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Bud blight (*Gemmamyces piceae*) in the eastern part of the Krušné hory Mountains

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Abstract: A survey was carried out in 2013–2019 on the life cycle of the bud blight *Gemmamyces piceae* (Borthw.) Casagrande and the trend assessment in the infestation of the Colorado blue spruce *Picea pungens* Engelm. stands. Four ecologically different plots were chosen in the Fláje region in the Eastern part of the Krušné hory Mountains. The pycnidia were formed at the beginning of June on buds infested the previous year. Later, in the second half of June, they formed on the spring attack buds. Conidia occurred in the first week of July on the buds of the old infestation, or later in the case of the spring attack buds, in the middle of July. The production of conidia lasted till the end of September. Ascospores occurred in the middle of July and were produced until the end of September. The ability of host trees to sprout became continually weaker, along with the defoliation progress. A distinct decline in the sprouting ability was noticed on stands with a dense crown canopy and on stands with competition of broad-leaved trees – with an admixture of birch.

Keywords: Colorado blue spruce; disease development; epidemic; *Gemmamyces piceae*

Since 2010, substitute forest stands of Colorado blue spruce – *Picea pungens* Engelm. (BS) in the Eastern part of the Krušné hory Mts. were strongly affected by the bud blight *Gemmamyces piceae* (Borthw.) Casagrande, (further just bud blight) (Soukup, Pešková 2009a; Pospíšil, Pospíšil 2011; Janů 2011; Šefl 2013; Mikoláš 2014; Schořálková 2015; Černý et al. 2016; Zýka et al. 2018). The beginning of the pandemic in the region was traced back to year 2000 (Černý at al. 2016) and the first documented findings are dated to 2007 (Pospíšil sec. Zýka et al. 2018). The pathogen life cycle is well known (Soukup, Pešková 2009a; Černý at al. 2016). It is also known that the fungus in the mountain conditions of Central Europe is greatly virulent in the BS stands. The fungus is immensely destructive for stands of this spruce species (Pospíšil, Pospíšil 2011; Černý et al. 2016; Zýka et al. 2018). This paper brings a survey on the fungus life cycle in the centre of its mountain pandemic in the Fláje region over the period of 2013–2019, and confirms the destructive impact of the infestation on the BS stands.

The purpose of the study was (1) to evaluate the extent of the damage to the BS trees by the bud blight depending on select environmental factors and (2) to study the life cycle of the fungus in the environment of its calamitous spread.

Hypotheses were formulated that the extent that the bud blight damage relates to (1) the elevation, (2) the stand density and competition from broad-leaved trees, and (3) the proximity to flowing water or a water reservoir.

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Susceptibility of spruce species planted in the Fláje region to bud blight

In forests stands, in proximity to the Fláje water reservoir (above 700 m a.s.l.), the pathogen covertly, although abundantly, occurs in the tree tops of Norway spruce *P. abies* (L.) H. Karst., especially in stands older than 50 years. On this taxon, stunted buds with fruiting fungus bodies, as well as malformed sprouts, are common. In locations lower than 700 m a.s.l. or those that were further from the water reservoir, the infestation of the Norway spruce was much sparser. In the vicinity of the Fláje region, there are plantations of White spruce *P. glauca* (Moench) Voss, Black spruce *P. mariana* (Mill.) Britton, Sterns & Poggenburg and Serbian spruce *P. omorica* (Pančić) Purk. The White spruce was strongly affected by the fungus, at least 5% of all the buds. The Norway spruce and White spruce are supposed to be susceptible to the pathogen (Soukup, Pešková 2009; Ferdinandsen, Jørgensen sec. Černý et al. 2016). The Black spruce and Serbian spruce were still without any symptoms of infestation in the studied area. These facts are in conformity with the findings of Soukup and Pešková (2009a). However, the Black spruce is currently being attacked by this pathogen in Alaska (Dubois, Wurtz 2016).

METHODS

Four forest plantations of BS in proximity to the Fláje water reservoir were selected in 2013. They differed in the altitude, distance from the water reservoir and in the stand density (see Figure 1, Table 1). The selected stands are comprised of at least 15% of BS, and they are older than 15 years. For the purposes of the study, BS stands less affected by the pathogen were chosen so that the development of the stand damage could be monitored across several vegetative seasons. The following number of BS trees on the plots were involved in the study: Vista/155, Pump/146, Crib/92 and Height/138.

