# RELATIONSHIP BETWEEN BEECH BARK WOUNDS AND BARK NECROSIS AND POSSIBLE METHODS TO CONTROL BARK NECROSIS

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Z laboratórnych a terénnych pokusov vidieť variabilitu v patogenite izolátov získaných z kôry nekrotických rán bukovej kôry. Agresívnejšie izoláty spôsobovali nekrózy poranenej kôry i na živých stromoch i na výrezoch a obmedzovali rašenie púčikov výrezov v blízkosti infekcie. Nespôsobovali však nekrózy kôry neporanenej. Menej agresívne izoláty spôsobovali nekrózy poranenej kôry výrezov, avšak nespôsobovali nekrózy poranenej kôry živých stromov ani neporanenej kôry živých stromov a výrezov, a taktiež neobmedzovali rašenie púčikov.

V laboratórnych podmienkach žiaden z izolátov neprejavoval makroskopické zmeny v raste mycélia na živnej pôde spolu s antagonistickou hubou *Trichoderma harzianum*. V terénnych podmienkach bol pozorovaný inhibičný vplyv biopreparátu Polyversum s účinným agens *Pythium oligandrum* a repelentu Pellacol, s účinnou látkou thiram 100 g/kg na tvorbu mikroskopických plodničiek na nekrotických ranách vzniknutých izolátom č. 26. Vplyv na rýchlosť šírenia sa nekrotickej rany nebol pozorovaný pri aplikácii žiadneho zo skúšaných prípravkov.

**Kľúčové slová**: *Fagus sylvatica* L., nekrotické ochorenie kôry buka, biopreparáty, infekčný test

More aggressive fungal isolates brought about necrosis of beech bark if it was infected through wounds of both short logs under laboratory conditions and living trees under field conditions. They also limited budding in the vicinity of necrosis. Less aggressive isolates brought about necrosis of beech bark infected through wounds but only of short logs under laboratory conditions. None of isolates caused bark necrosis of living beech trees under field conditions unless it was wounded.

There were no macroscopic symptoms on double cultures between antagonistic fungus *Trichoderma harzianum* Rifai agg. and particular isolates. Biopreparation Polyversum (with *Pythium oligandrum* Drechsler as an active ingredient) and repellent Pellacol (withn thiram 100 g/kg as an active ingredient) inhibited production of undetermined fungal fruiting bodies on necrotic lesions. Biopeparation Supresivit (with *Trichoderma harzianum* as an active ingredient) and fungicide Dithane M 45 (with 80% mancozeb as an active ingredient) as well as Polyversum and Pellacal showed no influence upon spreading of necrotic lesions.

## Introduction

Importance of beech bark disease has been growing in Carpathian Mountains within last few years (CHIRA, CHIRA 1997, 1999; ZÚBRIK *et al.* 1998; KUNCA *et al.* 1998; 2000). *Nectria sp.* is considered as an initiator of the disease and beech scale insect (*Cryprococcus fagi* Baer.) as a predisposing factor (HOUSTON *et al.* 1979).

Beech bark is protected against fungal infection by hard dead felem cells that are impregnated by suberin substance (POŽGAJ *et al.* 1997). Lenticels and bark wounds may be a significant entrance for fungal pathogens that cause necrotic lesions (KÚDELA 1989).

The aim of the work was to evaluate resistance of beech bark against fungal infection. In addition some biological preparations (JANČAŘÍK 1996), fungicide and repellent were tested in order to limit fungal growth and spreading bark necrotic lesions.

## Material and methods

### *Infectious test under laboratory conditions*

There were isolated 15 fungal strains out of necrotic lesions without their determination. They were cultivated on 2% malt agar at 24°C.

15 logs 30 cm long and 5 cm in diameter were transported from the forest to laboratory in February 1999. There was made a 0.8 $cm^2$ circle wound by a sharp knife on each log so that a bark was removed up to a cambium. Then such wound was infected by a fungal inoculum from nutrient substratum.

