



Infection levels of pathogens in the European spruce bark beetle *Ips typographus* (Coleoptera: Curculionidae) at managed and unmanaged forest locations: a meta-data analysis

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Abstract

Forest management greatly affects the population density of the European spruce bark beetle, *Ips typographus*. In this study, a meta-analysis was used to determine whether infection levels of pathogens of *I. typographus* differ between managed and unmanaged spruce stands. The analysis used data from 10 publications and a total of 61 locations. The results indicated that infection levels of the most common pathogens (ItEPV, *Gregarina typographi*, and *Mattesia schwenkei*) are higher in unmanaged than in managed locations. The only exception is the microsporidium *Chytridiopsis typographi*, which is more common in managed than in unmanaged locations. Our meta-analysis indicates that pathogen levels are generally higher in unmanaged than in managed locations.

Key words: pathogen infection levels; management; microsporidia; gregarines; ItEPV

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1. Introduction

Intensive forest management, which involves sanitation felling and logging, dramatically reduces the amount and diversity of woody debris; such debris is crucial for forest nutrient cycling and for the biodiversity of forest ecosystems (Harmon et al. 1986; Bobiec 2002; Jacobs et al. 2007; Langor et al. 2008; Lonsdale et al. 2008; Brunet et al. 2010). The loss of this debris and the subsequent loss of biodiversity undoubtedly alter ecosystem functioning (Fischer et al. 2010). In many forest regions, the quantity of deadwood is an order of magnitude smaller in managed than in unmanaged locations. The loss of deadwood at managed locations also affects thousands of saproxylic species (Seibold et al. 2015).

Saproxylic beetles, including the spruce bark beetle *Ips typographus* (Linnaeus, 1758), do not occur randomly in forest stands but choose their host trees based on volatile substances secreted by the trees and by beetle preference for host height (Gossner et al. 2013a, 2013b). The extent of a bark beetle outbreak is determined by a wide range of factors including beetle movement. Unmanaged and managed areas of spruce stands are very frequently located adjacent to each other, and there is seldom a clear boundary or sufficiently large distance that would prevent bark beetles from flying between the areas (Grodzki et al. 2006). The migration potential of *I. typographus* is substantial; individuals can fly several kilometres (Botterweg 1982; Forsse & Solbreck 1985; Otto & Schreiber 2001), and *I. typographus* has been captured in pheromone traps located considerable distances from the nearest spruce stand (Duelli et al. 1986).

Pathogens are closely tied to their hosts, and thus management interventions that affect their hosts can also influ-

ence the pathogens. Removal of bark beetle-infested trees, for example, will remove both bark beetles and their pathogens but could increase the release of pathogen spores from living beetles or from their remains. The species composition and infection level of pathogens in bark beetle populations differ not only between locations but also between years in the same location (Wegensteiner et al. 2014). The requirements for pathogen reproduction and the effects of pathogens on their hosts are poorly known for essentially all 10 pathogen species of *I. typographus* in Europe (Wegensteiner 2004; Lukášová & Holuša 2012; Wegensteiner et al. 2015). In the case of microbial insect diseases, the pathogens invade and multiply in insects and spread to infect other individuals (Wegensteiner 2004), but the pathogens lack the ability to actively move and actively seek hosts. Pathogens of bark beetles spread horizontally when healthy beetles contact and ingest spores associated with an infected beetle or when they contact and ingest spores carried by a vector (a non-host organism). For some pathogens, transmission can be vertical, i.e., the pathogen can be transferred from the parents to the offspring (Wegensteiner 2004).

In unmanaged areas, very large populations of bark beetles often develop but then collapse, and researchers have inferred that the collapse results from combinations of abiotic factors and natural enemies (Grodzki et al. 2006). This inference, however, has not been sufficiently supported by data and especially by data collected over long-term study.

In this research, we conducted a meta-analysis of the published literature to determine whether the infection levels in *I. typographus* populations are higher in unmanaged areas than in managed areas of forests.

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2. Materials and methods

Scientific articles on pathogens of *I. typographus* were obtained by searching the Web of Science, Scopus, Google Scholar, and regional journals. The search, which included papers published before 25 August 2016, was performed using the keywords “*Ips typographus*”, “management”, “pathogen”, and their combinations. Information on altitude, type of forest management, species spectrum of pathogens, and infection levels was then extracted from the published manuscripts.