In each growing season, from 2013 through 2019, the ability of the BS to sprout, the rate of the defoliation and the increment of the dead specimens were evaluated. The trees were ranked into four groups as for the extent of the sprouting, 0–25% – nearly all buds affected, 26–50% – low rate of sprouting, 51–75% – good sprouting and 76–100% – healthy trees or only some buds affected. The damage to the buds and sprouts may not be caused just by bud blight, but also by frost, insects, or by other fungal pathogens (Soukup, Pešková 2009b). Bud blight is, however, a fundamental factor in the health status of the BS buds, therefore, other factors in this evaluation were left out and the ability to sprout was related to the rate of the bud blight infestation. The extent of the defoliation was classified into four categories as well, 0–25% – low rate of defoliation, healthy trees, 26–50% – moderate defoliation, 51–75% – strong defoliation and 76–100% – very strong defoliation and dead trees. Even the defoliation, aside from natural needle senescence, can be caused by many other factors such as drought, nu-

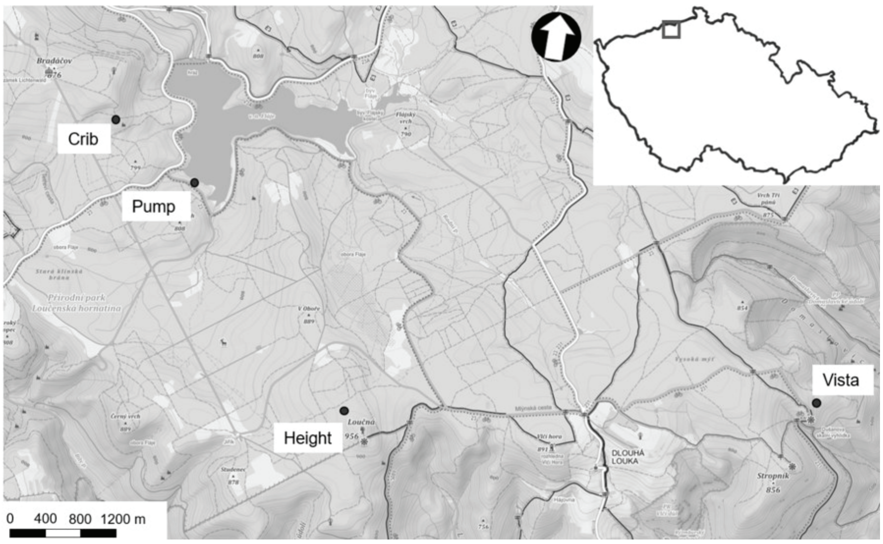

Figure 1. Location of the eastern part of Krušné hory Mts. in the Czech Republic and the placement of the study plots in Fláje region

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Table 1. Characteristics of the plots

Plot/stand[a]	Altitude (m a.s.l.)	Exposure	Distance from water reservoir	Stand age[a] (years)/ mean height[a] of BS (m)	Stand density according to administrative/subjective evaluation	Habitat/ average day temperature[b]/ average day RH[b] for period May–July, 2014–2016	Portion of BS in stand (%)
Vista/447A4	755	SE slope steeper than 10°	Beyond basin	34/7	9/dense canopy of broadleaved taxa, BS partly defoliated by shading	*Calamagrostio villosae-Fagetum*, sheltered habitat, advection wind may appear/12.8°C/82%	15
Pump/208A2	740	NE slope up to 10°	20 m	17/3	8/open park-like forest	*Calamagrostio villosae-Piceetum* /12.3°C/84%	85
Crib/122A3a	835	S gentle slope	780 m	30/6	8/locally dense canopy, sparsely birch, BS partly defoliated by shading	Wet variety of *Calamagrostio villosae-Piceetum*, amphitheatre in protracted slope towards reservoir, sheltered from N by steep terrain edge/12.2°C/82%	85
Height/224A4	920	ENE slope up to 5°	Beyond basin	31/5	8/open park-like forest	*Calamagrostio villosae-Piceetum*, summit exposed ridge/12.6°C/80%	85