Wounds were bounded with a non sticking tape. One end of the log was covered with a candle wax, second end was sunk into water. All such treated logs were placed in a shade of the laboratory at 20°C. The experiment was repeated two times.

### *Infectious test under field conditions*

The field experiment was established in the 2nd age class of beech trees with 100% canopy closure, stockings 1, north exposure, 25° slope of West Carpathian Mountains, Štiavnické vrchy hills.

Bark of 16 beech trees was infected by 11 fungal isolates on 24. 4. 1999 in two modifications: (i) 1 $cm^2$ of inoculum was transferred upon the non wounded bark surface (ii) 1 $cm^2$ of inoculum was transferred into 0.8 $cm^2$ circle wound which was made by a sharp knife so that the bark up to cambium was removed from the wound. Each of 16 trees also had one wound without inoculum as a control.

Response of beech bark was checked on 28. 5. 1999 (35 days after infection), 19. 7. 1999 (87 days after infection), 4. 8. 1999 (103 days after infection) and 19. 8. 1999 (118 days after infection).

Influence of *Trichoderma harzianum* Rifai agg. CCM 8148 pure culture upon particular isolates under laboratory conditions

The influence of *Trichoderma harzianum* Rifai agg. CCM 8148 upon particular isolates was tested on doubled cultures on 2% malt agar at 24°C.

Influence of biopreparations and fungicide against spreading of necrotic lesions under field conditions

Necrotic lesions brought about isolates No 24 and 26 were coated for the first time 87 days after inoculation (19. 7. 1999) and the second time 16 days after the first treatment (4. 8. 1999) by 0.5% Supresivit solution (*Trichoderma harzianum* Rifai agg. as an active ingredient), 0.5% Polyversum solution (*Pythium oligandrum* Drechsler as an active ingredient), 3% Dithane M 45 solution (containing 80% of mancozeb as an active ingredient) and Pellacol (thiram 100 g/kg as an active ingredient) repellent solution. Each treatment was repeated 3 times and there were 4 controls (untreated lesions). Spreading of treated necrotic lesions was controlled 15 days (19. 8. 1999) after the second treatment.

1. **Reaction of infected wounds of beech bark under laboratory and field conditions – Reakcia infikovaných rán bukovej kôry v laboratórnych a terénnych podmienkach**

| Number of isolate[1)] | Laboratory conditions[2)] | | | Field conditions[6)] | |
|---|---|---|---|---|---|
| | Necrotic bark[3)] | Callused wound[4)] | Neither necrotic nor callused wound[5)] | Necrotic bark* | Callused wound[4)] |
| 1 | | | | | 16 |
| 2 | 2 | | | | 16 |
| 3 | | 2 | | | |
| 5 | 1 | | 1 | | 16 |
| 6 | | | | | 16 |
| 15 | 2 | | | | |
| 16 | | 2 | | | |
| 17 | 2 | | | | |
| 18 | 2 | | | | 16 |
| 19 | | 2 | | | |
| 20 | 1 | 1 | | | |
| 21 | 2 | | | | |
| 22 | 2 | | | | |
| 23 | 1 | | 1 | | |
| 24 | 2 | | | 16 | |
| 25 | 2 | | | | |
| 26 | 1 | | 1 | 16 | |
| 27 | | | | | 16 |
| 28 | | | | | 16 |
| 29 | | | | | 16 |
| 30 | | | | | 16 |
| Total[7)] | 12 | 4 | 3 | 2 | 9 |

*počet infikovaných rán

*[1)]Číslo izolátu, [2)]laboratórne podmienky, [3)]nekrotizovaná kôra, [4)]kalusovaná rana, [5)]ani kalusovaná ani nekrotizovaná rana, [6)]terénne podmienky, [7)]počet izolátov spolu*

Measured data were evaluated by analysis of variance and Duncan test at the 1% and 5% significance level.

