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CANKER AND WILT OF BLACK LOCUST (*ROBINIA PSEUDOACACIA* L.) CAUSED BY *FUSARIUM* SPECIES

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From the pathological material of black locust trees showing symptoms of wilting of the foliage or canker of the bark the following *Fusarium* species were isolated: *Fusarium avenaceum* (Fr.) Sacc., *Fusarium lateritium* Nees., *Fusarium semitectum* Berk. & Rav., *Fusarium solani* (Mart.) Sacc., *Fusarium sulphureum* Schlecht. (syn.: *Fusarium sambucinum* Fuckel f. 6 Wollenw.) The results of the provocation infections of one-year-old black locust seedlings showed that all of the species – except *Fusarium solani* – are able to cause considerable necrosis in living bark and phloem. *Fusarium sulphureum* had by far the highest pathogenecity among the tested species. *Fusarium semitectum* isolated from withered black locust tree also caused necrosis on significant bark area. In the course of the penetration assay *Fusarium sulphureum* and *Fusarium avenaceum* were the most successful, and these species can cause cankers on the stem and twigs of black locust without frost effect.

Keywords: black locust, wilting, canker, *Fusarium avenaceum, Fusarium lateritium, Fusarium semitectum, Fusarium sambucinum, Fusarium sulphureum*

Introduction

The black locust (*Robinia pseudoacacia* L.) native in Atlantic North America was introduced in Hungary in 1710. It is actually one of the most important tree species for the forestry in Hungary, occupying about 20% of the forest area in the country and this rate will probably increase in the future thanks to the reprivatisation and the growing demand for its wood.

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During about the 300 years since its introduction more and more indigenous pests and pathogenes attacked and colonized this tree species, which process was facilitated by frost susceptibility of black locust and the establishment of pure stands on large areas regardless of the site conditions. Among the pests *Parthenolecanium corni* Bché. living on weak end shoots of black locust growing in dry sites caused the first panic in professional circles at the end of the 19th century. Nowadays the surprising fast spreading of two introduced leaf miner moth (*Parectopa robiniella* and *Phyllonorychter robiniella*) endangers the future healthy state of black locust stands. Among the pathogenes the following species were best known: *Robinia mosaic virus* and *Phloeospora robiniae* (Desm.) Höhn. on leaves, *Nectria cinabarina, Diaporthe oncostoma* and *Cucurbitaria elongata* on shoots and branches, *Perenniporia fraxinea* (Bull. Ex Fr.) Ryv., *Phellinus robustus* (P. Karst.) Bourd. Et Galz., *Laetiporus sulphureus* (Bull. Ex Fr.) Murill and *Armillaria* species as wood decaying agents in stem [1].

In recent years, some health problems have appeared in young afforestations and in certain stands of different age. The most typical symptoms are: foliage withering in the spring, die-back of the branches and the canker of the bark. The latter problem is the most frequent in the young black locust stands situated in terrain depressions and in valleys between hills. These bark die-off phenomena were earlier thought to be caused by frost, only. Recent studies on the causing agents of canker and dieback of black locust demonstrated the role of severeal fungi as *Phomopsis oncostoma, Fusarium avenaceum, Fusarium lateritium* and others in the appearance and development of the syndrome [2, 3]. Anyhow, according to the findings of my investigations (besides the *Phomopsis oncostoma* and *Nectria cinnabarina*) some *Fusarium* species also play a key-role in the development and aggravation of the symptoms. In the connection of the wilting of black locust *Fusarium* species were isolated as well.

Material and methods

The symptoms of the decline as well as the fungi associated with them and the factors influencing the decline are discussed on the basis of the findings of the investigations carried out in plantations, shelterbelts situated in Győr-Moson-Sopron, Pest, Somogy and Szabolcs-Szatmár-Bereg counties in the period of 2000–2001.

The fungi were isolated from diseased twigs and branches by placing surface-sterilized bark or wood chips taken from the margin of the dead and healthy tissue on PDA medium in agar plates. In other cases the branches bearing mature fungal fruit bodies were incubated in wet chamber for 2–4 days. The method of isolation was to transfer small section of fruit bodies or mycelia without the host tissue or place

inoculum from spore mass to the PDA. The identification of *Fusarium* isolates was based on morphological and cultural characters on PDA media under natural day-night rhythm in the laboratory at 20–22° [4, 5, 6].

Inoculation experiments were carried out on 1-year-old pot-grown seedlings from 17th of April in 2001. The plants were inoculated in groups of 15 with a total of 5 fungal strains. The plants were irrigated three times a week to ensure appropriate water availability. At the time of inoculation the mean plant size was 50 cm tall with a 8 mm stem diameter measured 10 cm above the substrate level. The superficial wound (5 mm in diameter) was made on the bark of each plant at 10 cm above the ground level with sterile scalpel. The mycelial plug obtained from the margin of a fungal colony of 8 days old was placed in the wound, with the mycelium facing the stem, and the wound was wrapped with Parafilm^{R}. Fifteen additional control plants were treated similarly with sterile PDA plugs. The lengths of vascular necrosis were measured upwards and downwards from the point of inoculation after three months.

The inoculation by contact was carried out on different bark surfaces (on green shoots, on greenish-brown shoots and on older brown bark surface in 5 replications) of 2-year-old seedlings in August of 2001. The evaluation of the experiment was made after 2 weeks.

Results

The symptoms and the morphological and cultural characters of the fungi

In the course of my investigation the following *Fusarium* species were isolated and identified:

Fusarium avenaceum (Fr.) Sacc.

