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Bark necrotic disease in a beech thicket

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Abstract: Symptoms of non-specific bark necroses in beech trees of all ages have lately been described. We investigated the occurrence of bark necrotic disease in beech thicket and health status of the trees. Our objective was to examine how bark necrotic wounds persist and the regenerative potential of young beech thicket. The research experiment was carried out at two isolated localities in the Western Carpathians in the period 2015–2017. A terminal shoot (stem) and a lateral shoot (branch) of each of the 30 sampled beech trees were examined. Young beech stands were affected by the necrotic disease in 87–94% cases (stems 69–83%, branches 33–56%). Shoot diameter and age had a significantly negative linear correlation ($P < 0.05$) with the development of all analysed disease characteristics on the beech bark. The encouraging finding is a positive regenerative capacity of young beech trees. Over the same period more annual shoots were healed in comparison with those that were newly infected (10.8% on stems and 0.6% on branches).

Keywords: annual shoots; beech bark disease; damage; *Fagus sylvatica* L.; fungal infection; young beech stand

At present, a number of phytopathological signs, mostly caused by various fungi, has been recorded at all developmental stages of the *Fagus sylvatica* L. stands, from natural regeneration up to maturity (Kacprzyk *et al*. 2017). Perhaps the beech bark necrotic disease is the best-known disease caused by parasitic fungi (Surovec 1992). A mass occurrence of bark necroses in young beech stands was recorded by Leontovyč (1992) as early as in 1988–1989, when the dieback of thickets younger than 20 years was observed, primarily in the industrial zone in Slovakia. Since the middle of the last century (throughout the period of acidic deposition), beech bark disease (BBD) has been observed on *Fagus grandifolia* Ehrh. in a large part of its distribution range (Houston 1994). In the northeastern USA and eastern Canada, beech trees are considered to be in the aftermath of the initial infection wave of BBD (Cale *et al*. 2015; Steiner *et al*. 2017).

The causality of the rise and induction of necroses as an external symptom of beech bark necrotic disease has been discussed ever since the beginning of the research on this issue (Yamada 2001; Yamaji & Ichihara 2012). Necrotic damage to beech bark tissues as well as to other woody plants is associated with the activity or interaction among various insect and fungal pathogens, mainly fungi of the genus *Anthostoma* Nitschke, *Ceratocystis* Ellis & Halst., *Cylindrocarpon*-like species, *Cytospora* Ehr.: Fr., *Diatrype* Fr., *Fusarium* Link, *Nectria* (Fr.) Fr., *Neonectria* Wollenw., *Ophiostoma* Syd., and fungal-like organisms *Phytoplasma*, *Phytophthora* de Bary, also *Phomopsis* Sacc., *Valsa* Fr., *Verticillium* Nees, etc. (Slippers & Wingfield 2010, Montecchio *et al*. 2011; Giencke *et al*. 2014; Saracchi *et al*. 2015 etc.). Recent reviews on the aetiology of beech bark necrotic disease by Orlikowski *et al*. (2006), Jung *et al*. (2013), etc. indicate that the primary *Phytophthora* infections of stem bark are triggered by climatic extremes that are a major factor in the beech bark disease complex in central Europe.

Cale *et al*. (2015) described the relation between parasitic fungi and chemical composition of the infected wood, e.g. findings suggest that *Neonectria ditissima* infection is facilitated by the native scale insect *Xylococculus betulae* Pergande in Hubbard &

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Pergande and low bark levels of phosphorus and the phenolic isorhamnetin. The most important causes of necrosis induction are generally bark damage by biotic factors (e.g. fungi, insects, wildlife), abiotic factors (e.g. frost, hail, fire) and other factors (e.g. human management activities) allowing the infection by the fungi that eventually kills the tree (Cale *et al*. 2015). The beech bark disease pathogenic fungi are *Neonectria coccinea* on *Fagus* in Europe, *N. faginata* on *Fagus* in North America and *N. ditissima* on hardwoods in North America, Europe and Asia (Castlebury *et al*. 2006; Hirooka *et al*. 2013).

In our contribution, we focus on the evaluation of the dynamics and interactions between the growth and the incidence of necrotic wounds on the bark of examined individual beech trees. In the research experiment, we wanted to verify the self-healing ability of young beech stands based on two working hypotheses: (*i*) from the point of ontogenetic development, during the healing process the calluses are formed more frequently on smallest necrotic wounds (stage 1) than on larger necroses, (*ii*) the morphometric parameters and age of individual beech trees correlate with the incidence of necroses and the intensity of necrotic disease.

MATERIAL AND METHODS

Study sites and analysed material. The three-year experiment was performed at two localities in the highlands of Central Slovakia (Kremnica Mountains and Štiavnica Mountains, Table 1), Western Carpathians, Central Europe. At each site, we selected 15 young individual beech (*Fagus sylvatica* L.) trees grown from natural regeneration. The trees were chosen so that they did not directly affect each other. Hence, the trees should not be adjacent and the distance between them should exceed their height. The analysed material consisted of 30 shoots from each site (n = 30; 15 terminal shoots – stems and 15 lateral shoots – branches). Supplementary information on the research sites can be found in Barna (2015), Gašová *et al*. (2017), and Kukla *et al*. (2017).