Data that were collected without reference to management type were excluded from the analyses. If certain information such as altitude was omitted in a source document, such information was obtained based on location names and GPS coordinates (Appendix 1). Local pathogen infection levels included in the analyses were always based on individual samples.

In an initial analysis, 219 manuscripts from the Scopus database and 183 from the Web of Science database were considered. After articles that lacked information on type of management at the study locations (managed vs. unmanaged) were discarded, we acquired and analysed data from the following 10 publications: Haidler et al. 2003; Händel et al. 2003; Wegensteiner et al. 2007, 2014, 2015; Holuša et al. 2009; Kereselidze et al. 2010; Lukášová et al. 2012, 2013; and Lukášová & Holuša 2015 (Table 1). Only data concerning living adult beetles from wind-thrown trees, horizontally lying trap trees, pheromone traps, trap logs, and standing trees were included in the analysis. Managed areas represented commercial forest stands where intensive and regular sanitation felling of trees infested by bark beetles was applied. Unmanaged areas were those without sanitation cutting, harvesting, or other bark beetle preventive measures. In one unmanaged area, spruce trees infested by bark beetles (presence of entry holes, boring dust, resin on the bark) were selected and felled as part of a research effort (Lukášová et al. 2012). Unmanaged areas were commonly in locations with a strict level of nature conservation, e.g., national parks and nature reserves.

The following pathogens were excluded from the analyses because of the small numbers of locations, low infection levels, and small numbers of references: the protozoan *Malamoeba scolyti* (Purrini, 1980), the neogregarine *Menzbiera chalcographi* (Weiser, 1955), and the microsporidia *Nosema typographi* (Weiser, 1955) and *Unikaryon montanum* (Weiser, Wegensteiner, Žižka, 1998). Because we lim-

ited our analysis to those pathogens with higher frequencies of occurrence, our analysis included only four species: the microsporidium *Chytridiopsis typographi*, the gregarine *Gregarina typographi*, the virus ItEPV, and the neogregarine *Mattesia schwenkei*. If a given study constituted multi-year research, average infection levels per location were calculated.

Because of a lack of normality in the data (as determined by the Shapiro-Wilk test), non-parametric tests (Kruskal-Wallis test) of pathogen infection levels were performed in the STATISTICA 12 program. We used these tests to determine whether pathogen infection levels in *I. typographus* differed between managed and unmanaged spruce stands.

3. Results

The 10 publications that were included in the meta-analysis contained data from 45 managed locations and 16 unmanaged locations. For each of the four pathogens, a total of at least 20,000 *I. typographus* specimens (adult beetles) had been dissected (Table 1).

At unmanaged locations, infections were most frequent for the gregarine *G. typographi* (Shapiro-Wilk test: $W = 0.54$, $p < 0.0001$; Kruskal-Wallis test: $H(1;64) = 8.75$, $p < 0.01$; Fig. 1). The average *G. typographi* infection level (\pm SD) across all unmanaged locations was $13.7 \pm 17.0\%$. At managed locations, *C. typographi* was dominant (Shapiro-Wilk test: $W = 0.55$, $p < 0.0001$; Kruskal-Wallis test: $H(1;63) = 5.64$, $p < 0.01$; Fig. 1). The average *C. typographi* infection level (\pm SD) across all managed locations was $14.5 \pm 21.9\%$.

The infection level averaged across all locations was $2.7 \pm 5.4\%$ for ItEPV and $0.9 \pm 5.3\%$ for *M. schwenkei*. For both of these pathogens, infection levels were higher in unmanaged than in managed locations: ItEPV – Shapiro-Wilk test: $W = 0.53$, $p < 0.0001$; Kruskal-Wallis test: $H(1;51) = 5.71$, $p < 0.01$ (Fig. 1); and *M. schwenkei* – Shapiro-Wilk test: $W = 0.49$, $p < 0.0001$; Kruskal-Wallis test: $H(1;40) = 7.98$, $p < 0.01$ (Fig. 1).

As noted earlier, some pathogens were excluded from the statistical analysis because of insufficient data, i.e., forestry management type was unspecified or the numbers of non-zero values were insufficient to support statistical evaluation. Among these other pathogens, the average infection level across all locations was $0.2 \pm 0.5\%$ for *Nosema typographi*, $1.8 \pm 3.6\%$ for *Unikaryon montanum*, $0.1 \pm 0.1\%$ for *Malamoeba scolyti*, and $0.001 \pm 0.001\%$ for *Menzbiera*

Table 1. Background information on the pathogens of *Ips typographus*, the number of analysed locations, and data sources included in the meta-analysis.

