results suggest that the nematodes in the hemocoel and the parasitoid *T. seitneri* have the highest potential for use in the biological control of the two bark beetles. In the case of nematodes, however, we recommend further research focusing on the accurate detection of species that reduce bark beetle fecundity. Conversely, the eugregarine *G. typographi*, whose presence apparently increases fecundity, should possibly be considered a symbiotic organism rather than a pathogen. A number of known

antagonists occurring in the adult stages were not detected in our research and deserve additional study. On the other hand, the prevalence of some pathogens is so low^{32,33,68} that we suspect that they are unlikely to influence the populations of the two bark beetles.

Declarations

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Author contribution

KR and JHol conceived and designed research. KR, JS, and JHol conducted the experiments. JHor analyzed the data. KR wrote the manuscript. All authors read and approved the manuscript.

Conflict of interest

All the authors declare that they have no conflict of interest.

Additional information

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author KR on reasonable request.

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Figures

Figure 1

Sampling sites in the Czech Republic where females were collected from 2009 to 2016. Sampling sites for *I. typographus* are indicated by black circles, and those for *I. cembrae* are indicated by white circles

Figure 2

Scatterplot for nonparametric correlation between maternal gallery length and the number of eggs in *I. typographus* galleries.

Figure 3

Scatterplot for nonparametric correlation between maternal gallery length and the number of eggs in *I. cembrae* galleries.