[a]stand coding, stand age and mean height of Colorado blue spruce *Picea pungens* Engelm. (BS) stated according to Forest Management Plan 2011–2020 of the State Forest Enterprise LS Litvínov, LČR s.p.; [b]data measured on the plots

tritional deficiencies or by fungal needle casts (Semelová, Vacek 2010; Soukup, Pešková 2009b). The ability of the trees to replace old defoliated twigs by new healthy sprouts is a sign of a bud's good health condition. In this study, a simplified schema is used – the greater the rate of defoliation, the greater and longer lasting the extent of the infestation from the bud blight is. The temperature and relative humidity (RH) of the air were measured on the study plots at 7:00, 15:00 and 23:00 UTC+2 (OMEGA OM-CP-RHTEMP101, Omega Engineering, Canada). The average daily temperature (T_m, Equation 1) and the average daily RH (RH_m, Equation 2) have been calculated for the periods May–July, 2014–2016.

$$T_m = T_7 + T_{15} + (2 \times T_{23})/4 \quad (1)$$

$$RH_m = RH_7 + RH_{15} + (2 \times RH_{23})/4 \quad (2)$$

The life cycle of the pathogen was observed in a 2-week periods from April 15th to October 15th, the infested buds with conidiomata of the spring infestation and buds of the previous year's infestation were collected. The samples were preserved in a solution of ethanol and glacial acetic acid (3:1), and were also cultivated in a wet chamber. A Burkard type (AMET, Czech Republic) spore trap was placed on the chosen plots in order to monitor the spore occurrence in the 2014 (Height, 920 m a.s.l.) and 2015 (Pump, 740 m a.s.l.) seasons. The device was placed under the canopy of heavily infested BS specimens, the orifice of the device was 0.3 m above the ground. The spore sampling took place from the beginning of June to the end of October.

The sampler operated at a flux of 0.57 l·s^{-1}, the speed of the air flow was ca. 18 m·s^{-1}. The ability of the spores to be distributed by the air was tested by the exposition of dry and wet fruiting bodies to the air flow of 5 m·s^{-1}.

A clean and Vaseline coated glass served as the impact surface. The spore size was measured by camera software (Motic Images Plus). The estimation of the previous course of the stand's defoliation was calculated by logistic function in the Statistica software (Statsoft, New York, USA).

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RESULTS

The BS started to sprout in the middle of May in the conditions of the altitude range of 755–920 m a.s.l. The first symptoms of the bud infestation were noted during this time. In the beginning of June, a grey granulous mycelium appeared on the buds. The juvenile pycnidia were recognisable around June 20th, and they started to get pointy in the place of the future ostiole in the beginning of July. Mature conidia were noticed from July 10th till the end of September. In this time span, the conidia production oscillated from copious numbers to their near absence. Later, just a scarce amount of conidia was noticed in the pycnidia spread up to the first ten days of October. The cultivation time for the conidia depended on the maturity of the pycnidia. It takes 2 days in the case of a pycnidia collected at the end of July, or 10 days when collected in the beginning of July (both are the case of the pycnidia on the buds in the spring infestation). The pycnidia formation and conidia production on the buds of the previous year's infestation was shifted three weeks earlier. On the buds attacked the previous year, colonies of both types of fruiting bodies were formed and scattered among the old withered pycnidia of the previous year's activity. Spores of both types from these buds were released in the first week of July, see Table 2.

Ascospores, as a casual catch on the surface excluding ascomata, were rarely registered from the middle of July till the middle of October. Numer-

Table 2. Life cycle of *Gemmamyces piceae* (Borthw.) Casagrande in the altitude range of 755–920 m a.s.l. in the Fláje region