We assessed the tree growth and health in the years 2014–2016, and the analyses were performed in the spring of the next year, before the start of the growing season (before budbreak). In 2015, we analysed annual shoots of the last 5 years (increments for the years 2010–2014). Each year, we expanded our research on another annual shoot. In 2016, we examined the last six years for each individual (2010–2015) and in 2017 the last seven years (2010–2016). In the first year, we analysed 75 annual shoots at each site, in the second year we analysed 90 shoots, and in the third year 105 annual shoots on the stem (altogether 270 annual shoots) and the same amount on the lateral branches. On each individual beech tree, we sought one branch with 5 annual shoots (5 years old). This was not possible everywhere, therefore some branches were younger (with fewer annual shoots). As beech crowns

Table 1. Characteristics of research sites

Characteristics	Štiavnica Mountains (S)	Kremnica Mountains (K)
Latitude	48°33'10"	48°38'03"
Longitude	18°56'47"	19°04'13'
Aspect	W	SW
Altitude (m a.s.l.)	575–600	480–500
Slope (°)	17–25	13–20
Geological substrate	andesite tuffit agglomerates	andesite tuffit agglomerates
Soil – subgroup	modal, slightly acid Cambisol	modal, saturated Cambisol
Soil – texture (%)	Clay 12.9 ± 1.9*	Clay 11.2 ± 1.08
	Silt 60.5 ± 3.6	Silt 66.5 ± 3.7
	Sand 26.6 ± 2.2	Sand 22.4 ± 5.0
Mean annual precipitation (mm)	850	660
Mean annual temperature (°C)	6.2	8.3
Plant association	*Dentario bulbiferae-Fagetum*	*Dentario bulbiferae-Fagetum*
Tree species composition (%)	beech 65, hornbeam 14, aspen 11, maple 9, oak 1	beech 76, hornbeam 19, linden 1, aspen 1, maple 1, willow 1, oak 1
Sample trees – height (m)	2.4[a] ± 0.6	2.3[a] ± 0.5

*mean ± SD

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expanded during the research experiment, some of the analysed branches died due to the lack of light and were dropped from the research. The number of stems also decreased, by two at each locality in the last year. Therefore, the final number of the analysed annual shoots was lower than the planned 2 × 270 annual shoots.

Measurements. We started the survey in April 2015 by selecting sample trees. To ensure that the same samples were measured every year, each selected tree was tagged with a tape on the stem and the branch we examined. We measured the total height of sample trees and the length and diameter of five consecutive annual shoots located behind the terminal bud. The analysed increments were therefore 1–5 years old. In addition to stems, we also examined lateral branches that grew out of the stem starting from the six-year annual growth. Thus, we obtained the same comparative material for stems and branches. The lengths of one-year annual shoots were measured from the terminal bud to the end of the scars on the bark, caused by dropped scales of the previous year's bud (Roloff 1999; Barna *et al*. 2009). The lengths of the following annual shoots were measured from the scars of one year to the start of the next year scarring (Figure 1). The diameters of the annual shoots were measured at their beginning, i.e. at their widest place. On each annual shoot, we visually detected the presence of necrotic wounds, their number (quantitative character), and the development stage (qualitative character). On the basis of the ontogenetic development of necroses, we categorised them into three stages according to their size: (*i*) small necroses, with unbroken bark; (*ii*) medium necroses, with cracked bark; (*iii*) large necroses, with cracked bark and exposed wood (Figure 1).

Sample trees were observed in 2016 and 2017. We measured the diameters of all annual shoots, but the lengths were measured only on the leader, as the length of older shoots does not change. We also evaluated the number of necroses and their progression stage. Hence, the number of analysed annual shoots increased by each year's increments (Figure 2). In 2016, we examined the annual shoots from the years 2010–2015 (6 to 1 year old) and in 2017 the annual increments from the years 2010–2016 (7 to 1 year old).

Stage of necroses. Data for the variable "stage of necrosis" was determined for each infected annual shoot infected by the highest degree of necrosis occurring on the annual shoot.

Necrotic disease severity. To evaluate the influence of measured annual shoot characteristics (diameter, length, age) on necrotic disease, we quantified the severity of the necrotic disease on individual annual shoots with an index (*I*). The index of disease was calculated for each annual shoot as a sum of quantitative and qualitative values of the disease:

$$I = n_1 \times 1 + n_2 \times 2 + n_3 \times 3$$

where: n_1 – number of necroses in the first stage; n_2 – number of necroses in the second stage; n_3 – number of necroses in the third stage; values 1, 2, 3 are weights of necroses according to their qualitative development (1 – 1st stage, 2 – 2nd stage, 3 – 3rd stage of necrosis progression)

