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ORIGINAL PAPER

Sphaeropsis sapinea (Fr.) Dyko & B. Sutton in *Pinus sylvestris* L. stands affected by long-term drought

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Abstract
In this study, the fruit bodies (pycnidial) colonization and spore presence of *Sphaeropsis sapinea* (Fr.) Dyko & B. Sutton on Scots pine (*Pinus sylvestris* L.) trees in stands affected by long-term drought in the Czech Republic were evaluated. A total of 520 cones at four sites were evaluated every 1.5 months from June 2019 to December 2020. The pycnidia of *S. sapinea* in relation to colonization by subcortical insects in inner bark and wood, and wood-decaying fungi a total of 340 trunks at 17 sites during the autumn of 2020 were also evaluated. Pycnidial colonization of *S. sapinea* on cones was significantly higher at the site with the highest air humidity and significantly lower in the sampling periods of June 2019, August 2019, and November 2019, which were characterized by low precipitation levels. *S. sapinea* spore presence on cones was significantly higher at sites in Bohemia compared to those in Moravia, in sites with higher air humidity, and in three consecutive sampling periods in March 2020–June 2020. Pycnidial colonization of *S. sapinea* on trunks was significantly positively dependent on the colonization of subcortical insects in both inner bark and wood, but not with the colonization of wood-decaying fungi. The results of this study show a positive relationship between high humidity and colonization by subcortical insects in inner bark and wood with *S. sapinea* on Scots pine.

Key words: *Diplodia* tip blight; fungal pathogens; Scots pine; subcortical insects; wood-decaying fungi

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1. Introduction

Sphaeropsis sapinea (Fr.) Dyko & B. Sutton, syn. *Diplodia sapinea* (Fr.) Fuckel, is an endophytic, ascomycetous fungus, plant saprophyte, and parasite that causes Diplodia tip blight (Bußkamp et al. 2020). Infection with *S. sapinea* can result in serious damage to conifers (Doğmuş-Lehtijärvi et al. 2014). The most common host trees of *S. sapinea* are pines (*Pinus* spp.), but *Abies* spp., *Cedrus* spp., *Larix* spp., *Picea* spp. *Pseudotsuga* spp., *Tsuga* spp., *Chamaecyparis* spp., *Cupressus* spp., *Juniperus* spp., *Platycladus* spp., *Fagus* spp., *Quercus* spp., *Alnus* spp. and *Arceuthobium* spp. can also serve as host trees (Zlatković et al. 2017; Smahi et al. 2017; Bußkamp et al. 2021; CABI 2021).

S. sapinea forms fruit bodies (pycnidis) that contain spores (conidia) on the trunks, branches, needles, and cones of the hosts (Zhou et al. 1997). Pycnidia are dark, immersed to erumpent, ovoid, and ostiolate. Conidia are ovoid to obovoid, rounded at the apex, initially hyaline to yellowish becoming dark brown, usually 0–1 septate, thick-walled, and approximately 30–45 µm × 10–16 µm (Swart et al. 1993). Colonization of pine cones by *S. sapinea* increases with higher winter temperatures and higher summer precipitation (Fabre et al. 2011). Germination of *S. sapinea* spores increases with high humidity (Capretti et al. 2013). The optimal temperature for mycelial growth of the pathogen is about 30 °C (Keen & Smith 1989). Significant factors that influence the outbreak of *S. sapinea* are temperature, altitude and land cover (Bosso et al. 2017). Subcortical insects (Whitehill et al. 2007; Davydenko & Baturkin 2020) and large pine weevil (*Hylobius abietis* L.) (Drenkhan et al. 2017) can be vectors of *S. sapinea* spores, making it easier for this pathogen to colonize the host's cambial tissues. (Zwolinski et al. 1995). *S. sapinea* is able to survive on dead host tissues for at least several months up to one year (Santini et al. 2008).

S. sapinea attacks cones, which reduces the amount of seed production and germination (Doğmuş-Lehtijärvi et al. 2014). *S. sapinea* also attacks trees from the seedling stage in nurseries to mature trees in ornamental planting, forest plantations and natural stands (Georgieva & Hlebarska 2016). *S. sapinea* has an endophytic life stage

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when it does not harm the host (Smith et al. 1996; Flowers et al. 2001; Terhonen et al. 2020; Blumenstein et al. 2021b). However, the fungus can accumulate unnoticed in healthy trees before the disease breaks out, representing a sudden threat to hosts (Stanosz et al. 2001; Blumenstein et al. 2021a). The pathogenicity of *S. sapinea* has increased due to climate change, climate warming, and longer or more frequent droughts (Desprez-Loustau et al. 2007; Vornam et al. 2019). *S. sapinea* has changed from a relatively harmless species to a virulent one in many countries (Spathelf et al. 2014). Pathogen-induced defoliation can act as predisposing and inciting factors for tree death, reducing the capacity of trees to survive short- or long-term stressing events, such as bark beetle attacks (Oliva et al. 2016).