Year	First bud bent	Gray granular mycelium	First juvenile conidiomata	Conidiomata get tip	Conidia juvenile	Conidia adult in spread/Ta, C	Ostiole	Ascospores (casual catch beyond perithecia)/ Ta, Ca	Conidial mass covering conidiomata
2012		9.6.	23.6.		23.6.	10.7., 31.7., 16.8., 13.9.			
2013	30.5.		(20.6.) 2.7.		30.7.	4.9., 24.9.	30.7.–20.8.		
2014		8.6.	5.7.	26.7.		5.7., 19.7. R, 26.7. R, 16.8., 23.8. R, 30.8. R	16.8.		s 19.7., s 26.7., s 2.8.
2015		7.6.	21.6.	11.7.	11.7.	1.8., 22.8., 5.9. R, 26.9. R, 10.10. R	22.8.	10.10. R	s 1.8., s 5.9.
2016		4.6.				3.7., 28.7.	28.7.	4.9.R, 9.10.R	s 4.9.
2017			1.7.			17.8. R	17.8.	17.8. R (locally copious), 10.9.R	
2018		3.6.	15.6.	18.7.	24.6.	8.8., 2.9., 8.8. output of conidial ribbon, rainy; watched in the field	15.8.	18.7. (locally copious)	s 8.8.
2019	5.6.	9.7.	16.7.	30.7.	9.7.	Ta: 9.7., Ca2: 9.7., Cs10: 9.7., Cas2: 30.7.; Cas2: 24.8.	24.8.	Ta: 9.7. copious; Ca2: 9.7., Ca2: 30.7.; Ca2: 24. 8.	a 30.7. s 24.8.
Date of highest frequency of stadium for 2012–2019	**30.5.**	**3.6.**	**20.6.**	**10.7.**	**20.6.**	**10.7.–15.8. rare till 10. 9. Swings from copious to rare**	**30.7.–17.8.**	**18.7. R- 17.8. copious; rare 10.9.–9.10.**	**s 19.7.–5.9. a 30.7.**
Date[a] according to Černý et al. (2016)			End May			Onset June – first rare in the field July – conidia production culminates		Onset July. Perithecia production culminates in span of 8th–9th month	

R – rare occurrence, date in brackets means that the attribute was noticed on one of plots only; Ta – fruit bodies abrasion of buds of the previous year's infestation; C2/C10 = 2day/10day cultivation in a damp chamber, date of collection in the field is stated; s – spring infestation; a – previous year's infestation; [a]Černý et al. (2016), the data presented there are related to altitude 530 m a.s.l.; in bold – date of the highest frequency of occurrence of stadium related to span of whole survey

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Table 3. Size of conidia and ascospores on plot Pump

Type of spore Date of sampling Conidia origin	Conidia 8. 8. 2018 spring infestation	Conidia 9. 7. 2019 previous year infestation	Ascospores 9. 7. 2019
Length (µm)	153.3 (**206.9** ± 21) 246.2[a]	286.1 (**316.6** ± 19.9) 345.7	29.2 (**48.00** ± 6.9) 63.9
Width (µm)	3.9 (**5.3** ± 0.6) 6.4	7.3 (**7.8** ± 0.5) 8.4	13.3 (**17.7** ± 1.8) 20.8
Septation (n)	11 (**22** ± 6) 32	19 (**22** ± 2) 25	5 (**7** ± 1) 8

[a]Values represent the minimum, (mean ± SD), maximum; in bold – mean

ous ascospores and even conidia were obtained by abrasion of the fruiting bodies from the previous year's infestation in the first half of July. The same material of the same date of collection released ascospores and conidia after 24 hours of cultivation in the wet chamber. The successful cultivation of both types of spores on the buds took place from the beginning of July until the end of September. There was an abundance of a conidial mass in the field, which then, when wet, formed a white film on the infested buds. The average conidia length was 207 µm, SD ± 21 in the case of the spring infestation and 317 µm, SD ± 20 in the case of the previous year's infestation. The conidia from the pycnidia of the previous year's infestation were also broader, reaching 7.8 µm, SD ± 0.5, whereas the conidia of the spring infestation were just 5.3 µm, SD ± 0.6. The average length of the ascospores was 48 µm, SD ± 6.9 and their width was 17.7 µm, SD ± 1.8, Table 3.