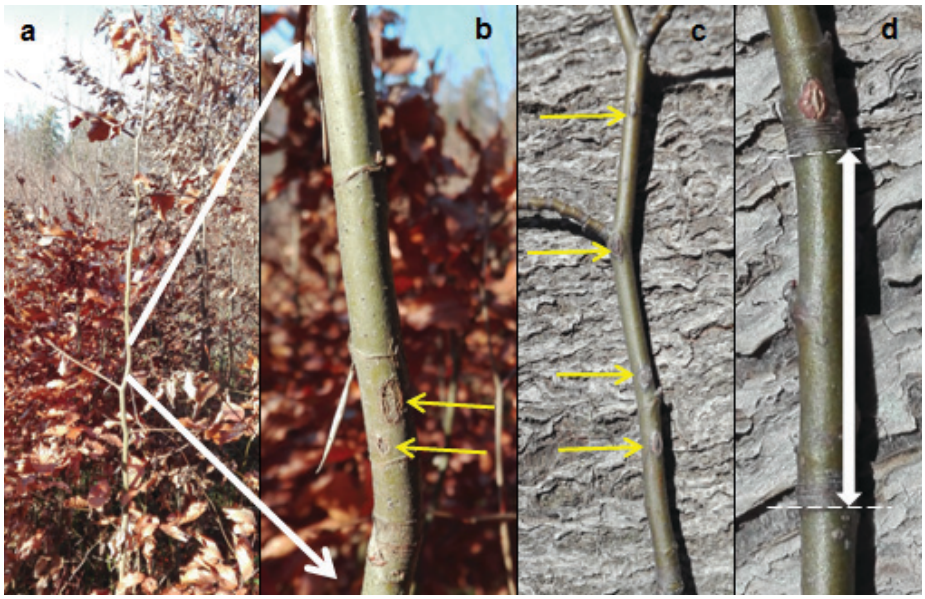

Figure 1. (**a**) The young beech sample tree; (**b**) Two necroses on the stem of the analysed sample tree; (**c**) Four necroses on the analysed branch; (**d**) The arrows indicate the length of the annual shoots which are identified by scale-scar rings

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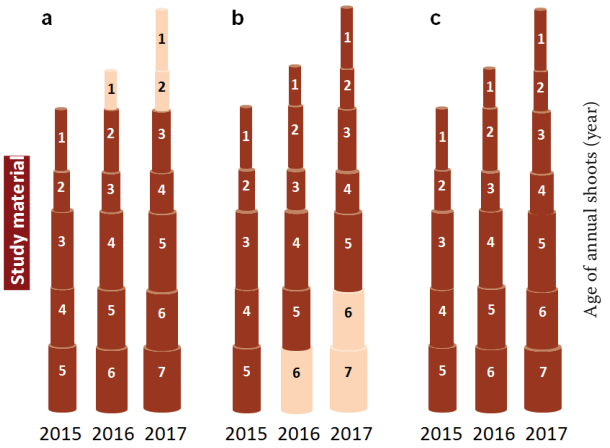

Figure 2. Diagram of the necrotic disease analysis by years of research experiment: (**a**) on the same annual shoots of 5 year-old sections, (**b**) 1–5 year-old annual shoots, and (**c**) on all annual shoots of stems and branches during a three-year research experiment

We determined the extent of the necrotic disease for each analysed file (for locality and year) as a sum of index values of all annual shoots, i.e. a qualitative sum of all identified necroses (in Results). Mean disease occurrence was determined as a ratio of the total disease occurrence and the number of affected rings in individual years for a particular locality and a part of the tree (stem, branch).

Diameter increment. Diameter increment was calculated as a difference in two diameters of the annual shoot in two subsequent years: $id = d_{l+1} - d_l$.

Statistical analysis. The mean percentage of infected annual shoots between experimental years was compared using the Kruskal-Wallis non-parametric test. Because the studied young trees were geographically split between two sites in the Kremnica Mountains and the Štiavnica Mountains, we looked for the differences between the regions and tree parts (stem and branch) for each dependent variable from the group of disease characteristics: the number of necroses, the stage of necroses and the severity of the disease. No significant differences were detected by the Mann-Whitney *U* test, and therefore the data from both regions were pooled for subsequent analyses using Spearman's correlation. To analyse the linear relationship between variables, we used the following approach: in the first step, Spearman's correlation was calculated for measured variables from the group of disease characteristics. In the second step, variables were adjusted to reflect the aspect of individual annual shoot health change in the course of the experiment. Similar to the diameter increment, we derived the data about the health change from disease characteristics by calculating the difference in their values in two subsequent years ($n_{t+1} - n_t$: $n_{2016} - n_{2015}$, $n_{2017} - n_{2016}$). In this second step of Spearman's correlation, we could analyse variables for the years 2016 and 2017. All analyses were carried out in Statistica 9 program (StatSoft Inc. 2009).

RESULTS

During the entire experiment in the Kremnica Mountains (locality K) we recorded the occurrence of bark necrotic disease on 94 annual shoots on stems and 35 annual shoots on branches. In the Štiavnica Mountains (locality S), we found the disease on 52 annual shoots on stems and on 22 annual shoots on branches.

We compared the state of the disease (number of necroses, stage of necroses and severity of the disease) between the investigated localities and between the tree parts (stems and branches) of the same locality by the Mann-Whitney *U* test; based on this test we could not confirm any significant differences between tested parameters of the disease in any case ($P > 0.05$; Table 2).

From the relative evaluation of necrotic bark disease over three years on the same annual shoots and the comparison of the disease between the years (Figure 3) we see that annual shoots of stems are more often infected with diseases than annual shoots on branches. In the last five years 69–83% of annual shoots on stems and 44–70% of annual shoots on branches were affected by necroses. We found 87–94% of necrotic damage on whole individuals (stem and branches) of young beech trees.

The analysis of necroses on the same annual shoots throughout the experiment (Table 3) showed that necroses persisted on more than 57% of infected annual shoots on stems at both localities. In the case of branches it was by 13% less. The largest proportion of necrosis persistence was found amongst less severe necroses (development stage 1 and 2). On annual shoots on stems which suffered from a necrotic disease throughout the whole research experiment the disease more frequently subsided (24.4%) than progressed (8.1%).