The occurrence of *S. sapinea* has been confirmed in a total of 128 countries in Europe, Asia, Africa, North America, South America, Australia and Oceania (CABI 2021). In Europe, *S. sapinea* is rapidly spreading to the north. Now, it is commonly seen in the Baltic Sea region (Adamson et al. 2015), Finland (Terhonen et al. 2020) and Scandinavia (CABI 2021) and is threatening to become a serious pathogen in these areas (Brodde et al. 2019; Adamson et al. 2021). In recent years, water balance disturbances in Poland have contributed to the spread of fungal pathogens, including *S. sapinea,* on *P. sylvestris* (Skrzecz & Perlińska 2018). In Slovakia, *S. sapinea* has been a serious pathogen on Austrian pine (*Pinus nigra* J.F. Arnold) since 2000 (Kunca & Leontovyč 2013; Leontovyč et al. 2020), including urban trees (Juhásová et al. 2006). In the Czech Republic, *S. sapinea* previously occurred mainly on non-native *P. nigra* (Jankovský & Palovčíková 2003; Novotný et al. 2012), but since 2015, it has also begun attacking native *P. sylvestris*, especially in stands weakened by drought and high temperatures (Pešková & Soukup 2016).

The most important measures to control the spread of *S. sapinea* are to provide and conserve water during drought seasons (Sinclair & Lyon 2005). Pruning and removal of excised shoots of both seedlings (Munck & Stanosz 2008) and mature trees may improve their appearance, but a new colonization may occur because conidia are also released from diseased cones on green branches (Sinclair & Lyon 2005). The persistence of pine-pathogenic species, such as *S. sapinea*, for up to one year in dead plant material questions the feasibility of leaving coarse woody debris (Santini et al. 2008). Planting of more resistant tree species and varieties can also be recommended (Gerhold et al. 1994; Iturritxa et al. 2013), but non-native species can be more susceptible to pathogens (Lombardero et al. 2008). In forest nurseries, it is recommended to apply chemical preparations during the period of spore dissemination (Georgieva & Hlebarska 2016). There is a link between the *S. sapinea* spore presence on cones and the occurrence of Diplodia tip blight (Peterson et Wysong 1968). Monitoring of fruit bodies and sporulation of *S. sapinea* on pine cones could therefore be a useful method for determining stands that are in danger of being afflicted with Diplodia tip blight.

The aims of this study, which was performed in forest pine stands affected by long-term drought in the Czech Republic, were to: 1) monitor the dynamics of *S. sapinea* pycnidial colonization and its spore presence on pine cones and assess how they are affected by air temperature and air humidity; 2) assess the link between the occurrence of *S. sapinea* pycnidial colonization on pine trunks and the colonization by subcortical insects intinner bark, subcortical insects in wood, and wood-decaying fungi.

2. Material and methods

After years with prolonged drought episodes culmitating in 2018 (Skalák et al. 2019; ČHMÚ 2021), 4 permanent research sites were established in the Czech Republic in pine forest with visible symptoms of decline due to drought in June 201t: Valtice, Příšťpo, Brodce, Vrbová Lhota (Table 1). Each research site was approximately rectangular in shape and consisted of 50 live *P. sylvestris* trees. A temperature-humidity logger (type S3631 from Comet System, s.r.o, Czech Republic) was installed on the trees in the centre of each site at a height of approximately 1.3 m above the ground and was protected against direct sunlight and rain by a plastic cup coated with dark adhesive tape. The loggers recorded the air temperature and relative air humidity at 1-hour intervals. Data was gathered from the sites between July 2019 and November with the exception of the Vrbová Lhota site. Only the period between January 2020 and November 2020 was considered for this site, as no data was gathered outside of this period (due to logger failure). The mean daily air temperature was calculated from the temperatures measured by the logger at 7:00, 14:00, and 21:00 Central European Time, according to the formula: $(T_7 + T_{14} + 2 \times T_{21}) / 4$. The mean daily air humidity was calculated as the arithmetic mean from the values measured by the logger. The mean monthly air temperature and humidity were calculated as the arithmetic mean from the mean daily values.

Every 1.5 months, 10 light-coloured pine cones without traces of decomposition were collected at each research site from the sampling sites (evenly distributed throughout the sites), from July 2019 to December 2020 (40 cones in each sampling period, 13 sampling periods, for a total of 520 cones). Pycnidial colonization of *S. sapinea* on each cone was evaluated using a stereomicroscope in 4 classes: 0 (no pycnidia), 1 (individual pycnidia), 2 (many pycnidia on some parts of cones only), and 3 (many pycnidia on the whole cone) (Fig. 1). *S. sapinea* spore presence in its pycnidia on each cone was subsequently evaluated using a laboratory microscope in two levels: 0 (no spores) and 1 (spores present). Other fungi on the cones were excluded from the evaluation.