Pathogen	Number of locations		Altitude		Total number of beetles dissected	Data sources
	Managed	Unmanaged	>600 m a.s.l.	≤600		
ItEPV (Weiser & Wegensteiner, 1994)	39	12	28	23	25,366	1, 2, 5, 6, 7, 9, 10
<i>Gregarina typographi</i> (Fuchs, 1915)	48	16	35	29	30,854	1, 2, 3, 4, 5, 7, 8, 9, 10
<i>Chytridiopsis typographi</i> [(Weiser, 1954) Weiser, 1970]	46	17	34	29	30,680	1, 2, 3, 4, 6, 7, 8, 9, 10
<i>Mattesia schwenkei</i> (Purrini, 1977)	32	8	20	20	20,952	2, 6, 9, 10

¹ Haidler et al. 2003, ² Händel et al. 2003, ³ Holuša et al. 2009, ⁴ Kereselidze et al. 2010, ⁵ Lukášová & Holuša 2015, ⁶ Lukášová et al. 2012, ⁷ Lukášová et al. 2013, ⁸ Wegensteiner et al. 2007, ⁹ Wegensteiner et al. 2014, ¹⁰ Wegensteiner et al. 2015; N = number of dissected beetles.

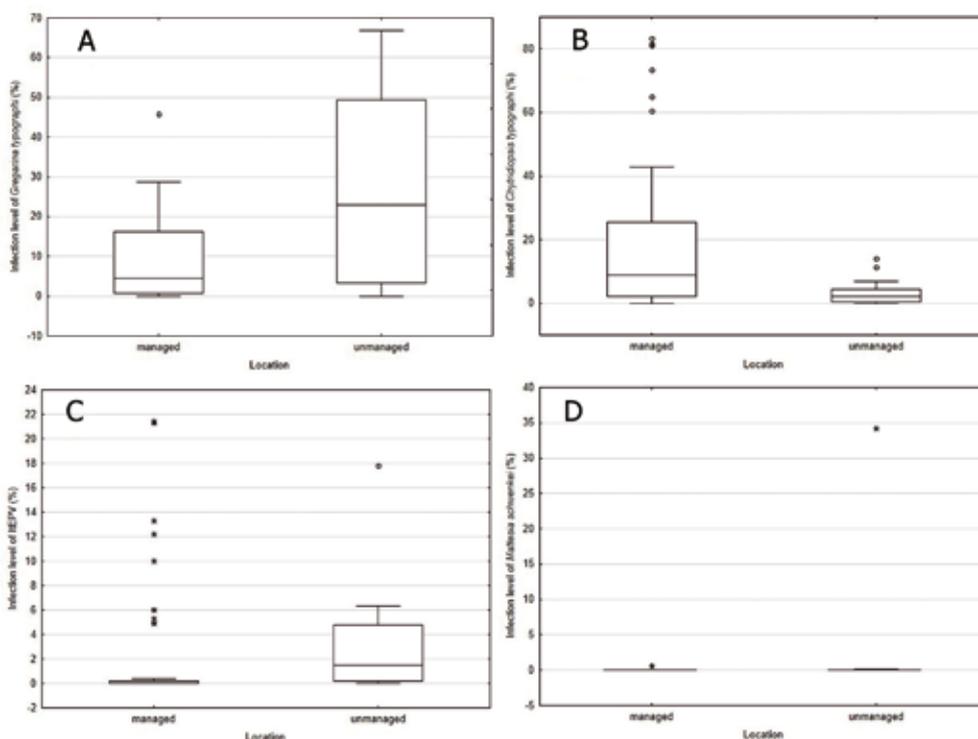


Fig. 1. Percentage of *Ips typographus* adults infected by four pathogens in managed vs. unmanaged locations. (A) *Gregarina typographi*, (B) *Chytridiopsis typographi*, (C) *Entomopoxvirus typographi*, or (D) *Mattesia schwenkei*. Box plots show median plus 25–75% quartiles. The upper and lower whiskers mark the non-outlier range. Circles show outliers, and stars indicate extreme values.

chalcographi. Regarding the frequency of occurrence of these other pathogens at the 45 managed locations, the frequency was highest for *U. montanum* (32 locations) and was lowest for *M. chalcographi* (4 locations). Among the 16 unmanaged locations, the frequency was highest for *N. typographi* (8 locations) and lowest for *M. chalcographi* (0 locations) (Table 2).