Information about the healing of wounds, i.e. about the process of callus formation over necrotic wounds (Figure 4), is important for healthy development and growth of young beech trees. It is interesting that necroses occur on stems to a lesser extent (10.4%)

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Table 2. Comparison of disease state between groups: Kremnica Mountains (K) locality versus Štiavnica Mountains (S) locality and stems versus branches based on Mann-Whitney *U* test

Disease characters	Valid *n*		Z	*P*	Valid *n*		Z	*P*
	K	S			stems	branches		
	Stems				**Kremnica Mountains**			
Number of necroses	94	52	0.830	0.407	94	35	0.757	0.449
Stage of necroses	94	52	–1.661	0.097	94	35	1.271	0.204
Index of disease	94	52	–0.159	0.872	94	35	1.274	0.204
	Branches				**Štiavnica Mountains**			
Number of necroses	35	22	0.410	0.678	52	22	0.373	0.712
Stage of necroses	35	22	–1.205	0.230	52	22	0.940	0.350
Index of disease	35	22	–0.025	0.981	52	22	1.005	0.314

n – number of analysed infected annual shoots

and subside more (21.2%) than in the case of branches (15.4 and 16.0%, respectively). In the course of the experiment, we found also completely new necrotic wounds. In view of the future health status of the stand it is very important that the healing of wounds was recorded to be twice higher than the occurrence of newly developed necroses. Necroses more often occurred on younger annual shoots (the average age was 2.7 years) and disappeared on older ones (4.0 years; Mann-Whitney *U* test: Z = –3.016, *P* = 0.002). The situation on branches was very similar (2.9 and 3.4 years, Mann-Whitney *U* test: Z = 0.807, *P* = 0.451). The occurrence and healing (callus formation) of the wounds were most often recorded for the lowest severity of the disease (stage 1 of the necrosis development).

The summary qualitative value of stem disease according to the index of disease declined (Table 4) for the last five-year shoots, while no significant changes occurred for all analysed shoots. In the case of branches we showed that there is a significant decline of the disease manifestation (sum of index of disease values) in the second and third year of experiment over the first year (Figures 2b and 2c). As to the differences in the disease between stems and branches according to the index of disease, our outcomes from Table 2 confirmed that the differences were not significant. The only statistically significant difference between stems and branches was in the age of the infected shoots in 2015. We found no differences either on stems or on branches for the emergence of new wounds and healing of old wounds. A significant difference was found only in the shoot age on the stem. Healing takes place on older shoots. Since the total number of healed shoots was greater than the number of newly infected ones (stems: 43 vs. 36, branches: 13 vs. 12), the sum of the disease index was, on the contrary, smaller (stems: 107 vs. 131, branches: 26 vs. 32). This means that shoots with a lower degree of illness heal first. Differences between the occurrence and healing of qualitative bark disease in the index of disease were not found

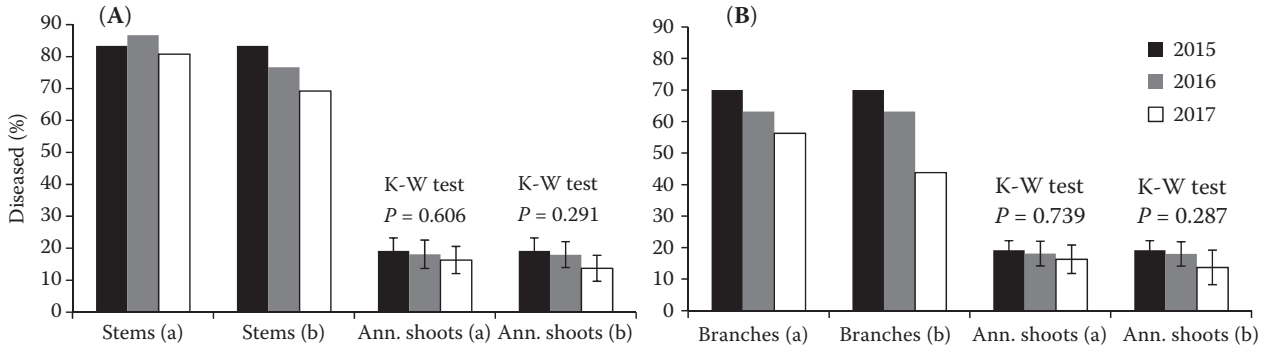

Figure 3. The mean percentage occurrence of necrotic disease (**A**) on stems (n_{2015} = 30, n_{2016} = 30, n_{2017} = 26) and the annual shoots on stems (a, b material analysed according to Figure 2) and (**B**) on branches (n_{2015}=30, n_{2016} = 19, n_{2017} = 16) and the annual shoots on branches (annual shoots: mean ± SE)

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Table 3. Temporal development of the same necrotic disease and its persistence on the annual shoots of 5 years old section of stems and branches in percent (analysed according to Figure 2a)

	Stems	Branches
Course of disease		
1st	22.4	16.8
2nd	24.5	21.4
3rd	10.7	6.3
Total	57.5	44.4
Age*	3.1[a]	2.8[a]
Progression of the disease		
In 2nd year	5.6	2.6
In 3rd year	2.5	0.0
Total	8.1	2.6
Subsidence of the disease		
In 2nd year	13.8	0.0
In 3rd year	10.6	0.0
Total	24.4	0.0

*mean age of necrotic annual shoots (years) at the beginning of the experiment

even at median values for whole trees (Figure 5; Mann-Whitney *U* test: Z = 1.510, *P* = 0.131).

