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SUSCEPTIBILITY OF WOUNDS OF THE EUROPEAN BEECH BARK TO INFECTION BY *NEONECTRIA COCCINEA* AND EFFICIENCY OF THE WOUND TREATMENT

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Abstract: Intact bark of European beech is not an infectious court for *Neonectria coccinea* (Pers. : Fr.) Rossman & Samuels. The fungus is not able to overcome mechanic barrier of bark. Wounds are infectious courts for the fungus and the result of the infection is the beech bark disease. Deeper wounds are more susceptible to infection than shallow wounds. Infection periods of wounds last approximately 0 days in spring, 1 day in summer, 7 days in autumn and 3 months in winter. However, fresh wounds are the most susceptible to infection in autumn. Wound treatment should be applied while wounds are susceptible to infection. If wounds are coated by fungicidal suspension prior to infection, necrotic lesion will not set in. It is the most effective in autumn and on the same day the wound was made. Curing treatment has little reason.

Key words: *Fagus sylvatica*, canker, necrosis, infectious court, *Cylindrocarpon*, pathogen, fungi, Pellacol

Introduction

It is well known that wounded bark is the infectious court for many facultative pathogens and the same applies to species of *Neonectria* Wollenw. as well as *Nectria* (Fr.) Fr., which are pathogens of European beech (EHRLICH 1934, PARKER 1974, HOUSTON 1975, OSTROFSKY BLANCHARD 1983, KUNCA 2000). *Nectria* spp. can not overcome intact bark that include corky layer with suberin ingredient (BIGGS 1992). Different agents, however, such as beech scale insect, deer, frost or forest management activities during silvicultural treatments can hurt bark in any season of the year and thus overcome the corky layer. *Nectria* spp. spores are present in most of beech forests of Slovakia (CICÁK, MIHÁL 2000) during the whole year. Thus, the infection of beech bark mostly depends (i) on the presence of wounds in the beech bark and (ii) on environmental conditions that are suitable for distribution and germination of *Nectria* or *Cylindrocarpon* Wollenw. spores. At the same time, the environmental conditions should be unsuitable for active defense mechanisms of the European beech trees (BIGGS 1992).

In my experiments I wanted to prove that intact bark is not an infectious court for *Neonectria*. Then, I wanted to give the information for foresters about the variability of wound susceptibility to infection during a year. Moreover, I tested treatment of wounds to check the efficiency of wound coating, which is a practical advice to foresters.

Materials and methods

Isolate AR 3711 of *Neonectria coccinea* was chosen to realize pathogenecity tests in the field. Applied inoculum was 3 weeks old mycelium cultured on 2% malt agar and isolated from perithecia growing on beech bark. This isolate comes from central Slovakia, Kremnické vrchy mountains, locality Štagiar and it is deposited in the American Culture Collection of the USDA-ARS, Beltsville, MD, USA. Beech stand is situated in central Slovakia, Štiavnické vrchy mountains, and belongs to Kysihýbel working-plan area of the School Forest Enterprise Banská Štiavnica (Tab. 1).

Table 1 Description of the beech stand, where inoculation tests were realized

Number of the stand	Area [ha]	Age [years]	Altitude [m]	Expo-sition	Slope [%]	Forest type	Propor-tion of beech [%]
113b	2,2	15	600	North	60	3311	100

There were chosen 160 beech trees. Forty of them were wounded in each season respectively. Each tree had 8 wounds. The wound was circular with diameter of 1,0 cm. The scheme of the experiment in one of the seasons looked like this:

a) This experiment was to test the efficiency of curing treatment of wounds against the infection. One tree was wounded 8 times by a cork borer up to cambium with a distance of 10 cm between each other. All wounds were immediately inoculated. Then the first wound was covered with Pellacol suspension 1 hour after inoculation, and consequently next wounds on the 1st day, 3rd day, 7th day, 14th day, 28th day and after 3 months. The eighth wound remained inoculated without Pellacol treatment. This version was repeated 10 times and tested the influence of late wound treatment in different seasons of the year.

b) This experiment was to test the efficiency of preventive treatment of wounds against the infection. One tree was wounded 8 times by a cork borer up to cambium with a distance of 10 cm between each other. All wounds were immediately treated with Pellacol suspension. Then the first treated wound was inoculated 1 hour after treatment and consequently next wounds on the 1st day, 3rd day, 7th day, 14th day, 28th day and after 3 months. The eighth wound remained treated with Pellacol without inoculation. This version was repeated 10 times and tested the influence of early wound treatment in different seasons of the year.