In 2013-2018, the number of sprouting buds continually decreased. The number was slightly different in the different localities. Plots with a closed canopy (Vista) and with competition of broad-leaved trees (Crib) showed a lesser ability to sprout, than those with open canopy plots (Pump, Height). The decreasing trend of the BS sprouting level lasted until 2016. Then, in 2017, an increased ability to sprout appeared on all the plots. Furthermore, in 2018, the ability to sprout decreased again, but not to the level of 2016. In the span of the whole survey time (i.e., 5 or 6 years depending on the particular plot), the ability of the buds to sprout decreased from 60%, resp. 57%, to 23–30% on the closed canopy plots, whereas on the open plots, the sprouting decline went from 56%, resp. 42% of the sprouting buds, to only 33%, resp. 36%, Figure 2.

The reverse trend that occurred in the sprouting in 2017, Figure 2, can be induced by the temperature dynamics during the BS budding time (April to June). The dynamics of the sprouting may be one of the important factors in the ability to resist the pathogen. In low temperatures (2014), sprouting can be assumed to be slow and the buds remain in a vulnerable stage for a longer period of time, resulting in greater damage caused by the pathogen. On the other hand, in the case of a rapid transition to warm weather (2017), sprouting can be assumed to be fast and the duration of the vulnerable budding stage is shorter, and the pathogen, therefore,

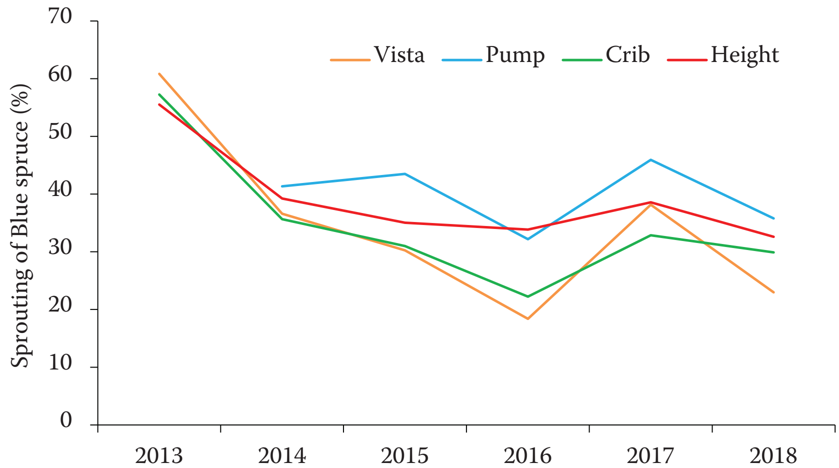

Figure 2. Mean values of the Blue spruce – *Picea pungens* Engelm. (BS) sprouting on the plots

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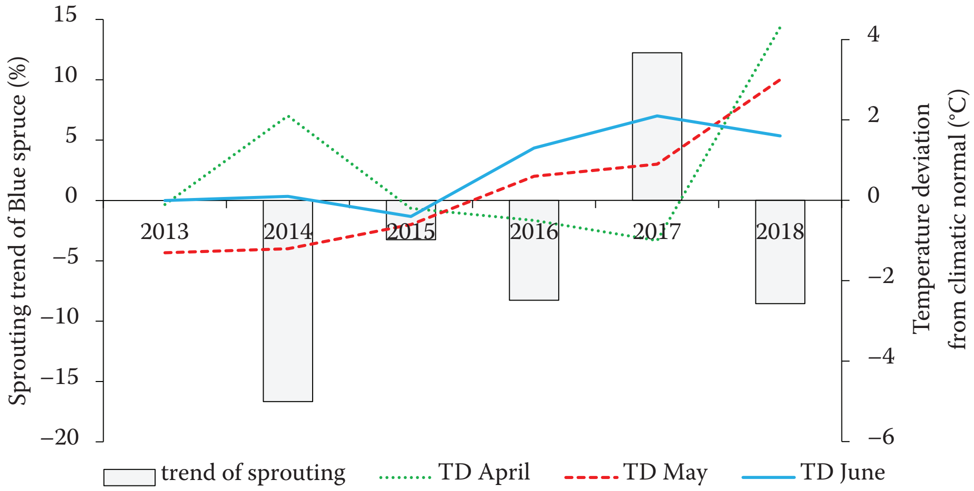