4. Discussion

The meta-analysis of *I. typographus* pathogens reported here was based on data from 61 locations in 10 studies. Most studies concerning *I. typographus* have been done in Europe, where forests are commonly managed to suppress *I. typographus* outbreaks. One limitation in our analysis is that specific information on the nature of the management at managed locations was often lacking. It is reasonable to assume, however, that logging and removal of the infested

spruces were regularly conducted at all managed locations.

The rapid removal of bark beetle-infested wood and of material suitable for reproduction can considerably reduce the local abundance of *I. typographus* during outbreaks (Wermelinger 2004; Stadelmann et al. 2013). On the other hand, deterioration of conditions for successful development of *I. typographus* populations resulting from landscape fragmentation in managed areas causes pioneering beetles to fly substantial distances and seek new locations (Valeria et al. 2016). This movement of beetles can result in the transport of associated pathogens to new areas and can also support increases in pathogen numbers in unmanaged areas, which serve as disease reservoirs and places with highest pathogen infection rates and species spectra. The infection levels of most pathogens in unmanaged stands are probably maintained over the long term, in part because spores in beetle-infested trees remain viable for long periods (Wegensteiner 2004).

Table 2. Background information on four pathogens of *Ips typographus* that were not included in the meta-analysis.

Pathogen	Number of locations		Altitude		Total number of beetles dissected	Data sources
	Managed	Unmanaged	>600	≤600		
<i>Malamoeba scolyti</i> (Purrini, 1980)	9	4	6	7	17,193	2, 9
<i>Menzbiera chalcographyi</i> (Weiser, 1955)	4	0	4	0	1,368	3
<i>Nosema typographi</i> (Weiser, 1955)	18	8	12	14	21,821	2, 3, 5, 7, 9
<i>Unikarion montanum</i> (Weiser, Wegensteiner, Žizka, 1998)	32	7	19	20	20,724	2, 9, 10

¹ Haidler et al. 2003, ² Händel et al. 2003, ³ Holuša et al. 2009, ⁴ Kereselidze et al. 2010, ⁵ Lukášová & Holuša 2015, ⁶ Lukášová et al. 2012, ⁷ Lukášová et al. 2013, ⁸ Wegensteiner et al. 2007, ⁹ Wegensteiner et al. 2014, ¹⁰ Wegensteiner et al. 2015; N = number of dissected beetles.

Vertical pathogen transmission (trans ovum or in the course of maturation feeding) from beetles of the parental generation to beetles of the filial generation can also occur to a limited degree. Pathogens transmitted to eggs evidently remain unapparent until the beetles mature into adults. Natural infection of beetle larvae is unlikely because all of the economically important bark beetle species normally feed in phloem (which is sterile), and larvae of most of these species avoid contact with each other (Wegensteiner 2004). For pathogens that do spread vertically, a generally low percentage of infected individuals persists in local populations, especially considering the isolated life cycle of *I. typographus*.

The diversity and infection levels of *I. typographus* pathogens can vary considerably in managed and unmanaged forest stands (Wermelinger 2004; Wegensteiner et al. 2007, 2015; Lukášová et al. 2012). Dispersal mechanisms also differ considerably among pathogens of *I. typographus*. The pathogen development cycle is frequently synchronized to that of the host to ensure the pathogen's successful infection and subsequent distribution (Massey 1956; Thong & Webster 1973; Wegensteiner 2004). Microsporidian species in the genera *Nosema* and *Unikaryon* are often vertically transmitted via eggs, and infection develops only in adults (Weiser et al. 2000). Horizontal transfer of microsporidians is possible during the maturation feeding of adults in the galleries, where adults encounter other adults; a second opportunity occurs during contact between the male and female in the primary mating gallery (Žižka et al. 1996, 1997, 1998; Weiser et al. 2000). Pathogens like *Chytridiopsis typographi* that develop only in the gut wall and that do not infect other organs have only one way to invade the host: horizontal transmission by peroral infection (Weiser et al. 2000; Wegensteiner 2004). The risk of infection is most probably influenced by other, primarily abiotic factors and in particular by temperature, UV radiation, or relative humidity. These conditions also greatly influence the suitability of trap trees and fallen trees for pathogen infection in that pathogens more frequently infect bark beetles in open and sun-exposed areas than in closed, shaded areas (Wegensteiner et al. 2014).