Out of the twelve analysed relations, statistically significant relationships were found only in two cases (Table 5). To confirm or to reject the relation between the investigated parameters, in further analyses we used the values of interannual differences

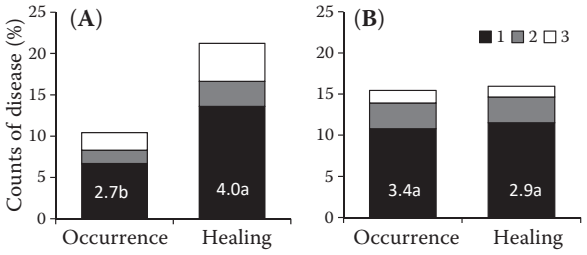

Figure 4. Occurrence and healing (callusing) of the necrotic wounds on all annual shoots of stems (**A**) and branches (**B**) during a three-year study (analysed according to Figure 2c)

The numerical value indicates the average age (year) of all infected annual shoots. Letters indicate statistically significant differences by Mann-Whitney *U* test ($P < 0.05$). 1, 2, and 3 – stages of necroses

in the health status (change of disease) of affected annual shoots (more details in Statistical analysis). In this case, we confirmed a significant negative influence (Spearman's correlation) of the diameter and age of annual shoots on interannual changes in necrotic characteristics. This means that older and thicker shoots were more affected by necroses. And conversely, the average increment and length of annual shoots did not affect the necrotic disease.

DISCUSSION

During our experiment, the necrotic disease affected 81–87% (Figure 3) of the same annual shoots

Table 4. The course of the disease according to index of disease

Course of disease	Stems				Branches			
	sum	mean	age	count	sum	mean	age	count
1–5 year annual shoots								
2015	204	4.1[a] ± 0.5	3.3[a] ± 0.2	50	82	2.9[a] ± 0.5	2.6[b] ± 0.2	28
2016	176	3.7[a] ± 0.7	3.1[a] ± 0.2	47	43	2.5[a] ± 0.6	3.5[a] ± 0.3	17
2017	153	4.8[a] ± 0.9	3.5[a] ± 0.2	32	50	3.8[a] ± 0.7	3.9[a] ± 0.3	13
All annual shoots								
2015	204	4.1[a] ± 0.5	3.3[a] ± 0.2	50	82	2.9[a] ± 0.5	2.6[b] ± 0.2	28
2016	210	4.4[a] ± 0.7	3.8[a] ± 0.2	48	43	2.5[a] ± 0.6	3.5[a] ± 0.3	17
2017	198	4.7[a] ± 0.8	4.8[a] ± 0.2	42	51	3.6[a] ± 0.7	4.6[a] ± 0.3	14
Occurrence of wounds	131	3.1[A] ± 0.5	3.8[B] ± 0.3	36	32	2.7[A] ± 0.6	3.7[A] ± 0.4	12
Healing of wounds	107	2.6[A] ± 0.4	4.4[A] ± 0.2	43	26	2.0[A] ± 0.3	3.8[A] ± 0.4	13

Occurrence and healing of wounds were detected in the 2nd and 3rd year of experiment; sum – sum of the values of index of disease; mean – mean value of index of disease; age – age of infected annual shoots; count – number of infected annual shoots; mean ± SE – letters indicate significant differences by Mann-Whitney *U* test ($P < 0.05$) between stems and branches (lowercase), occurrence and healing of wounds (capital letters)

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Table 5. Spearman's correlation between shoot parameters and actual necrotic disease (n = 134), and annual change in necrotic disease (n = 167) on stems and branches

Shoot parameters	Disease characters	Actual		Annual change	
		Spearman *R*	*P*	Spearman *R*	*P*
Diameter	number of necroses	–0.017	0.845	–0.249	**0.001**
	stage of necrosis	0.207	**0.016**	–0.204	**0.009**
	index of disease	0.119	0.171	–0.241	**0.002**
Diameter increment	number of necroses	–0.037	0.668	0.106	0.175
	stage of necrosis	0.053	0.541	0.133	0.087
	index of disease	0.017	0.847	0.094	0.225
Length	number of necroses	0.053	0.541	–0.064	0.413
	stage of necrosis	–0.048	0.580	0.076	0.327
	index of disease	0.052	0.551	0.003	0.971
Age	number of necroses	0.042	0.632	–0.234	**0.002**
	stage of necrosis	0.194	**0.025**	–0.211	**0.006**
	index of disease	0.134	0.124	–0.211	**0.006**

(Figure 2a) on stems of young beech stands, which is more than on branches (57–70%). In the stands with different stand density, Mihál *et al.* (1997) found that the disease frequency in the regeneration growth decreases continually with increasing density (from 97% on the clear-felled area to 35% in the stand with closed canopy). This could indicate that terminal shoots on the stems of young beech trees growing in open spaces are affected by necroses more often than annual shoots of branches growing in closed canopy stands, where they are better protected from adverse climatic factors or fungal spores transmitted by wind. This suggests that the disease progression on stems and branches may not be related (Table 3).

