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Short communication

New data on the host specificity of *Larssoniella duplicati*

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Abstract

Background and Purpose: Ips duplicatus *and* I. typographus *are two serious pests of spruce in Europe. They have similar bionomics and are likely to occur and meet on the same host trees. One could hypothesize that the two species support similar levels of similar pathogens, but* Larssoniella duplicati *is species-specific for* I. duplicatus.

Materials and Methods: *To test this hypothesis, we collected mature beetles from two felled s pruce trees and determined their pathogen infection levels.*

Results and Conclusions: *In total, 857 mature* I. duplicatus *beetles and 349 I. typographus were dissected. Two microsporidian pathogens were detected.* Chytridiopsis typographi *was found in both species of bark beetles, while* Larssoniella duplicati *was found only in* Ips duplicatus. *Therefore, it may still be considered that* L. duplicati *is species-specific for* I. duplicatus. *There were no significant differences between sample trees in pathogen infection levels in bark beetles.*

INTRODUCTION

Ips typographus (Linnaeus, 1758) and *I. duplicatus* (Sahlberg, 1836) have similar tree hosts and similar interactions with their hosts *(1)*. Norway spruce is the main host of both species in Europe *(2, 3)*. The spatial distributions of these species on the trunk often overlap, although *I. duplicatus* generally occurs higher in the tree than does *I. typographus (4)* or prefers thinner bark for its development *(1, 5)*.

In Europe, four protozoan, four microsporidia, and one viral pathogen of the genus *Ips* have been determined *(6, 7)*. The only known species-specific pathogen is the microsporidium *Larssoniella duplicati* Weiser, Holuša, Žižka 2006 (Fungi, Microsporidia), which occurs in the intestinal muscles of *I. duplicatus (8–10)*. *Larssoniella duplicati* has been observed in *I. duplicatus* in eastern Czech Republic as well as in its area of origin in north-eastern Poland *(8–10)*.

In eastern Czech Republic, an outbreak of both species had begun in 2003 *(11)*, and the beetles had been already collected and analysed for pathogens in 2002 *(8)*. It is therefore interesting to determine if there is evidence of *L. duplicati* being transmitted into *I. typographus* or if *L. duplicati* is truly species-specific for *I. duplicatus*.

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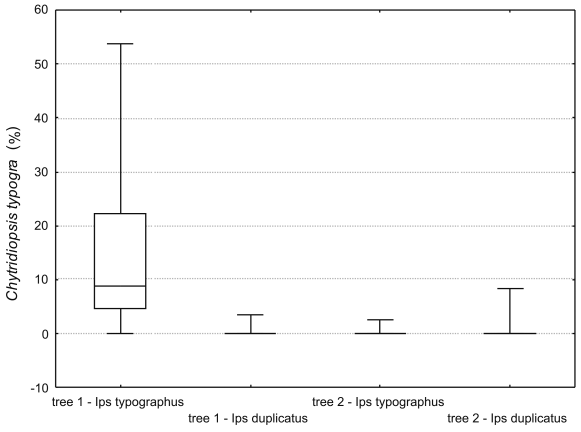

Figure 1. *Percentage of* Ips duplicatus *and* Ips typographus *infected by* Chytridiopsis typographi. *Box plots show median plus upper and lower quartiles for all samples on trap trees 1 and 2. Minimum and maximum values are shown by the upper and lower whiskers.*

The objectives of the current study were to identify and compare the pathogens of these two bark beetles at one locality in long-term outbreak status.

MATERIALS AND METHODS

I. typographus and *I. duplicatus* were studied at the locality of Hlubocec in the Czech Republic (GPS 49°50'31.5"N, 17°56'38.2"E; 441 m a.s.l.) in an area of long term outbreak of both species (> 10 years). On 8 August 2009, two sample trees (*Picea abies* (Linnaeus) Karsten; diameters at chest height = 18 and 20 cm, respectively; heights = 16 and 18 m, respectively) were cut. We collected all maternal beetles from the individual galleries at consecutive sample areas 2 m long along the entire length of the tree (about 0.5 m wide; width was equivalent to half the tree circumference). The offspring generation occurred in the stages of pupae and callow beetles. The individual beetles were placed into 2 cm^3 Eppendorf micro test tubes, and a piece of damp gauze was added to maintain 100% relative humidity. The beetles were immediately frozen and stored at –5°C. All internal organs and the fat body were dissected in a water drop using surgical tweezers. Each dissected beetle was examined using an Arsenal LPE 5013i-T light microscope (Arsenal s.r.o., Prague, Czech Republic) at 40–400x magnification to determine whether it was infected with one or pathogens and, if so, which.