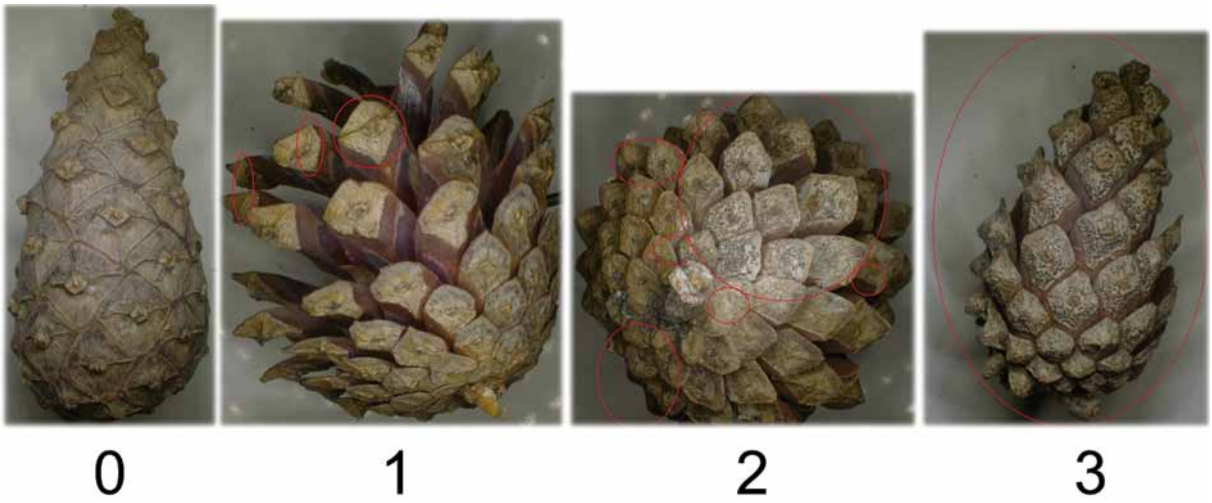


Fig. 1. The examples of pycnydial colonization of *Sphaeropis sapinea* classes on cones: 0 – no pycnidia, 1 – individual pycnidia, 2 – many pycnidia on some parts of cones only, 3 – many pycnidia on the whole cone.

Table 1. Summary information about research sites for evaluations on pine cones. Altitude – values in meters above sea level; Soil type according to CENIA (2010–2020).

Site	GPS	Altitude	Soil type
Valtice	48.7596542N, 16.8160897E	185	Arenic Cambisols
Příšťpo	49.0505056N, 15.9360144E	450	Dystric Cambisols
Brodce	49.8422722N, 14.5976958E	305	Eutric Cambisols
Vrbová Lhota	50.1180942N, 15.0869150E	190	Calcic Chernozems

In 2020, a one-time evaluation was carried out at 17 sites in the Czech Republic (Table 2) that had a significant proportion of pine stands (ÚHÚL 2021), on this year's fallen *P. sylvestris* trunks due to windfall, windbreak or cutting (based on informations from forest owners). Trunks which fall down before 2020 were not evaluated due to high colonization of saprotrophic fungi. Twenty trunks were evaluated per site (total 340 trunks). Pycnidial colonization of *S. sapinea* on trunks, colonization by subcortical insects in inner bark, subcortical insects in wood, and wood-decaying fungi were evaluated, all in 3 levels: 0 (absent), 1 (low occurrence in a limited part of the trunk), 2 (strong or widespread occurrence). Pycnidial colonization of *S. sapinea* was related to upper (crown) parts of trunk only, because the pycnidia were totally absent on the thick bark in the other parts of the trunks. Colonization of wood-decaying fungi was evaluated as a presence of visible symptoms of the colonization of these fungi: wood rot, fruit bodies on the trunk, mycelium, syrrocium or rhizomorphs under bark. In unclear cases, samples were taken from the trunks and examined in a laboratory using a microscope.

The obtained data was statistically tested in SPSS software. Generalized linear models (GLM) were used (Denis 2019).

3. Results

The dynamics of air temperature and air humidity during the measurement period (July 2019–November 2020) were similar in all 4 permanent research sites. The highest mean monthly temperatures were recorded at the Valtice site and then at the Vrbová Lhota site. Above-average temperatures (compared to the normal from 1981–2010) were recorded in all months at all sites, except for May

Table 2. All sites for evaluation of *Sphaeropsis sapinea* on pine trunks, their positions and mean ± standard deviation (range 0–2) of assessed variables. Number of sampled trunks for each site: 20.