Figure 3. Blue spruce (*Picea pungens* Engelm.) sprouting trend and temperature deviation (TD) from the climatic normal in April, May and June for the Ústecký kraj region, climatic data ČHMÚ (2020); climatic normal of 1981–2010

has a limited opportunity to inflict serious damage, see Figure 3. The average weekly temperatures and average weekly RH during the first two weeks of the conidia release did not have any effect on the sprouting ability of the buds in the following year. In the season of 2016, which preceded the season of the increased sprouting ability of the buds, weekly temperatures in time of the conidia production were similar to the 2014 season, the season which conversely preceded the year of a strong drop in the sprouting ability. The average weekly RH values were similar and steady in both seasons (2014 and 2016) and remained around 85%.

The extent of the defoliation steeply increased on all the plots in the years 2013–2016. In the following years, 2016–2018, the defoliation trend turned less progressive, Figure 4, apparently mitigated by the successful proliferation of new sprouts in the vegetation period of 2017. The largely open and younger stand of the Pump plot showed a steep defoliation trend too, but compared to the other plots, it kept a 10% lower defoliation rate. Based on the three plots located further away from the water reservoir, an average trend of the defoliation was constructed and was fitted by the logistic function (Equation 3). On its basis, we can suppose that the defoliation and disease expansion may have started before 2008, Figure 5.

$$y = 68.97/1 + \exp\,[-0.72 \times (x - 2013.43)] \qquad (3)$$

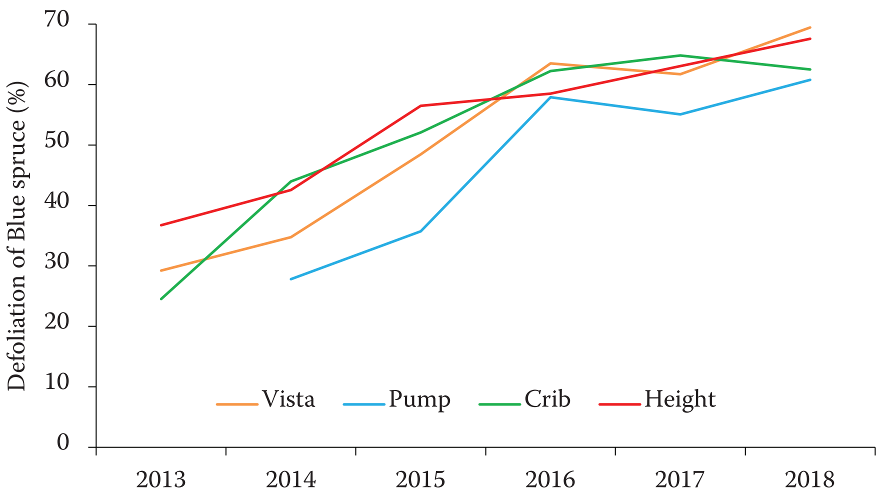

Figure 4. Mean values of the defoliation of the Blue spruce (BS) trees on the plots

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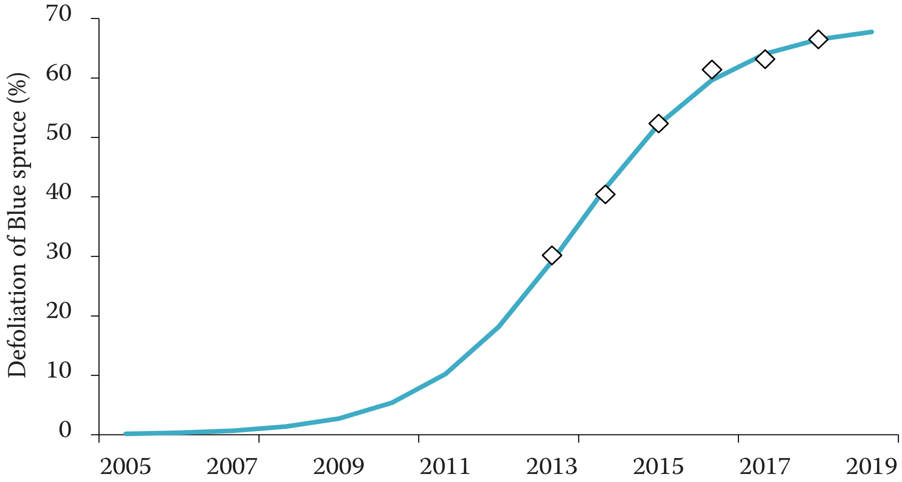