The gregarine *G. typographi* is a frequent pathogen of bark beetles and regularly occurs with other pathogens in *I. typographus* populations; the effect of *G. typographi* on *I. typographus* populations is disputed (Bjornson & Schütte 2003; Takov et al. 2011). Trophozoites and gamonts of *G. typographi* develop extracellularly in the digestive tract of their hosts, where the gamonts undergo syzygy and form zygotes enclosed in thick-walled gamont cysts that are excreted into the environment. After a relatively long maturation period (lasting at least from the time of parental gallery initiation to the maturation feeding of the offspring generation, i.e., approximately 2 months), the cysts are ingested by a suitable host to start a new parasitic cycle (Wegensteiner et al. 2010). The infection level of *G. typographi* within an *I. typographus* population increases several times during the breeding season (Lukášová & Holuša 2011). Our finding that *G. typographi* infection levels are higher in unmanaged than in managed area is consistent with a previous report (Kereselidze et al. 2010).

Chytridiopsis typographi was the only pathogen that did not have higher infection levels in unmanaged than in managed areas. The explanation may relate to the chronic character of the disease, which remains latent for a relatively long time; symptoms appear only in adults (Wegensteiner & Weiser 1996). *Chytridiopsis typographi* infection levels in *I. typographus* are usually about 10% (Wegensteiner 2004; Wegensteiner & Weiser 2004; Holuša et al. 2009; Wegensteiner et al. 2010). Infection destroys the midgut cells of *I. typographus* (Wegensteiner et al. 1996). The entire developmental cycle up to the mature spore stage occurs in the cytoplasm of midgut epithelium cells; once spores mature, they can initiate infection of new cells. Spread between hosts can occur when mature spores (enclosed in sporophorous vesicles) are released into the environment (Wegensteiner et al. 2010).

Insect viruses are obligate pathogens, and most are relatively host specific. The only known virus of *I. typographus*—ItEPV—is also host specific and occurs in the cells of the midgut epithelium (Weiser & Wegensteiner 1994; Wegensteiner & Weiser 1995). ItEPV infections are usually very contagious and cause mortality (Wegensteiner 2004). Discoidal inclusion bodies of ItEPV are released with faeces, and the infection destroys the gut epithelium of adult beetles (Wegensteiner 2004). ItEPV infection levels in *I. typographus* populations frequently range from 0.0 to 3.6% (Wegensteiner et al. 1996) and averaged 2.7% in the current meta-analysis. The virus is transferred during gallery formation and after the death of the host. Infection levels are higher in unmanaged areas than in managed areas probably because dead hosts and associated spores are not removed during sanitation cutting. A similar situation occurs with the neogregarine *M. schwenkei*, which also infects the bark beetle's fat body. Transfer of *M. schwenkei* between individuals occurs only through cannibalism or ingestion of part of a deceased, infected host (Wegensteiner 2004; Lukášová & Holuša 2012). *I. typographus* mortality caused by *M. schwenkei* occurs most frequently during the overwintering period (Lukášová & Holuša 2012).

The infection levels of some pathogens of bark beetles can be very low and even zero at many locations (Lukášová & Holuša 2012). This may help explain why we did not detect differences in infection levels between managed and unmanaged locations for the following species: *U. montanum*, *M. scolyti*, *M. chalcographi*, and *N. typographi*. Very low infection levels can result from inefficient transfer of propagules within the bark beetle population and also from low bark beetle population densities, such that individuals only meet within the family gallery and mortality occurs early during development. At low population densities, bark beetles of the new generation avoid older galleries and the associated spores of these pathogens.

Pathogens of bark beetles have been studied for almost 100 years (Wegensteiner 2004) with the goal of understanding and developing biological control. The value of pathogens as biological control agents against *I. typographus* is still unclear. Analysis of the effect of management on infection levels should help clarify this question. Although the current meta-analysis provides useful information, the number of publications reporting the effects of forest intervention on *I. typographus* natural enemies is small, and additional research is needed.

5. Conclusion

Whether or not spruce stands are managed can greatly influence the infection levels of *I. typographus* pathogens. Pathogen species that occur infrequently and with low levels of infection tend to remain in the old foci of *I. typographus* in the absence of forest management. We can therefore assume that pathogen levels and diversity will probably be higher in unmanaged than in managed locations because hosts and pathogen propagules are removed by management practices. Despite the greater pressure exerted by pathogens on *I. typographus* populations in unmanaged than in managed areas, their ability to suppress bark beetle outbreaks in unmanaged area is unclear and requires additional study.