Site	GPS	S. sapinea	Insect inner bark	Insect wood	Fungi wood
Býšť	50.1519389N, 15.8684200E	0.60 ± 0.94	1.30 ± 0.98	0.00 ± 0.00	0.20 ± 0.62
Horšovský Týn	49.5410833N, 12.9355556E	1.10 ± 0.91	1.90 ± 0.45	1.00 ± 0.97	0.85 ± 0.99
Chramosty	49.6632256N, 14.3142286E	1.85 ± 0.49	2.00 ± 0.00	0.85 ± 0.93	1.35 ± 0.93
Jemčina	49.1109722N 14.8534881E	0.30 ± 0.73	1.55 ± 0.83	0.00 ± 0.00	2.00 ± 0.00
Kladruby	49.7170089N, 13.0268167E	0.55 ± 0.89	0.80 ± 0.85	0.00 ± 0.00	0.10 ± 0.45
Kryry	50.2112686N, 13.4334769E	0.45 ± 0.69	1.40 ± 0.82	0.40 ± 0.75	0.05 ± 0.22
Nový Ples	50.3105811N 15.9525286E	1.65 ± 0.49	1.55 ± 0.83	1.50 ± 0.76	1.55 ± 0.83
Přelovice	50.0657689N, 15.6119669E	1.10 ± 0.91	1.70 ± 0.73	0.90 ± 0.91	1.30 ± 0.98
Rakov	49.3475000N, 14.3336111E	0.55 ± 0.89	1.45 ± 0.83	0.15 ± 0.49	0.50 ± 0.89
Rozkoš	49.0263511N 15.9537692E	0.40 ± 0.82	2.00 ± 0.00	1.30 ± 0.86	0.00 ± 0.00
Srní u České Lípy	50.6411639N, 14.6103083E	0.50 ± 0.83	1.80 ± 0.52	0.30 ± 0.66	1.60 ± 0.82
Strážnice	48.9428686N, 17.2704803E	1.40 ± 0.82	1.50 ± 0.76	0.00 ± 0.00	0.00 ± 0.00
Třeboň	49.0021900N, 14.8220656E	0.70 ± 0.98	1.70 ± 0.66	0.40 ± 0.75	0.20 ± 0.41
Tvořihráz	48.9092297N, 16.0999289E	0.50 ± 0.89	2.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.45
Valtice	48.7663567N, 16.7900678E	0.05 ± 0.22	0.65 ± 0.93	0.00 ± 0.00	0.00 ± 0.00
Vrbová Lhota	50.1139339N, 15.0938819E	0.60 ± 0.88	0.60 ± 0.94	0.50 ± 0.89	0.10 ± 0.45
Ždírec	49.4648753N, 15.6571569E	1.10 ± 1.02	1.00 ± 1.03	0.00 ± 0.00	0.00 ± 0.00

2020 (Fig. 2). The highest air humidity was recorded at the Brodce site, which was higher compared to other sites, especially during June 2020–December 2020. The lowest air humidity was recorded at the Vrbová Lhota site (in all measurement months), and in terms of the period, from March 2020 to May 2020 with a minimum in April 2020 at all sites (Fig. 3).

On cones, pycnidial colonization of *S. sapinea* was significantly higher at the Brodce site (Table 3, Fig. 4), and in the sampling periods June 2019, August 2019, and November 2019 (Table 3, Fig. 4). *S. sapinea* spore presence differed among all sites, ranked in decreasing order: Brodce, Vrbové Lhota, Příšťpo, Valtice (Table 3, Fig. 4). Pycnidial colonization was also significantly higher in successive sampling periods March 2020, April 2020, and June 2020 (Table 3, Fig. 4).

On pine trunks, *S. sapinea* pycnidia were recorded in low occurrence (level 1) on 32 trunks (9%) and in strong occurrence (level 2) on 118 trunks (35%). Pycnidial colonization of *S. sapinea* on trunks was significantly positively dependent on the colonization by subcortical insects in inner bark (Table 3, Fig. 5) and by subcortical insects in wood (Table 3, Fig. 5), but independent from the colonization by wood-decaying fungi (Table 3, Fig. 5). White rot on wood occurred on most trunks colonized by wood-decaying fungi, along with white syrrocium and/or black cord-like rhizomorphs of *Armillaria* sp.

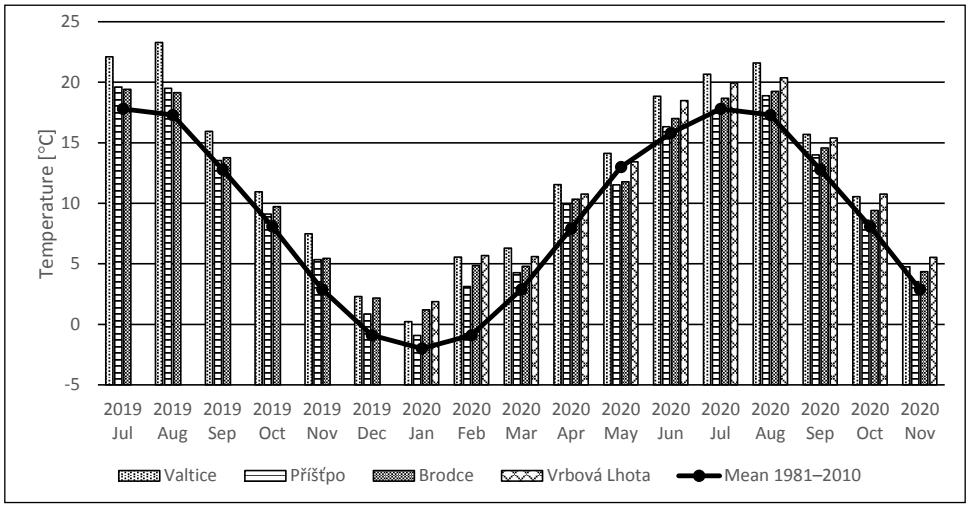


Fig. 2. Mean monthly air temperature at research localitiessites for the evaluation of pine cones during the research period (own data), and mean monthly temperature for the Czech Republic in 1981–2010 (ČHMÚ 2021).