c) This experiment was to test the susceptibility of aging wounds upon the infection. One tree was wounded 8 times by a cork borer up to cambium with a distance of 10 cm between each other. Then the first wound was immediately inoculated and consequently next wounds on the 1st day, 3rd day, 7th day, 14th day, 28th day and after 3 months. The eighth wound remained without inoculation. This version was repeated 10 times.

d) This experiment was to test the susceptibility of aging shallow wounds upon the infection. One tree was slightly wounded 8 times by a cork borer just beyond the periderm layer with a distance of 10 cm between each other. Then the first wound was immediately inoculated and consequently next wounds on the 1st day, 3rd day, 7th day, 14th day, 28th day and after 3 months. The eighth wound remained without inoculation. This version was repeated 10 times.

In addition, each of the 160 trees mentioned above, was inoculated once on the intact bark to test it as an infectious court.

Pellacol is a brown suspension that contains thiram as a fungicidal active ingredient. It is registered as a repellent and wound coat in Slovakia.

Field experiments started in summer on 29. 7. 2000, in autumn on 21. 10. 2000, in winter on 5. 1. 2001 and spring on 18. 4. 2001. Wounds were checked one year after wound initiation in each season.

Change of the surface bark color was considered to be necrotic. Height and width of necrotic lesion around wounds were measured. It included 1 cm of the initial wound and so if measurements were less than 1 cm that wound was considered callusing.

Hypotheses on differences of averages among variants were tested by analysis of variance and Tukey test.

Results

There was an elliptical shape of necrotic lesions with longer vertical than horizontal length (Tab. 2). As high correlation between height and width of the lesions ($r = 0.9$; $P < 0.01$) was observed, just height of lesions was considered in further statistical calculations.

Table 2 Description of necrotic lesions

Description		**Height**	**Width**
Mean	[cm]	0.9	0.5
SD		0.8	0.4
Min		0	0
Max		4.3	3.7
N		1 440	1 440

Depth of wounds significantly influenced the height of lesions. No necrotic wound originated from inoculation of intact bark. Intact bark was not an infectious court for *Neonectria coccinea*. There were larger necrotic lesions around deeper wounds than around shallow wounds (Tab. 3).

Table 3 Height of necrotic lesion after inoculation of different types of infectious courts; mean ± SD followed by different letters differ significantly within a column, $P < 0.05$

Type of infectious court	**Height of necrotic lesion [cm]**	**N**
Intact bark	0 ± 0 a	160
Shallow wound	0.31 ± 0.59 b	320
Deep wound	1.20 ± 0.53 c	320

Susceptibility of wounds to infection varied during a year. The largest necrotic lesions resulted from wounds inoculated in autumn and winter. The smallest necrotic lesions resulted from wounds inoculated in spring (Tab. 4).

Table 4 Height of necrotic lesion after inoculation of deep wounds in different seasons of the year; mean ± SD followed by different letters differ significantly within a column, P < 0.05

Season	Height of necrotic lesion [cm]	N
Spring	0.78 ± 0.30 a	80
Summer	1.16 ± 0.44 b	80
Winter	1.36 ± 0.36 c	80
Autumn	1.47 ± 0.67 c	80

Wounds were at the same level of susceptibility from their initiation to over 3 months in spring (Tab. 5). Their susceptibility was, however, very low from the beginning. It might have been caused by efficiency of active defending mechanism of trees, which starts its functioning in spring. The infectious period lasted 0 days in spring.

Table 5 Height of necrotic lesions after inoculation of deep wounds in spring; mean ± SD followed by different letters differ significantly within a column, P < 0.05

Inoculation after	Mean ± SD	N
7 days	0.58 ± 0.20 a	10
3 days	0.72 ± 0.29 a	10
1 day	0.77 ± 0.22 a	10
without inoculation	0.79 ± 0.28 a	10
14 days	0.80 ± 0.35 a	10
0 days	0.81 ± 0.45 a	10
3 months	0.83 ± 0.25 a	10
28 days	0.97 ± 0.23 a	10

Susceptibility to infection of aging wounds varied in summer (Tab. 6). Wounds were the most susceptible on the day they were initiated. The next day the susceptibility decreased to the second level and this status of wounds lasted more than 3 months.