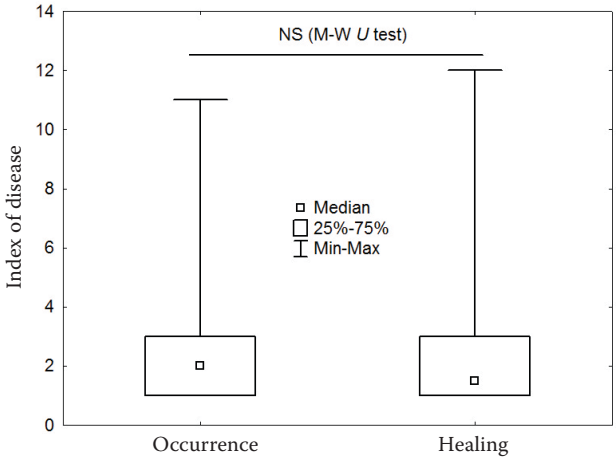

Figure 5. Occurrence (n = 48) and healing (n = 56) of the necrotic wounds by index of disease at a level of whole trees (stems and branches). Research material according to Figure 2c

Most plant pathogens are unable to penetrate the healthy bark directly, but they can successfully invade wounded bark tissues (Kacprzyk *et al.* 2017). Minor cortical injuries during the cambium electrical resistance measurement may also cause the induction of necroses. Cicák and Mihál (2005) evaluated 2 904 mechanical wounds, out of which 155 were caused by the necrotic induction. Since the process of parallel wound healing (callus formation) was carried out after mechanical injury, not every bruising was the cause of necrotic induction. During our research, we found that the healing process of wounds and subsequent callus formation took place also on branches and stems of beech trees at both localities (mostly on stems in the Štiavnica Mountains 44%, least on branches in the Kremnica Mountains 26%). Most of the healed wounds were in the qualitatively least severe stage of disease (stage 1 of the necrosis development, Figure 4), on average on 14% of af-

Table 6. Relationship between diameter and length of one-year shoot and necrotic disease (n = 8) according to Spearman's correlation

Shoot parameters	Disease characters	Spearman *R*	*P*
Diameter	number of necroses	0.791	**0.019**
	stage of necroses	0.082	0.846
	index of disease	0.843	**0.009**
Length	number of necroses	0.791	**0.019**
	stage of necroses	-0.247	0.555
	index of disease	0.690	0.058

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fected annual shoots on stems and 12% on branches. This is two to three times more than in the case of necroses in stages 2 and 3 together. Thus, we have confirmed our hypothesis that the calluses are more likely formed at the least severe stage of the disease (stage 1) than on the necroses in stage 3. We have found that some necroses disappeared immediately in the next year after they emerged. Apart from one exception, they were always in the 1st stage of disease and of varying intensity (from 25% to 50%). A similar pattern also applies to the occurrence of new necroses. Most necroses occurred in the 1st qualitative stage, always more than in the 2nd and 3rd stages together. In this case, there were more necroses on branches compared to stems. An important result for the health status of stands is that the proportion of newly infected shoots has never exceeded the healed ones. This difference in stems was almost 50%, in the case of branches it was approximately balanced. Based on these results, it appears that although the stems are relatively more affected by the necrotic disease than branches, they have a better ability to form calluses over necroses. The data may indicate strong vitality and regeneration capabilities of the evaluated young beech stands.

The index of disease suggests that young beech stems were more affected throughout the experiment than branches and had also a higher average disease rate of infected annual shoots. This applies to all three methods of analyses (Figure 2). The disease of branches was more or less even for both methods of analyses. This indicates that the age of annual shoots does not have a clear effect on the current state of necrotic disease. We confirmed this also by Spearman's correlation (Table 5), based on which a significant relationship between the age and the investigated health indicators with the necrosis stage was confirmed only in one case (P = 0.025). Similarly, diameter increment positively correlated with the necrosis stage (P = 0.016). There was no relation between the studied disease characteristics and diameter increment or the length of annual shoots. Therefore, for a more thorough reassessment of relations, we used the values of the year-to-year differences (between 2016 and 2015, 2017 and 2016) in the health status of the affected annual shoots, instead of the current health status. In this case, Spearman's correlation clearly confirmed the negative influence of the diameter and age of annual shoots on the year-to-year changes in the examined necrotic characteristics (P < 0.01), i.e. on the disease progression. It should be added that even in the case of significant relationships (P < 0.01) these correlations were quite low (R^2 = 0.062–0.042). This indicates that other important factors also affect the studied disease characteristics, but they were not analysed in this work. Cicák *et al.* (1995) studied this issue in the young beech coppice stand and found a significant relationship between the incidence of necrotic wounds on the bark of beech branch terminal shoots and the annual length growth. To verify this result (the effect of shoot length on the necrotic disease), we had to select only one-year annual shoots from the analysed data because the shoot length does not change after the first year. The results of the supplementary analysis confirmed a significant impact of the length and diameter of the annual shoot on the disease (Table 6). From this calculation we can find out that the given relationship (R^2 = 0.71 and 0.63) is more significant than the previous ones in Table 5, because the examined parameters explain 60–70% of disease variability. Kintl *et al.* (2010) indicates that the length and diameter of branches are also critical for the self-cleaning process. Longer and thicker branches die less often than short and thin ones that are mostly suppressed.