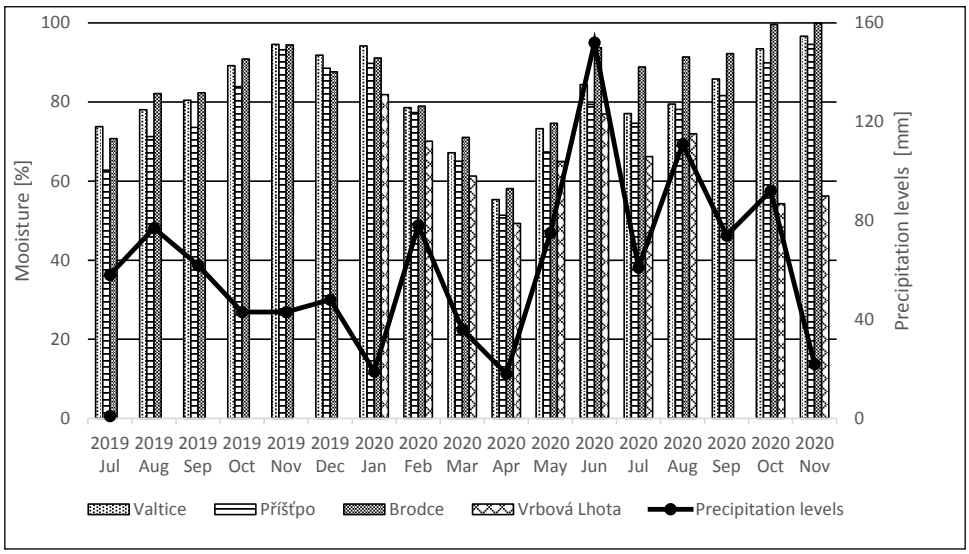


Fig. 3. Mean monthly moisture at research sites for the evaluation of pine cones during the research period (own data) and precipitation levels for the Czech Republic (ČHMÚ 2021).

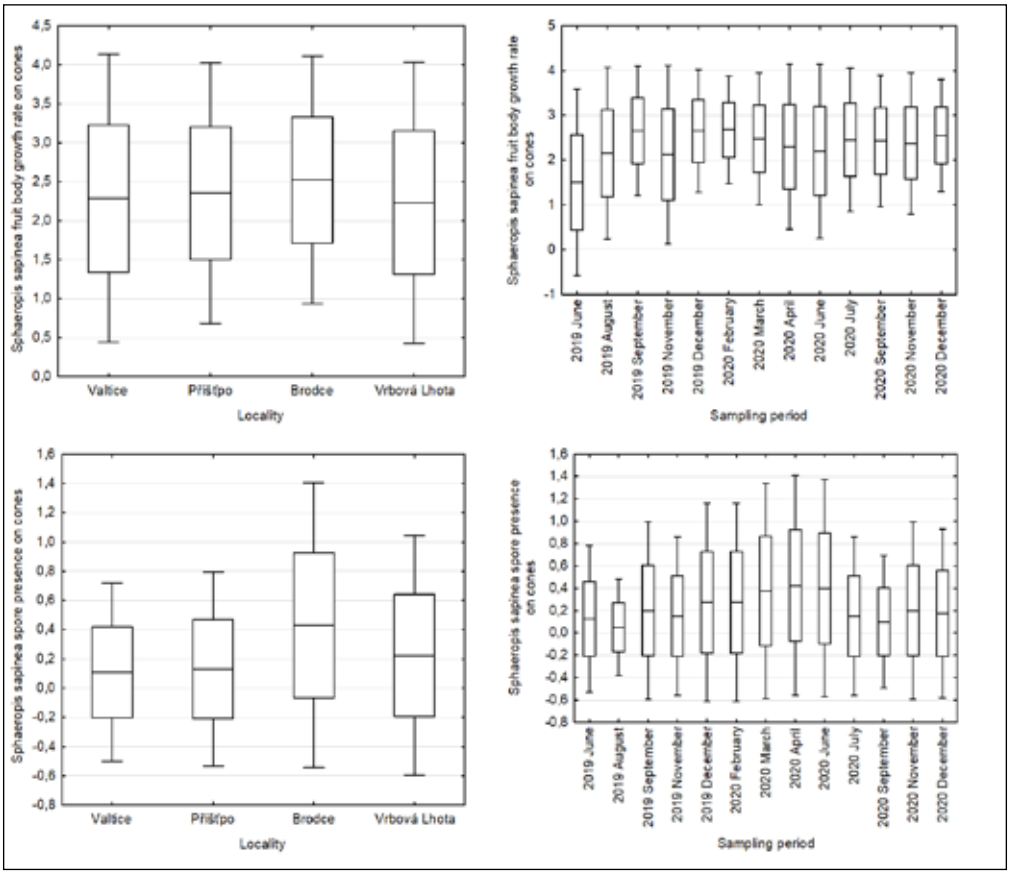


Fig. 4. Box plots of pycnidial colonization of *Sphaeropsis sapinea* and its spore presence on pine cones. Centre line – mean, box – standard deviation, whiskers – 1.96 * standard deviation. 130 samples per site box, 40 samples per sampling period box.