Table 6 Height of necrotic lesions after inoculation of deep wounds in summer; mean ± SD followed by different letters differ significantly within a column, P < 0.05

Inoculation after	Mean ± SD	N)
28 days	0.85 ± 0.42 a	10
without inoculation	0.97± 0.43 ab	10
3 months	0.99 ± 0.37 ab	10
14 days	1.21 ± 0.57 ab	10
1 day	1.26 ± 0.35 ab	10
3 days	1.27 ± 0.26 ab	10
7 days	1.30 ± 0.44 ab	10
0 days	1.46 ± 0.43 b	10

Susceptibility to infection of aging wounds varied in autumn more than in other seasons of the year (Tab. 7). Wounds were the most susceptible to infection on the day they were initiated. The next day susceptibility decreased to the second level and it lasted through the seventh day. The third level started on the 14^{th} day and lasted more than 3 months. A longer infectious period in autumn than in other seasons of the year bears mostly on decreased active defense mechanisms of trees at the end of the growing season. As for the fungal pathogen, it can react very quickly at higher temperatures in autumn and so it can infect and colonize nearly dormant host tissues. The length of infectious period in autumn lasted for 7 days.

Table 7 Height of necrotic lesions after inoculation of deep wounds in autumn; mean ± SD followed by different letters differ significantly within a column, P < 0.05

Inoculation after	Mean ± SD	N
without inoculation	0.94 ± 0.28 a	10
3 months	1.04 ± 0.39 ab	10
28 days	1.21 ± 0.51 ab	10
1 day	1.34 ± 0.19 ab	10
14 days	1.36 ± 0.33 ab	10
3 days	1.67 ± 0.41 b	10
7 days	1.69 ± 0.53 b	10
0 days	2.54 ± 0.93 c	10

Susceptibility to infection of aging wounds did not vary in winter (Tab. 8). It was on the same level from the day of initiation until more than 3 months. The infectious period lasted over 3 months in winter. It might have resulted from the growing characteristics of the *Neonectria coccinea*. Its mycelium can grow on 2% malt agar at 5 °C and does not die after 14 days under frost conditions (at -20 °C). After this low temperature it can continue to grow without slower growing of mycelium (author's unpublished laboratory experiments). Temperatures above 0 °C occur several times in winter, and this is the right time for infecting and colonizing host tissues by *Neonectria coccinea* infectious agents.

Table 8 Height of necrotic lesions after inoculation of deep wounds in winter; mean ± SD followed by different letters differ significantly within a column, $P < 0.05$

Inoculation after	**Mean ± SD**	**N**
without inoculation	1.20 ± 0.18 a	10
3 months	1.28 ± 0.21 a	10
3 days	1.28 ± 0.13 a	10
7 day	1.29 ± 0.43 a	10
1 day	1.30 ± 0.24 a	10
28 days	1.37 ± 0.24 a	10
14 days	1.51 ± 0.26 a	10
0 days	1.64 ± 0.70 a	10

Based on these results we can state that the longest infectious period of wounds lasted in winter, the shortest in spring (Tab. 9).

Table 9 Length of infectious period of beech bark for *Neonectria coccinea*

Season	**Length of infectious period**
Spring	0 days
Summer	1 days
Autumn	7 days
Winter	3 months

Table 10 Influence of different variants in different seasons of the year on the height of necrotic lesions; mean ± SD followed by different letters differ significantly within a column, $P < 0.05$

Description of variants	Spring					Summer					Autumn					Winter				
	x		s_x		n	x		s_x		n	x		s_x		n	x		s_x		n
Only wounded to periderm	0	±	0	a	10	0.16	±	0.26	a	10	0.77	±	0.71	a	10	0.10	±	0.23	a	10
Only treated	0.54	±	0.28	b	10	0.69	±	0.30	b	10	0.86	±	0.16	a	10	1.09	±	0.27	b	10
Preventive treatment	0.59	±	0.26	b	70	0.94	±	0.47	bc	70	1.12	±	0.44	a	70	1.30	±	0.24	c	70
Only wounded to cambium	0.79	±	0.28	c	10	0.97	±	0.43	bc	10	0.94	±	0.28	a	10	1.20	±	0.18	bc	10
Only inoculated	0.82	±	0.23	c	10	1.08	±	0.42	c	10	2.63	±	0.62	b	10	1.19	±	0.26	bc	10
Curing treatment	0.88	±	0.20	c	70	1.21	±	0.41	c	70	2.75	±	0.69	b	70	1.20	±	0.18	bc	70

Spring is the season when trees are the most resistant to infection through wounds (Tab. 10). Even inoculated wounds without any treatment callused. Resistance of the whole tree system is really strong in spring. Wounds treated with Pellacol without subsequent infection callused well, even better than wounds without treatment. It means than Pellacol treatment increased callusing process. Wounds infected both prior to treatment or after treatment callused too, but more slowly.