Fungal parasites that cause necrotic diseases of beech bark have an important role in the relationship between necrotisation and annual increments of beech. Cicák *et al.* (1995) considered the unequal trophic possibilities of the fungal parasite as a significant factor, which may be different for each individual beech tree – in this case its annual shoots. This is also confirmed by a significant increase in the nitrogen/potassium ratio found in the leaves of shoots which were infected with the mycelium of *Neonectria ditissima* compared to non-infected shoots (Flückiger & Braun 1998). Beech was significantly more infected by pathogens and pests when trees were fertilised with nitrogen. After artificial inoculation with the mycelium of *Neonectria ditissima* an increased dieback of branches and an increased proportion of branches with small and yellow leaves distal from the infection point were observed due to the development of the pathogen attack with increasing N fertilisation. There was also a significant correlation between the size of bark necroses and N/K ratio in the leaves. It can be assumed that the requirements of *N. ditissima* for mineral and nitrogen nutrition that is provided to this fungus by longer annual shoots with greater growth can play an important role in this correlation. Similarly, Jönsson (2000) studied

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the soil treatment effects on bark lesions and frost sensitivity of beech in relation to the application of different treatments in beech stands (liming, wood ash, nitrogen). She reported that bark lesions on beech are mostly caused by frost damage and/or insect and fungal infections. Trees fertilised with nitrogen had significantly more lesions than trees from other treatments – this indicates that susceptibility to lesions is related to the nutrient status.

In connection with the above, it should be added that at the locality of the Kremnica Mountains, Mihál *et al.* (2000) found the occurrence of the species *Neonectria ditissima* (anamorph: *Cylindrocarpon willkommii* /Lindau/ Wollenw.) by *in vitro* cultivation from a young beech stand. Also Kunca (2005) identified *Neonectria coccinea* on *Fagus sylvatica* at the second locality (Štiavnica Mountains). Some pathogens are widespread and are practically ubiquitous, with minimal impacts on the host health (Stergiopoulos & Gordon 2014). Other, more aggressive, native pathogens may become widespread following stress, climate changes, soil salinity, etc. (Pšidová *et al*. 2013; Maringer *et al*. 2016; Woods *et al*. 2016). In contrast, diseases caused by invasive pathogens are often characterised by the spatial distribution of disease risk. Populations of the beech bark disease pathogen complex all share this strong signature of spatiotemporal variation in the distribution of disease risk (Garnas *et al*. 2011; Cobb & Metz 2017).

Our results show that the necrotic disease on the stem and branch bark is quite common in young beech stands and has its dynamics and progression order. The analysis of the problem highlights the complexity of mutual relations between the growth of individual young beech trees and the necrotic disease on the bark of their stems and branches in the complex of a number of integrating factors and processes. It is necessary to emphasise the important fact of the regenerative capacity of beech individuals in relation to necrotic disease. Annually, on average 21.2% of stem wounds and 16.0% of branch wounds were healed, this is by 10.8 and 0.6% more than the occurrence of new stem and branch necroses during the same period, respectively. This means that under normal conditions (optimal growth conditions), the regeneration processes in a young beech stand are so intense that a significant recovery from necroses can gradually take place. This is also true of branches because in thick young beech stands, shaded and infected branches gradually die and fall off due to the competition. By finding that the occurrence and healing of the wounds were most often seen in the lowest severity of the disease, we have confirmed the hypothesis that in the regeneration process trees are more likely to form calluses over the wounds caused by the smallest necroses (stage 1) than on larger necrotic wounds (stage 3). We also confirmed our second hypothesis: diameter and age have a significant negative linear relation to the development of all analysed disease characteristics on beech bark.

References

Barna M. (2015): Productivity and functioning of the beech ecosystem: Ecological Experimental Station – Kremnické vrchy Mts. (Western Carpathians). Lesnícký časopis - Forestry Journal, 61: 252–261.

Barna M., Schieber B., Cicák A. (2009): Effect of post-cutting changes in site conditions on the morphology and phenology of naturally regenerated beech seedlings (*Fagus sylvatica* L.). Polish Journal of Ecology, 57: 461–472.

Cale J.A., Teale S.A., Johnston M.T., Boyer G.L., Perri K.A., Castello J.D. (2015): New ecological and physiological dimensions of beech bark disease development in aftermath forests. Forest Ecology and Management, 336: 99–108.

Castlebury L.A., Rossman A.Y., Hyte A.S. (2006): Phylogenetic relationships of *Neonectria*/*Cylindrocarpon* on *Fagus* in North America. Canadian Journal of Botany, 84: 1417–1433.

Cicák A, Mihál I. (2005): Can artificial wounding of beech stems induce necroses? Journal of Forest Science, 51: 559–563.

Cicák A., Mihál I., Štefančík I. (1995): Methodical contribution to the assessment of bark necroses in non-mixed beech wood. Zprávy lesnického výzkumu, 40: 26–29.

Cobb R.C., Metz M.R. (2017): Tree diseases as a cause and consequence of interacting forest disturbances. Forests 8(5): 147. doi: 10.3390/f8050147

Flückiger W., Braun S. (1998): Nitrogen deposition in Swiss forests and its possible relevance for leaf nutrient status, parasite attacks and soil acidification. Environmental Pollution, 102: 69–76.

Garnas J.R., Ayres M.P., Liebhold A.M., Evans C. (2011): Subcontinental impacts of an invasive tree disease on forest structure and dynamics. Journal of Ecology, 99: 532–541.

Gašová K., Kuklová M., Kukla J. (2017): Contents of nutrients and arsenic in litterfall and surface humus in mature nudal beech stands subjected to different emission-immission loads. Folia Oecologica, 44: 11–19.

Giencke L.M., Dovčiak M., Mountrakis G., Cale J.A., Mitchell M.J. (2014): Beech bark disease: spatial patterns of thicket formation and disease spread in an aftermath forest in the

https://doi.org/10.17221/117/2018-PPS

north-eastern United States. Canadian Journal of Forest Research, 44: 1042–1050.