Table 3. Generalized linear models (GLMs) of pycnidial colonization of *Sphaeropsis sapinea* and its spore presence on pine cones, and pycnidial colonization on pine trunks. Effect – tested variables, df – degrees of freedom, Wald – value of the Wald Chi-square test; p – significance level (number non-significant * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$).

Model	N	Effect	Wald	df	P	Significantly different groups
Pycnidial colonization on cones	520	Site	9.26	3	*	higher: Brodce
		Sampling period	70.27	12	***	lower: 2019 June, 2019 August, 2019 November
Spore presence on cones	340	Site	58.54	3	*	Brodce > Vrbová Lhota > Příšťpo > Valtice
		Sampling period	47.02	12	***	higher: 2020 March, 2020 April, 2020 June
Pycnidial colonization on trunks	340	Insect inner bark	12.95	2	**	positive dependence
		Insect wood	12.12	2	**	positive dependence
		Fungi wood	4.04	2	0.13	

4. Discussion

Higher temperatures at the Valtice and Vrbová Lhota sites were related to their lower altitude (Table 1). During our study, temperatures at all sites were higher than mean temperatures in all months except May 2020. Low humidity at the Vrbová Lhota was probably due to it being located on the edge of the forest next to a meadow, whereas the other sites were within the forest stand. The high humidity at the Brodce site during June 2020–November 2020 compared to the other sites in our study was probably due to extremely high precipitation in June 2020 (171% compared to the mean from 1981–2020 for Prague and the Central Bohemian Region) (ČHMÚ 2021). A subsequent period with sufficient precipitation (ČHMÚ 2021) and the microclimatic conditions at the site were also causes of the high humidity.

Pycnidial colonization of *S. sapinea* on cones was significantly higher at the Brodce site, which was characterized by the highest air humidity (Fig. 3). Zwolinski et al. (1990) recorded a higher intensity of dieback by *S. sapinea* on *Pinus radiata* in South Africa in areas with lower altitudes, enclosed valley sites, and sheltered stands that are all characterised by higher air humidity and smaller temperature variation. Pycnidial colonization of *S. sapinea* in our study was significantly lower in the sampling periods June 2019, August 2019, and November 2019, which were characterized by high temperatures compared to the mean from 1981–2020 (Fig. 2) and by low precipitation (ČHMÚ 2021; Fig. 3). High summer precipitation has a positive effect on the colonization of pine cones by *S. sapinea* (Fabre et al. 2011). In the Czech Republic, lower precipitation lev-