## Results

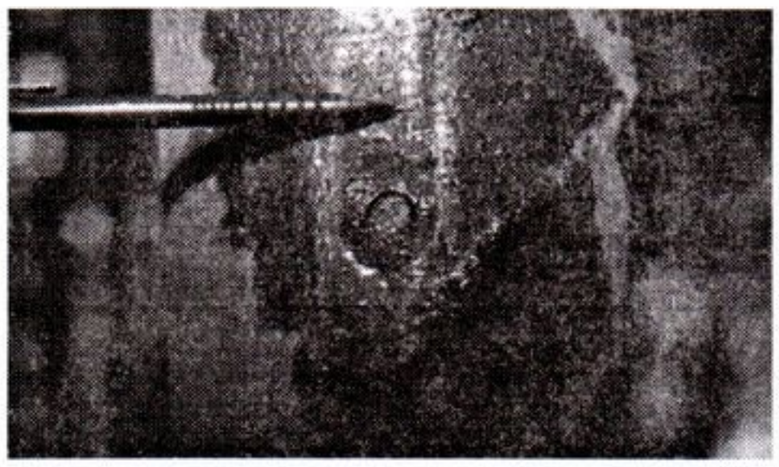
### *Infectious test under laboratory conditions*

Infected wounds were callused without any necrotic symptoms in 7 cases, neither callused nor necrotic in 3 cases and necrotic in 20 cases out of 30 cases 46 days after inoculation (Tab. 1).

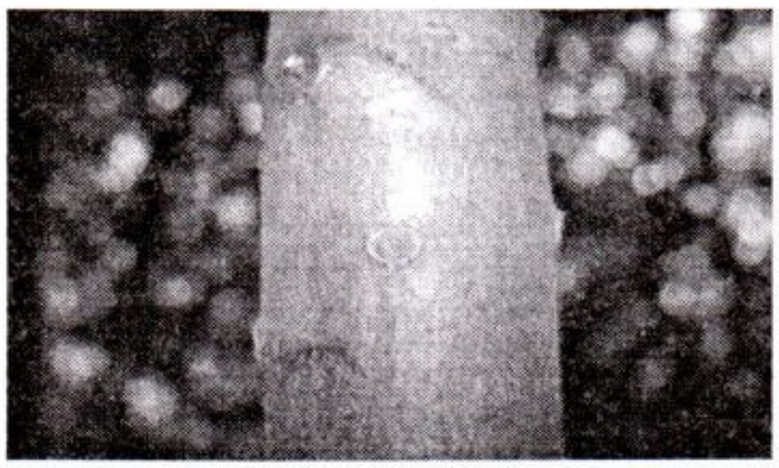
Colour of necrotic bark was the most visible symptom of the disease. It changed from healthy brown–green to dark–purple–black colour, well delimited and in many cases also slightly immersed in tangent direction.

The callus growth process was accomplishing by different speed from edges to the centres of wounds.

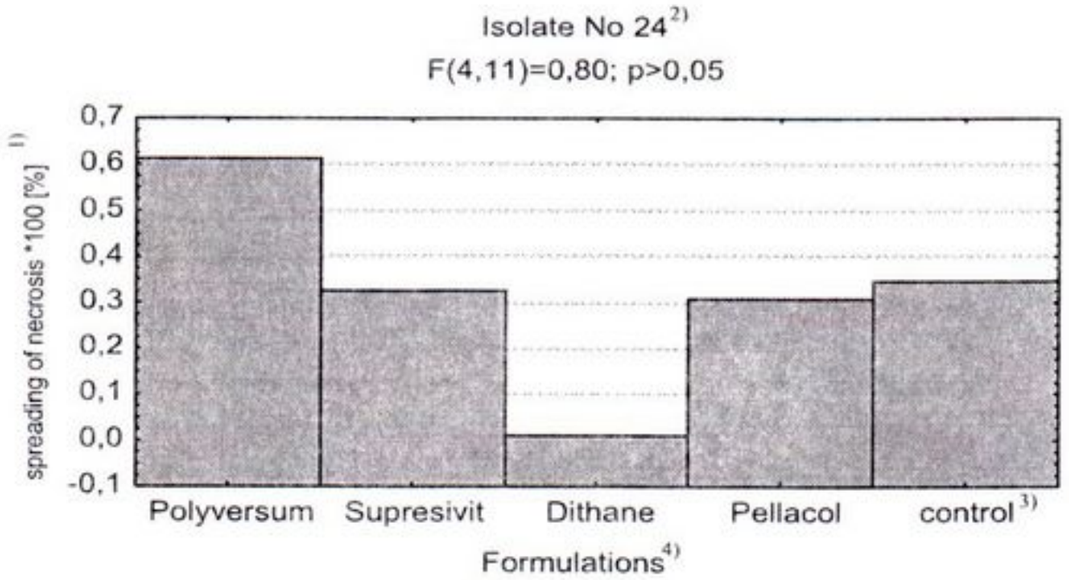
Wounds that were neither callused nor necrotic, had their xylem dry and colour of the bark in the vicinity was unchanged.