In summer the defense mechanism is still quite strong. Shallow wounds deep up to periderm callus pretty quickly. Wounds only treated callused faster than wounds without any treatment, so Pellacol helps wound callusing in summer as well as in spring. Preventive treatment can increase wound resistance too. Curing treatment did not stop bark necrosis development and wounds were as much infected as if they were not treated at all, just inoculated.

In autumn bark resistance is the weakest. Shallow wounds up to periderm callused but not as well as during other seasons of the year. Anyway, shallow wounds callused. Wounds treated with Pellacol callused better than wounds without any treatment. Pellacol helped wound callusing in the autumn as well as in other seasons of the year. Preventive treatment did not stop bark necrosis development, but significantly slowed this process. Curing treatment did not stop bark necrosis at all. Necrotic lesions were as large as around wounds just infected without any treatment.

In winter just shallow wounds callused. However, Pellacol increased callusing process of wounds, which were without infection. Infected wounds as well as wounds without treatment were susceptible to infection.

Discussion

In these experiments we found out that susceptibility of wounds to infection by *Neonectria coccinea* varies during a year and it seems to be consistent with variability of active defense mechanism efficiency of trees during a year (Biggs 1990). The longest infectious period lasts in winter, but the biggest necrotic lesions resulted from infections in autumn (Tab. 10). These are the seasons when active defense mechanism is weak due to tree dormancy. If trees are wounded during the tree dormancy, they cannot actively resist necrotic lesions. This is visible from wounds only infected and from the speed of shallow callusing wounds. However, pathogenic fungus like *N. coccinea* can grow even in cold weather conditions. In our own laboratory experiments, *N. coccinea* mycelium grew under 5 °C and did not die after 2 weeks storage under minus 20 °C. Thus *N. coccinea* can attack wounds in the bark during whole year. In general, the susceptibility of wounds to infection might depend not only on tree dormancy, but also on extreme stress conditions of trees such as soil water deficiency, late frost, large wounds, water excess, etc. We have an experience with infecting wounds in May during raining days, after which several trees died within next 3 years (not published data). So, what we mentioned above is not necessarily the only true finding. Active defense mechanisms of trees even in spring may not be strong enough to resist infectious pressure.

There are various opinions on wound dressing of trees (Chalker-Scott 2001). If we accept the theory that the wounded bark provides an infectious court for more pathogens than intact bark, then we may expect that proper treatment of wounds may improve their protection against infection. We can build a new mechanic barrier, which could compensate the function of real bark. Thus, we do not help active defense mechanism but mostly passive defense mechanism. It means, if passive defense mechanism is broken down by wounding and active defense mechanism does not work, because of various reasons, e.g. trees are out of growing season in autumn or winter (Biggs 1992), then wounds treated at that period of the year might be protected from being infected by pathogens.

Wound treatment in the season, when active defense mechanism is very effective, like in spring or summer, is less important than treatment of wounds after growing period like in autumn. Anyway, if wounds are treated with Pellacol and no infection arises, these wounds callus very well during whole year. Pellacol treatments applied prior to infection as protective treatment did not initiate new necrotic lesions in spring and summer, and still had its reason in autumn. At least this is the reason why wounds should be covered with Pellacol, but as soon as possible, because curative treatment is not effective at all during the whole year.

On the basis of this experiment we recommend to foresters to do covering of wounds but only fresh wounds, e.g. 0 days old, with preparation like Pellacol. Applications may be done during whole year but to be economic we strongly recommend to do it in autumn. It is not reasonable to treat shallow wounds, only deeper wounds.

Conclusion

Wounds are the most susceptible to infection in autumn and winter. Wounds covered with Pellacol suspension callused much better than wounds without any treatment in all seasons. Treated wounds subsequently infected with pathogenic fungus (preventive treatment) callused worse, but still inhibit development of necrotic lesions in spring, summer and autumn. However, necrotic lesions developed if wounds were treated with Pellacol after infection (curing treatment) and necrotic lesions were as large as if wounds were not treated at all. Thus treatment and preventive treatment of wounds are recommended for whole year, but mainly in autumn. Curing treatment is not recommended for forester at all.

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