The STATISTICA 9 software program was used for statistical analyses. Infection level was calculated as the percentage of beetles from the sample area (> 30 beetles) with positive infection. Infection levels per sample were compared between beetle species (Wilcoxon test) and between sample trees (ANOVA, Kruskal–Wallis test).

RESULTS

Totals of 857 *I. duplicatus* and 349 *I. typographus* beetles were dissected. The mean density of entry holes for both species exceeded 1 per dm^2 throughout the stem. From both sample trees, the microsporidian pathogen *L. duplicati* was found only in *I. duplicatus*. The mean infection levels on the sample trees were 14.6 ± 15.8% and 26.4 ± 10.5%, respectively. Infection level did not differ significantly between the two trees at the study locality (Shapiro–Wilk test for normality: $W = 0.89$, $p > 0.05$; ANOVA: $F = 1.92$, $p > 0.05$).

Both species of bark beetles contained the microsporidium *Chytridiopsis typographi* [(Weiser, 1954) Weiser, 1970]. *Chytridiopsis typographi* was more abundant in *I. typographus* than in *I. duplicatus* (Fig. 1). For this pathogen, infection levels were significantly higher in *I. typographus* than in *I. duplicatus* on the first sample tree (Shapiro-Wilk test for normality: $W = 0.57$, $p < 0.0001$; Wilcoxon test: $z = 2.02$, $p < 0.05$; Fig. 1), but there was no statistically significant difference between the two species on the second sample tree (Shapiro–Wilk test for normality: $W = 0.42$, $p < 0.000001$; Wilcoxon test: $z = 1.34$, $p > 0.05$; Fig. 1). There was no significant difference in infection level in *I. typographus* (Shapiro–Wilk test for normality: $W = 0.70$, $p < 0.01$; Kruskal–Wallis test: $H = 3.38$, $p > 0.05$) or in *I. duplicatus* (Shapiro–Wilk test for normality: $W = 0.01$, $p > 0.05$; ANOVA: $F = 0.30$, $p > 0.05$) between sample trees.

DISCUSSION

Microsporidium *C. typographi* was detected in both *I. typographus* and *I. duplicatus*. This pathogen is known to overlap in host range and has been observed in various bark beetle species *(6, 7, 12–14)*. Therefore, it is apparently a non-specific pathogen in the intestine of a number of pests in coniferous stands *(6)*. In our study, *C. typographi* was confirmed in both analysed trees and in both bark beetle species, with infection levels ranging among samples from 0 to 8.3% in *I. duplicatus* and from 0 to 53.8% in *I. typographus*. The infection level of this pathogen often varies greatly *(10, 12, 15–17)*.

Larssoniella duplicati was not found in *I. typographus* and therefore can still be regarded as species-specific for *I. duplicatus (8–10)*. Nor was it found in *Ips cembrae* (Heer, 1836) or *Ips amitinus* (Eichhoff, 1871) studied at nearby localities *(12, 13)*. Unfortunately, so far no manipulative experiments with *L. duplicati* were conducted under laboratory conditions. In this research area will be necessary to continue.

Larssoniella duplicati can attain high infection levels in *I. duplicatus*, frequently reaching 10–30% *(8–10, this study)*. Infection levels did not show any trend in changes of population density, over time of sampling *(9, 10)*, or between bark beetle generations *(10)*. This corresponds with equal infection level at the studied locality, excludes horizontal transmission, and supports the theory of vertical transmission *(8, 9)*. This is supported, too, by the fact that although both bark beetles have long been in outbreak status at the studied area no case of *I. typographus* infected by *L. duplicati* has been identified.

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