Hirooka Y., Rossman A.Y., Zhang W.Y., Saldago-Salazar C., Chaverri P. (2013): Species delimitation for *Neonectria coccinea* group including the causal agents of beech bark disease in Asia, Europe, and North America. Mycosystema, 32: 485–517.

Houston D.R. (1994): Major new tree disease epidemics: beech bark disease. Annual Review of Phytopathology, 32: 75–87

Jönsson A.M. (2000): Mineral nutrients of beech (*Fagus sylvatica*) bark in relation to frost sensitivity and soil treatments in southern Sweden. Annals of Forest Science, 57: 1–8.

Jung T., Vettraino A.M., Cech T.L., Vannini A. (2013): The impact of invasive *Phytophthora* species on European forests. In: Lamour K. (ed.): Phytophthora: A Global Perspective. Wallingford, CABI: 166–177.

Kacprzyk M., Matsiakh I., Musolin D.L., Selikhovkin A.V., Baranchikov Y.N., Burokiene D., Cech T., Talgø V., Vettraino A.M., Vannini A., Zambounis A., Prospero S. (2017): Damage to stems, branches and twigs of broadleaf woody plants. In: Roques A., Cleary M., Matsiakh I., Eschen R. (eds): Field Guide for the Identification of Damage on Woody Sentinel Plants. Institut National de la Recherche Agronomique, Unité de Zoologie Forestière, Orleans, CAB International: 104–134.

Kintl V., Hein S., Campioli M., Muys B. (2010): Modelling self-pruning and branch attributes for young *Quercus robur* L. and *Fagus sylvatica* L. trees. Forest Ecology and Management, 260: 2023–2034.

Kukla J., Bublinec E., Schieber B., Kellerová D., Bičárová S., Janík R. (2017): Immission-load-related dynamics of S-SO_4^{2-} in precipitation and in lysimetric solutions penetrating through beech ecosystems. Folia Oecologica, 44: 96–106.

Kunca A. (2005): Susceptibility of wounds of the European beech bark to infection by *Neonectria coccinea* and efficiency of the wound treatment. Lesnícky časopis - Forestry Journal, 51: 21–29.

Leontovyč R. (1992): Importance of the genus *Ophiostoma* in the current ecological conditions in Slovakia. In: Holubová V., Prášil K. (eds): Ophiostomatales – Results of Present Taxonomic and Phytopathological Research. Prague, ČSVSM: 35–49. (in Slovak)

Maringer J., Ascoli D., Küffer N., Schmidtlein S., Conedera M. (2016): What drives European beech (*Fagus sylvatica* L.) mortality after forest fires of varying severity? Forest Ecology and Management, 368: 81–93.

Mihál I., Cicák A., Čunderlík I. (1997): Bark necrosis damage to beech in the growth stage of advance regeneration. Lesnictví – Forestry, 43: 201–205.

Mihál I., Cicák A., Juhásová G., Ivanová H. (2000): Fungy of the genus *Nectria* as a cause of beech bark necrotic disease. Folia Oecologica, 27: 189–196.

Montecchio L., Scattolin L., De Battisti R. (2011): Dormouse injuries predispose beech to infection by *Neonectria ditissima.* Forest Pathology, 41: 114–119.

Orlikowski L.B., Oszako T., Szkuta G. (2006): First record of *Phytophthora* spp. associated with the decline of European beech stands in south-west Poland. Phytopathologia Polonica, 42: 37–46.

Pšidová E., Ditmarová L., Jamnická G., Váľka J., Gömöry D. (2013): Assessment of the impact of drought stress on particular biochemical and physiological characteristics of beech saplings leaves from different provenances. Folia Oecologica, 40: 97–106.

Roloff A. (1999): Tree vigor and branching pattern. Journal of Forest Science, 45: 206–216.

Saracchi M., Sardi P., Kunova A., Cortesi P. (2015): Potential host range of *Anthostoma decipiens* and *Endothiella* sp., agents of hornbeam blight. Journal of Plant Pathology, 97: 93–97.

Slippers B., Wingfield M.J. (2010): Emerging cryptic diseases of forest trees. Journal of Plant Pathology, 92 (4 Suppl): S4.53

Surovec D. (1992): Tracheomycoses of young beech stand from natural regeneration. Acta Instituti Forestalis Zvolenensis, 8: 97–105.

Steiner K.C., Westbrook J.W., Hebard F.V., Georgi L.L., Powell W.A., Fitzsimmons S.F. (2017): Rescue of American chestnut with extraspecific genes following its destruction by a naturalized pathogen. New Forests, 48: 317–336.

Stergiopoulos I., Gordon T.R. (2014): Cryptic fungal infections: The hidden agenda of plant pathogens. Plant-Microbe Interact, 5: 506.

Woods A.J., Martín-García J., Bulman L., Vasconcelos M.W., Boberg J., La Porta N., Peredo H., Vergara G., Ahumada R., Brown A., Diez J.J. (2016): Dothistroma needle blight, weather and possible climatic triggers for the disease's recent emergence. Forest Pathology, 46: 443–452.

Yamada T. (2001): Defense mechanisms in the sapwood of living trees against microbial infection (review). Journal of Forest Research, 6: 127–137.

Yamaji K., Ichihara Y. (2012): The role of catechin and epicatechin in chemical defense against damping-off fungi of current-year *Fagus crenata* seedlings in natural forest. Forest Pathology, 42: 1–7.

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