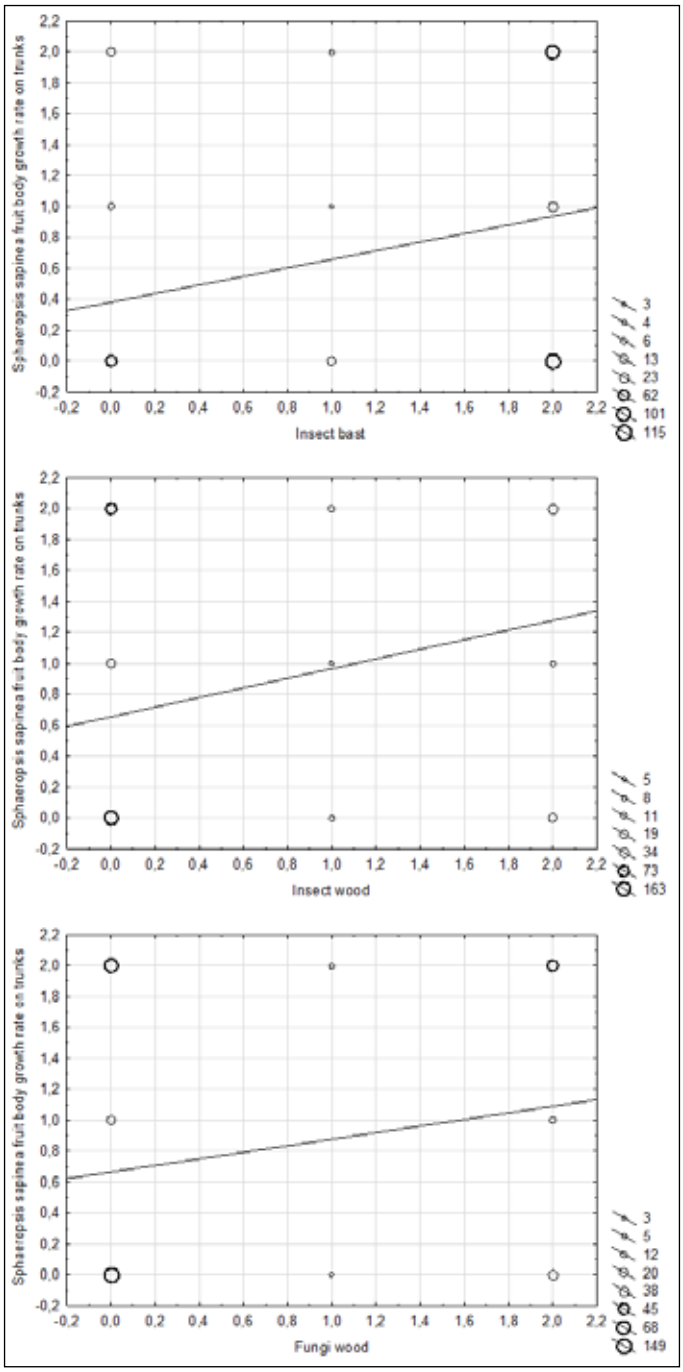


Fig. 5. Scatterplots of pycnidial colonization of *Sphaeropsis sapinea* fruit body growth rate on pine trunks. Numbers next to circles – number of its samples. Total 340 samples in each scatterplot.

els were recorded in 2019 compared to 2020 during our study (ČHMÚ 2021, Fig. 3). Blumenstein et al. (2021b) recorded a significantly higher abundance of *S. sapinea* on *P. sylvestris* branches in Germany in June compared to September. Contrary, pycnidial colonization of *S. sapinea* on cones showed seasonal dynamics in our study, probably due to negative effect of low humidity, respectively low precipitations, and high summer temperatures. *S. sapinea* spore presence on cones in our study differed significantly between all sites, ranked in decreasing order: Brodce, Vrbové Lhota, Příšťpo, Valtice. The Brodce and Vrbová Lhota sites were in Bohemia, while the remaining 2 sites were in Moravia. These results are consistent with surveys of the Forest Protection Service in the Czech Republic, where the colonization of *S. sapinea* in dying pine stands was higher in Bohemia than in Moravia (Liška et al. 2018), although these differences were less pronounced in the period of our study (2019–2020) than in previous years (Lorenc 2021). Of the pairs of sites both in Bohemia and Moravia, *S. sapinea* spore presence was

higher at those with higher altitudes, which were characterized by lower air temperature (Fig. 2). In contrast, the intensity of *S. sapinea* colonization (presence) (Fabre et al. 2011) as so as infection (as a pathogen) (Zwolinski et al. 1990) on pines decreased with increasing altitude in previous studies (Zwolinski et al. 1990; Fabre et al. 2011). In Bohemia, the lower *S. sapinea* spore presence at the Vrbová Lhota site could be due to lower air humidity (Fig. 3). In Moravia, the differences in air humidity measurements between sites were small. According to ČHMÚ (2021), precipitation levels in the same period were 967 mm in the South Moravian Region (Valtice) and 1120 mm in the Vysočina region (Příšťpo). Thus, increased precipitation could be the reason for the higher *S. sapinea* spore presence in Příšťpo compared to that seen in Valtice. Overall, *S. sapinea* spore presence in our study significantly differed between regions (higher in Bohemia than in Moravia), higher humidity and precipitation affected it positively, while the effects of both temperature and altitude were not significant.

Spore presence of *S. sapinea* on cones in our study was highest from March 2020–June 2020. Results from previous studies are different. Palmer et al. (1988) recorded the largest dispersion of *S. sapinea* spores on red pine (*Pinus resinosa* Aiton) cones from April–June in both research years 1981 and 1982. Kalder (2015) recorded the peak of *S. sapinea* sporulation on trunks of 20-year-old pines in Estonia in early November. Brookhouser & Peterson (1971) found the largest amount of releasing *S. sapinea* spores on *P. nigra* in Nebraska from June–August in research year 1969, but only if precipitation occurred during the relevant sampling period. Capretti et al. (2013) reported a positive effect of air humidity on germination of *S. sapinea* spores. In our study, the period with the highest presence of spores was first characterized by low humidity (March, April), which then increased due to high precipitation (May, June) (Fig. 3). Thus, the seasonal dynamics of *S. sapinea* sporulation may vary significantly between years depending on weather conditions, especially precipitation and air humidity.