Figure 5. Progress of the Blue spruce (BS) defoliation on the Vista, Crib and Height plots. The mean values of the defoliation (spots) and supposed previous course estimated by the logistic model

The number of dead trees within the survey period gradually increased up to 38% on the plot of the lowest altitude (755 m a.s.l.) with a dense canopy and competition of broad-leaved trees, as well as on the highest altitude (920 m a.s.l.) with an open plot, Figure 6. On the contrary, smaller increase in the dead trees was on the plots closer to the water reservoir (Crib, Pump), these plots were of dissimilar density in their stands.

The pycnidia and conidia occurred in all the locations at the same time, also the average temperature in May-July 2014–2016 was very similar; see Table 1. In the years 2014, 2016 and 2017, the occurrence of adult conidia in the fruit body spread was registered along with the mature pycnidia. In other seasons, 2013, 2015 and 2018, there was a shift of 6–7 weeks between the first pycnidia and the first conidia.

The spore sampler registered two specimens of conidia 120 μm long on July 9th and August 23rd 2014, both dates fit the time of culmination of the conidia occurrence in the fruit body spread. Spore sampler registered one ascospore, July 30th 2014, which was 30 μm long, i.e., 15 μm shorter than the average length of the pathogen ascospores on the plots.

The ability of the spore distribution by air, tested by air speed of 5 $m \cdot s^{-1}$, did not prove the ability of the spores to spread themselves into the environment, neither did the dry nor wet fruiting body

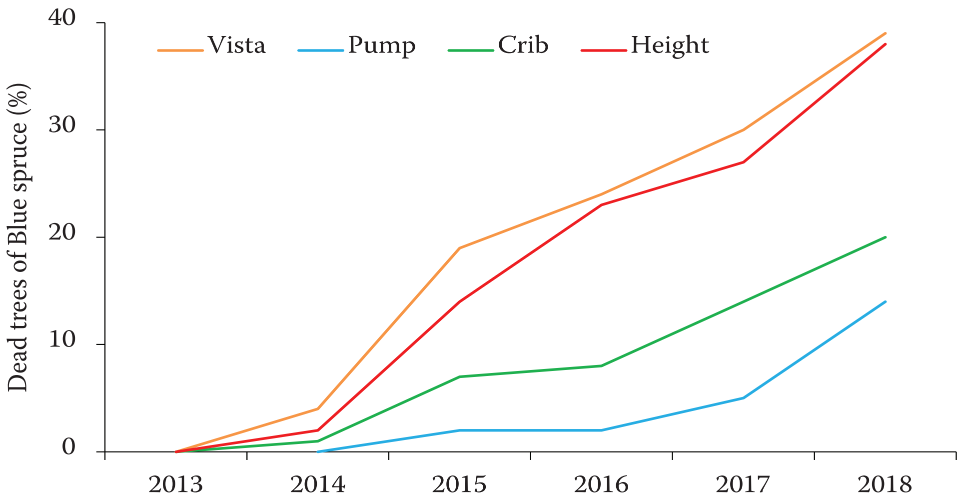

Figure 6. Dead trees of the Blue spruce (BS) on the plots

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with an emerged spore mass. The successful drift of the conidia was only possible, if a stream of air took away a droplet of water from the wet fruit body. The drift of the ascospores in this way was much scarcer. Aside from the droplet transport, spores were not noticed. A difference between the clean and Vaseline treated impact glass was not observed. The conidial mass is sticky, hydrophilous, and quickly dissolves when watered.

On each plot, 1–4 pathogen unaffected BS trees were noticed, i.e., 1–3% of total count of each plot. These specimens were placed out of the tree canopy.