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Appendix 1. Study locations from Table 1.

Locality*	Forest management	GPS coordinates	Country
Aigen-Schlögl	Y	48.665 N, 13.973 E	AUT
Białowieża I	Y	52.701 N, 23.886 E	POL
Białowieża II	Y	52.739 N, 23.786 E	POL
Białowieża III	Y	52.704 N, 23.899 E	POL
Białowieża IV	Y	52.666 N, 23.759 E	POL
Białowieża V	Y	52.670 N, 23.770 E	POL
Białowieża VI	Y	52.727 N, 23.842 E	POL
Białowieża VII	Y	52.683 N, 23.785 E	POL
Białowieża VIII	Y	52.730 N, 23.831 E	POL
Block N71	N	41.860 N, 43.234 E	GEO
Block N78	N	41.839 N, 43.283 E	GEO
Block N79	N	41.873 N, 43.334 E	GEO
Borjomi	Y	41.782 N, 43.494 E	GEO
Bystřice na Olši	Y	49.601 N, 18.717 E	CZE
Combloux	Y	46.470 N, 07.054 E	CHE
Edelwies	N	47.759 N, 15.056 E	AUT
Forêt de la Haute-Joux	Y	47.157 N, 06.106 E	FRA
Grünau	Y	47.767 N, 13.933 E	AUT
Horní Maršov	Y	50.670 N, 15.813 E	CZE
Hubhof	Y	48.392 N, 15.353 E	AUT
Hundsau I	N	47.779 N, 15.043 E	AUT
Hundsau II	N	47.778 N, 15.042 E	AUT
Hundsau III	N	47.777 N, 15.042 E	AUT
Chaux-des-Crotenay	Y	47.098 N, 06.569 E	FRA
Jánské Koupele	Y	49.733 N, 17.717 E	CZE
Kechkhobi	Y	41.782 N, 43.469 E	GEO
Keprník	N	50.181 N, 17.116 E	CZE
Kobernaußer Wald I	Y	48.088 N, 13.381 E	AUT
Kobernaußer Wald II	Y	48.087 N, 13.381 E	AUT
Kobernaußer Wald III	Y	48.088 N, 13.382 E	AUT
Kobernaußer Wald IV	Y	48.088 N, 13.380 E	AUT
Kozlov	Y	49.633 N, 17.504 E	CZE
Kreisbach	Y	48.097 N, 15.629 E	AUT
Kremsmünster	Y	48.086 N, 14.132 E	AUT
Lacelle	Y	46.063 N, 02.400 E	FRA
Lambach	Y	48.101 N, 13.872 E	AUT
Les Tenelles	Y	46.024 N, 01.622 E	FRA
Libani	Y	41.784 N, 43.462 E	GEO
Melk	Y	48.240 N, 15.393 E	AUT
Millevaches	Y	46.079 N, 02.144 E	FRA
Na Ztraceném, Ptačí potok	N	48.986 N, 13.502 E	CZE
Nasswald	Y	47.737 N, 15.664 E	AUT
Nová Pec	N	48.784 N, 13.951 E	CZE
Ossiach	Y	46.677 N, 14.003 E	AUT
Pec pod Sněžkou	N	50.703 N, 15.730 E	CZE
Prášily	N	49.107 N, 13.371 E	CZE
Pustá Polom	Y	49.868 N, 18.006 E	CZE
Puy de Montchal	Y	45.822 N, 03.476 E	FRA
Rothenburg	N	47.101 N, 08.233 E	CHE
Rothenburg Wald	Y	47.113 N, 08.284 E	CHE
Rothwald	N	47.749 N, 15.084 E	AUT
Siedlce	Y	52.188 N, 22.163 E	POL
Smrk Mt.	N	49.505 N, 18.378 E	CZE
St. Georgen/ Lavanttal	Y	46.724 N, 14.954 E	AUT
Staré Oldřůvky	Y	49.717 N, 17.609 E	CZE
Tamsweg	Y	47.117 N, 13.629 E	AUT
Tamsweg, Salzburg	Y	47.115 N, 13.849 E	AUT
Tösstock	N	47.306 N, 08.962 E	CHE
Treffen	Y	46.674 N, 13.862 E	AUT
Tsagveri	Y	41.794 N, 43.484 E	GEO
Václavovice	Y	49.733 N, 18.350 E	CZE

*Y = managed area, N = unmanaged area.