On trunks, pycnidial colonization of *S. sapinea* in our study was significantly positively dependent on colonization by subcortical insects both in inner bark and wood. Davydenko & Baturkin (2020) observed an association between *Ips acuminatus* Gyllenhal and *S. sapinea* on *P. sylvestris*. An association between *S. sapinea* colonization and subcortical insects was also observed in other hosts. Monterey pines (*Pinus radiata* D. Don) infested with *Pissodes neotensis* Germar and *Orthotomicus erosus* Wollaston were more frequently infected with *S. sapinea* (Zwolinski et al. 1995). Bezos et al. (2018) found that, on Monterey pines, *S. sapinea* spores were found abundantly in the galleries of bark beetles *Tomicus piniperda* L. (on branches), *Ips sexdentatus* Börner, *Hylastes attenuatus* Erichson, *Hylastes ater* Paykull, *H. angustatus* Herbst, *Hylurgops palliatus* Gyllenhal (in these 5 species in inner bark and wood), and *Orthotomicus erosus* Wollaston (in wood). The colonizationof *S. sapinea* was also associated with infestation from the genus *Dioryctia* in *P. resinosa* (Feci et al. 2003) and from pine cone bugs (*Gastrodes grossipes* De Geer) in *P. nigra* (Feci et al. 2002), which indicates the role of various groups of insects in spreading this fungus. Therefore, subcortical insects in both inner bark and wood could contribute to *S. sapinea* colonization of pine trunks in our study. *S. sapinea* pycnidial colonization on trunks in our study was not dependent on the colonization of wood-decaying fungi. White wood rot was observed on most of the colonized trunks, along with white syrrocium and/or black cord-like rhizomorphs. These symptoms are typical signs of infection by honey fungus (*Armillaria* spp.), which is a very serious pathogen of woody plants, such as *P. sylvestris* (Příhoda 1959). The virulence of this fungus increases with high temperatures and low precipitation during the growing season (Lindner et al. 2008). Hurel et al. (2021) recorded a lower susceptibility to *S. sapinea* infection in populations of marine pine (*Pinus pinaster* Aiton) from areas with high temperatures and frequent droughts, although a higher susceptibility to *Armillaria* ostoyae (Romagn.) Herink was recorded as well. Jankovský & Palovčíková (2003) recorded *S. sapinea* on a mass scale on shoots of declining *Pinus nigra* trees in the southern Moravia (Czech Republic), whereas *Armillaria* sp. occurred only sparsely and only proliferated as a response to the tree dieback, not as causal agents of the decline. It follows that pycnidial colonization of *S. sapinea* on pine trunks is not in direct positive relationship with colonization by wood-decaying fungi.

Increase in the drought frequency as a stress factor causes a reduction in the assimilation apparatus, which reduces the carbon content in woody plants and subsequently weakens their resistance to biotic pests (Oliva et al. 2014), including fungal pathogens (Desprez-Loustau et al. 2006) and subcortical insects (Økland & Berryman 2004; Netherer et al. 2015). Secondary colonization by cambiophagous insects of trees infected by *S. sapinea* can increase infection of *S. sapinea* and consequently the onset of dieback through girdling of branches and the bole (Zwolinski et al. 1995). According to meta-analysis by Jactel et al. (2012), primary pests and pathogens living in wood (including *A. ostoyeae*) caused significantly lower damage to the water-stressed trees compared with the control, whereas primary pests and pathogens living on foliage caused more damage to water-stressed trees, all irrespective of stress severity. Damage by secondary pests and pathogens (including *S. sapinea*) increased with water stress severity (Jactel et al. 2012). In our study, colonization (presence) of *S. sapinea* without its relation to damage was evaluated. *S. sapinea* can be present on tree mass like a saprophyte, without harming the host (Bußkamp et al. 2020; Blumenstein et al. 2021b). So, relationship between colonization and infection of *S. sapinea* may not be always positively correlated. How-

ever, *S. sapinea* has ability to accumulate unnoticed as an endophyte in healthy trees before the disease breaks out, representing a sudden threat to *P. sylvestris* in the future, especially with increasing drought conditions experienced by pines (Blumenstein et al. 2021b).

5. Conclusion

On cones, *S. sapinea* pycnidial colonization and its spore presence was positively affected mainly by air humidity. Although long periods of drought lead to the weakening of trees that resulted in reduced resistance infection by pathogens (Oliva et al. 2014), including *S. sapinea* (Vornam et al. 2019), the direct effect of drought on *S. sapinea* is negative (Desprez-Loustau et al. 2006; Hurel et al. 2021). Relationship between colonization and infection of *S. sapinea* may not be positively correlated. However, due to ability of *S. sapinea* to accumulate as an endophyte in healthy trees before the disease breaks out (Blumenstein et al. 2021b), pycnidial colonisation and spore presence of the fungus can be useful indicators of possible threat of pine stands decline and dying in the coming years. Climate change, manifested by rising temperatures and more frequent extremes that include longer and stronger droughts (Lindner et al. 2008), currently leads to the increased infections of *S. sapinea*. However, reversing this trend cannot be ruled out due to the ambivalent relationship between *S. sapinea* and drought.

The results of our study further confirm the relationship between colonization by subcortical insects both in inner bark and wood with the colonization of *S. sapinea* on *P. sylvestris*. Subcortical insects can be vectors od *S. sapinea* spores (Zwolinski et al. 1995; Whitehill et al. 2007; Davydenko & Baturkin 2020). Moreover, colonization by cambiophagous insects may extracerbate the infection of *S. sapinea* and consequently onset to dieback of pines (Zwolinski et al. 1995). Thus, forest protection measures preventing colonization by subcortical insects (including bark beetles) can also be useful for reduction of *S. sapinea* colonization and infection of pine trees.

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