**1.** Spreading necrotic lesion from the wound infected by the strain No. 26 118 days after inoculation – Šírenie sa nekrózy kôry od rany infikovanej kmeňom č. 26 118 dní po inokulácii



**2.** Callused wound infected by the strain No. 29 118 days after inoculation – Zakalusovaná rana infikovaná kmeňom č. 29 118 dní po inokulácii




**3.** Spreading of bark necrosis caused by isolate No. 24 31 days after the first treatment – Rozšírenie sa nekrózy kôry buka spôsobenej izolátom č. 24 31 dní po prvom ošetrení
*[1)]Šírenie nekrózy, [2)]izolát č. 24, [3)]neošetrená kontrola, [4)]prípravky*

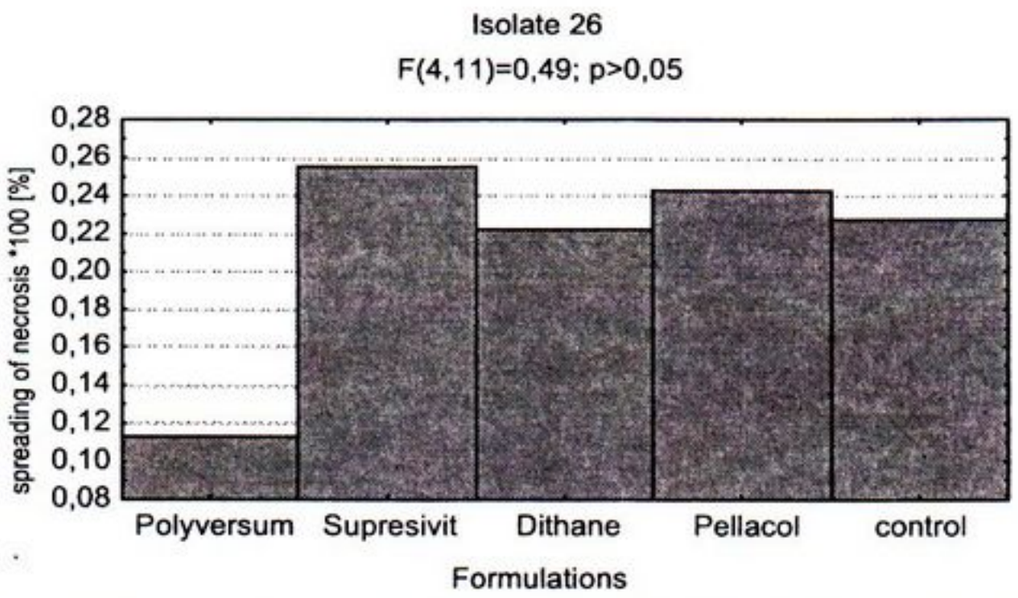
There was no immersed bark or callused tissue on the edge of wounds.

There was found out that buds which were close to the wounds infected by isolates No. 24 and 26, did not burst in leaves at all and remaining buds of those logs budded slower than other logs.