DISCUSSION

Bud blight extensively attacks BS in the eastern part of the Krušné hory Mts. above 520 m a.s.l. (Šefl 2013). Under the mentioned altitude plantations of this species in the region, the plantations were without symptoms of infestation or much less attacked. Zýka et al. (2018) also found that the intensity of the infestation grew along with an increased rate of precipitation and a temperature drop. However, in the studied elevation range of 740–920 m a.s.l., no difference was found in the infection rate of the BS stands. Likewise, no significant difference in the average daily temperature was found between the studied areas. The sprouting ability was reduced due to higher stand density and competition of the broadleaved trees, as Zýka et al. (2018) also states. A rapid transition to higher temperatures at the time of spruce sprouting may have a positive effect on the success of its sprouting due to the rapid transition from the most vulnerable budding stage to a more resilient stage. The BS defoliation was in progress at all times during the survey, with the exception of its slowdown in 2017, the season of the sprouting improvement. The logistic model, applied to the trend of the defoliation, showed the evolvement of the disease before 2008. Černý et al. (2016), with a similar logistic function, modelled the inception of the disease in the Krušné hory Mts. before 2000. This date shift could be caused by the fact that the chosen plots of the study (Vista, Crib and Height) were in better health conditions than the other stands of the Eastern Krušné hory Mts. region.

No difference in the timing of the life cycle of the pathogen was registered in the altitude range of 740–920 m a.s.l. In 2013–2018, the pycnidia occurred at the summit plot (920 m a.s.l.) at the same time as on the plots at a lower altitude. However, the presence of the adult conidia at the summit plot was three weeks late in half of the cases (in the years 2013, 2014, 2018). The life cycle stages of the pathogen are outlined well in the season, the same was noticed by Černý et al. (2016).

The average conidia length of pycnidia of the previous year's infestation was 316 μm. This size corresponds with the lower elevation collecting, surveyed by Černý et al. (2016). The conidia of the pycnidia of the previous year's infestation were longer than the conidia of the spring infestation, Table 4. There was also variation in the length of the conidia coming from different pycnidia of the same bud, although almost a similar conidia length was shown in the case of the same pycnidia. The length of the ascospores, 48 μm, collected from the fruiting bodies was in conformity with Černý et. al (2016).

The spore sampler was not successful in the pathogen spore trapping, as trapped spores were very rare. Černý et al. (2016) also reported a very small amount of captured ascospores. The inability of the spore sampler to catch any type of spores of the pathogen may have been caused by another pathogen spreading strategy, different from the anemochorous strategy. An indirect indication for the non-wind spread of the pathogen is the compact spore mass released on the surface of the fruit bodies. The hydrophilic properties and stickiness of the conidial mass indicates distribution by rain or by insect. Scolecoid spores (the case of the conidia of the pathogen) are adapted for distribution by water (Deacon 2006; Watkinson et al. 2016). A stronger infestation in the lower part of the tree crowns may be caused by the heavier spore impact as they drop down from the upper parts of the trees. On the other hand, the spore mass, when dry, becomes brittle and can be easily spread by the wind. The plentiful release of both types of spores is realised by the abrasion of the infested buds. This fact could be a hint of another way of the pathogen spread. The scarce presence of BS trees standing alone unaffected by bud blight is also mentioned by Černý et al. (2016).

CONCLUSION

In 2013–2018, the bud blight caused a significant decrease in the sprouting capability in the studied

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locations in the BS stands older than 35 years; in the younger stands (ca. 20 years), the decrease in the sprouting capability was less significant. During the studied period, the defoliation of the BS trees significantly increased regardless of their age.

The BS infection by the fungus was more severe in dense stands and in stands with competition from broad-leaved trees. The less severe infection of the BS trees in 2017 is likely related to the rapid transition to warmer temperatures during the sprouting period, whereby the rapid sprouting shortens the most vulnerable budding phase. In the 740–920 m a.s.l. elevation zone, no significant difference in the extent of the BS stand damage was recorded. No link between the extent of the BS stand infection and the proximity to a water reservoir could be established in our study.

The average weekly temperature and the average weekly RH at the time during the first two weeks of the conidia production had no effect on the success of the sprouting in the following year.

The spore production (both spore types) in the mountain environments started at the beginning of July and continued until mid-August; in the noted elevation zone, both the fruit body formation and spore production occurred at the same time. The pathogen likely spreads via a different mechanism than wind; this conclusion is based on the fact that spore capture by a spore sampler was not successful.

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