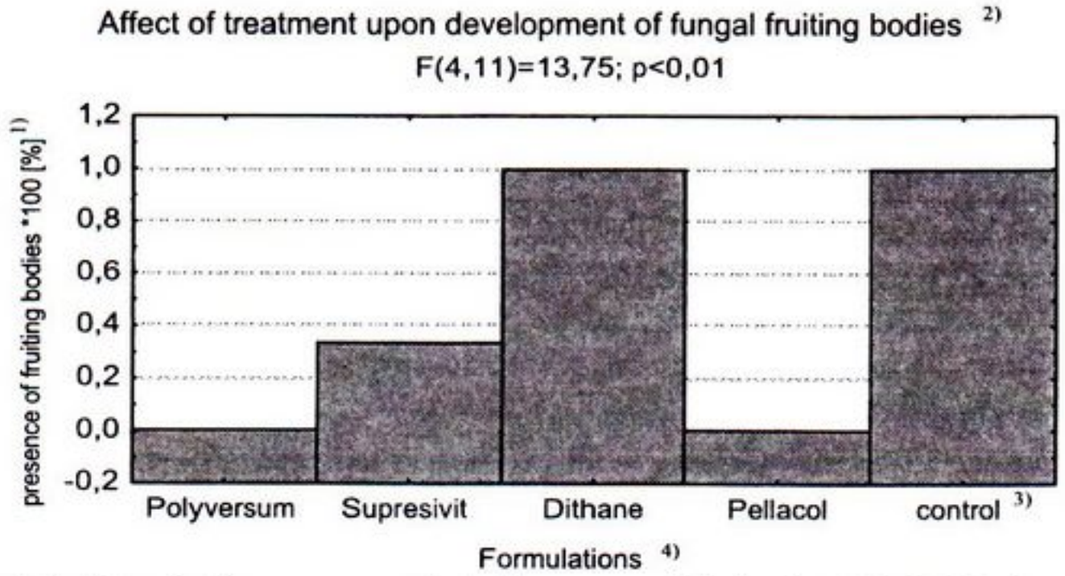
***Infectious test under field conditions***

If inoculum was put in the wound, only isolates No. 24 and 26 (out of 11 tested isolates) were able to start a bark necrosis developing on all of 16 infected trees (Tab. 1, Fig. 1). The rest 9 isolates were callused from the edge of the wound (Fig. 2). If the inoculum was put on the surface of unwounded bark, no bark necrosis developed on that spot at all (Tab. 1).

The sunk bark in longitudinal direction as well as change of colour was observed in connection with necrotic le-




**4.** Spreading of bark necrosis caused by isolate No. 26 31 days after the first treatment – Rozšírenie sa nekrózy kôry buka spôsobenej izolátom č. 26 31 dní po prvom ošetrení




**5.** Presence of fungal fruiting bodies on necrotic lesions caused by isolate No 26 31 days after the first treatment by particular formulations – Prítomnosť na nekrózach spôsobených izolátom č. 26 31 dní po prvom ošetrení jednotlivými prípravkami

*[1)]Prítomnosť plodníc, [2)]vplyv ošetrenia na vývoj plodníc, [3)]neošetrená kontrola, [4)]prípravky*

sions. It was impossible to measure them due to no sharp edges.

Influence of *Trichoderma harzianum* Rifai agg. CCM 8148 pure culture upon particular isolates under laboratory conditions

There was observed no reaction zones on doubled cultures between *T. harzianum* and particular isolates. Mycelia grew through each other without any macroscopic symptoms that could indicate any inhibition.

## Influence of biopreparations and fungicide against spreading of necrotic lesions under field conditions

None of the tested formulations had significant influence upon decrease of bark necrosis spreading caused by isola-

2. **Significant differences between affect of particular formulations upon fruiting bodies development caused by isolate No. 26 31 days after the first treatment. (ANOVA; p<0.01; Duncan test) – Významné rozdiely medzi vplyvom jednotlivých prípravkov na vývoj plodníc na nekrózach spôsobených izolátom č. 26 31 dní po prvom ošetrení (ANOVA; p<0,01; Duncanov test)**

| | Polyversum | Supresivit | Dithane | Pellacol |
|---|---|---|---|---|
| Supresivit | | | | |
| Dithane | ** | ** | | |
| Pellacol | | | ** | |
| Control | ** | ** | | ** |

tes No. 24 and 26. However, Dithane M 45, although not significantly, limited bark necrosis spreading caused by isolate No. 24 (Fig. 3) and Polyversum alike limited bark necrosis caused by isolate No. 26 (Fig. 4).

All controlled necrotic wounds which were infected by isolate No. 26 (4), all necrotic wounds which were infected by isolate No. 26 and treated by Dithane M 45 (3) and one wound which was infected by isolate No. 26 and treated by Supresivit were covered by several small fungal fruiting bodies. They were orange, 0.1 × 0.1 cm big and they burst out through the felem cracks but nobody determined them.

No fruiting body was observed on the necrotic wounds which were infected by isolate No. 24 or by isolate No. 26 and treated by Polyversum and Pellacol (Fig. 3, Tab. 2).

## Discussion

Living trees are less susceptible to fungal infection than tree logs because wounds on the living trees were callused even if they were infected by the same isolate which caused developing of necrotic lesions on the logs. It is obvious that those logs were under greater stress. I suppose that alike stress caused by drought or leaf–eating insect may increase susceptibility to less aggressive fungal species as initiators of bark necrosis. CHIRA and CHIRA (1997, 1999) describe bark necrosis mainly on the stands which are stressed by summer drought and late spring frost.

It is interesting that it was impossible to initiate bark necrosis by the pathogenic fungi if intact bark was inoculated under both laboratory and field conditions.

One of the most frequent species occurring on logs belong to genus *Trichoderma* (VESELÝ 1996, JANČAŘÍK 1996). They are in antagonistic relationship to more than 30 fungal species (OSHIMA 1966). However, these experiments did not prove any effect of *T. harzianum* upon 15 isolates under laboratory conditions and 11 isolates under field conditions. This result may be due to used evaluating method as we considered only macroscopic inhibition symptoms (ŠVECOVÁ *et al.* 1998). There are also microscopic symptoms, which were not considered in the experiment, pointing out the antago-

nistic relationship such as swelling of hyphae, production of resting spores (chlamydospores or sclerotia), extrusion of cytoplasm from hyphal tips and bursting and destruction of mycelium (YANG *et al.* 1993).

*Pythium oligandrum* Drechsler parasites on more than 30 fungal species and up to 19 of them belong to the genus *Pythium* (ŠVECOVÁ *et al.* 1998, JANČAŘÍK 1996). This mycoparasite inhibited production of fruiting bodies on the necrotic lesions caused by one of the tested isolate but this affect was not investigated under laboratory conditions.

Pellacol is a brown viscous liquid, repellent formulation which is applied upon wounds by coating. Because applied spot is coloured for a long time it is not possible to recognise the bark necrosis under the coat. Therefore it is not exactly known if this formulation had an effect upon bark necrosis. However, fruiting bodies production can be seen quite good and in my cases it limited production of fruiting bodies.

Dithane M 45 is a contact fungicide with mancozeb as an active ingredient. It is known that contact fungicides act only on the applied surface and they hardly penetrate into inner tissues. So they are not generally recommended for curing or preventing inner tissues from infection. On the other hand effectiveness of biopreparations still need more experiments.

**Summary**

Laboratory and field experiments show different pathogenicity of tested isolates. More aggressive isolates brought about necrosis through wounded bark on both logs and living trees and some of them limited budding of logs. Less aggressive isolates caused bark necrosis through wounds only on logs and budding of logs was not limited. None of the isolates initiated bark necrosis unless it was inoculated through wounded bark.

*Trichoderma harzianum* Rifai. exhibited no effect upon tested isolates either laboratory conditions or field conditions. Polyversum and Pellacol limited production of fruiting bodies on necrotic lesions caused by one of the isolate. None of tested formulations had a significant effect upon spreading of necrotic lesions.

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