This species was the fungus most frequently isolated from the *Fusarium* cankers of young (1–3 year old) black locust stem. The cankers mostly occurred around the stipule-thorns and at the basal section of lateral branches. These tissues of black locust are very susceptible for the frost damage, so the fungus probably infects the trees mainly through the lesions caused by frost. In some cases on the young green shoots I observed the symptoms of infection through lenticels as well. The first symptoms are the sunken bark with a brownish-red discoloration. Sometimes some orange coloured sporodochia can occur on the edge of the necrosis and at the base of the lateral shoots. Later on the diseased bark longitudinal splits developed, revealing the xylem underneath. On the surface of the infected wood the pinkish-red mycelia of the fungus

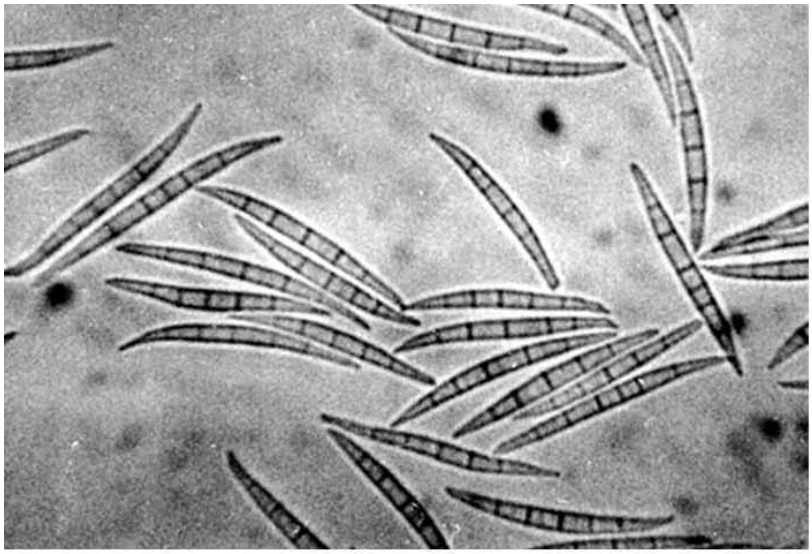

Figure 1. The macroconidia of *Fusarium avenaceum*

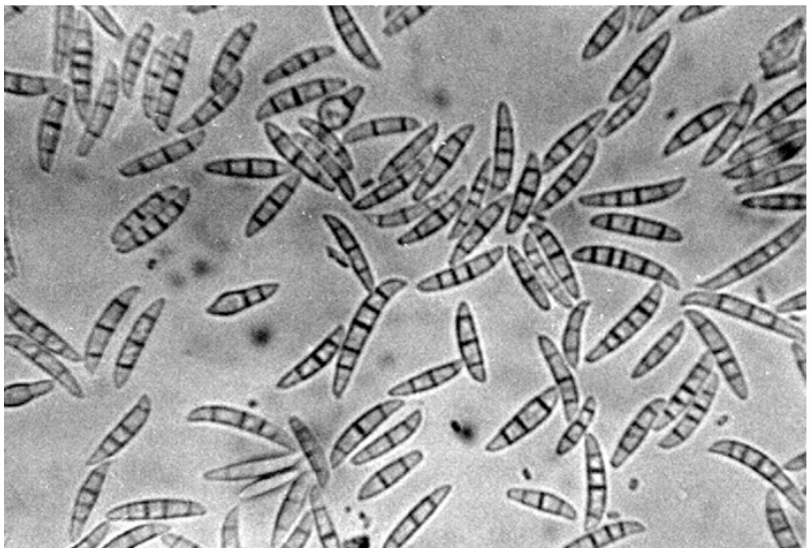

Figure 2. The macroconidia and the intercalary clamydospores of *Fusarium sulphureum*

can be rarely seen. On the older stems the canker sometimes occurred as a flattened areas but the bark remained intact. The diseased tree tries to heal up the lesion with the callus tissue year by year, but the fungus prevents it, causing swelling of the stem at the periphery of the canker.

The colony is moderately fast-growing, reaching a 35–55 mm diameter in 4 days. The aerial mycelium is abundant loosely, fluffy to cottony. The pigmentation is pink, rose to carmine with yellowish tinges. Sporulation seldom can be seen, mainly after 4–6 weeks from bright orange sporodochia. The mesoconidia are fusoid, 1–3-septate and often very variable in size. The macroconidia are uniform, narrowly fusoid, curved, 4–13-septate and with an elongated apical cell and a well-marked foot cell. They measure 32–110 × 2.5–4 μm. The clamydospores are absent.

Fusarium sulphureum Schlecht. (syn.: Fusarium sambucinum Fuckel f. 6 Wollenw.)

This fungus occurs often together with the *Fusarium avenaceum*. The canker caused by this species is similar to the canker caused by the aforementioned species, but often affects larger areas of the bark. However, this fungus attacks the xylem as well. The characteristic symptoms of the infection are the whitish-yellow mycelia developing on the surface of the infected bark and xylem. This fungus was also isolated from an older tree died shortly after the bursting of buds in the spring. In this case the fungus killed the phloem and the cambium at the base of the stem all around, causing dark brown discolouration of the affected tissues. In the wet chamber also yellowish-white mycelia had grown from the vessels of the xylem of this tree. The bark of this tree was attacked by moth larvae as well.

The culture grows very fast, reaching 58–78 mm in 4 days. The aerial mycelium is very abundant in the beginning, later becomes powdery by the scattered masses of conidia. The pigmentation is whitish, cream, yellowish, peach to ochraceous, finally slightly cinnamon coloured. The sporulation starts very quickly. In the old cultures a few cauliflower-shaped sclerotial bodies may occur. The macroconidia are thin-walled, curved, fusiform, with a curved pointed apical cell and a distinctly pedicellate foot cell, which is mostly 3–5-septate and 24–44 × 3.5–5.5 μm. The clamydospores are not very abundant, mainly intercalary.

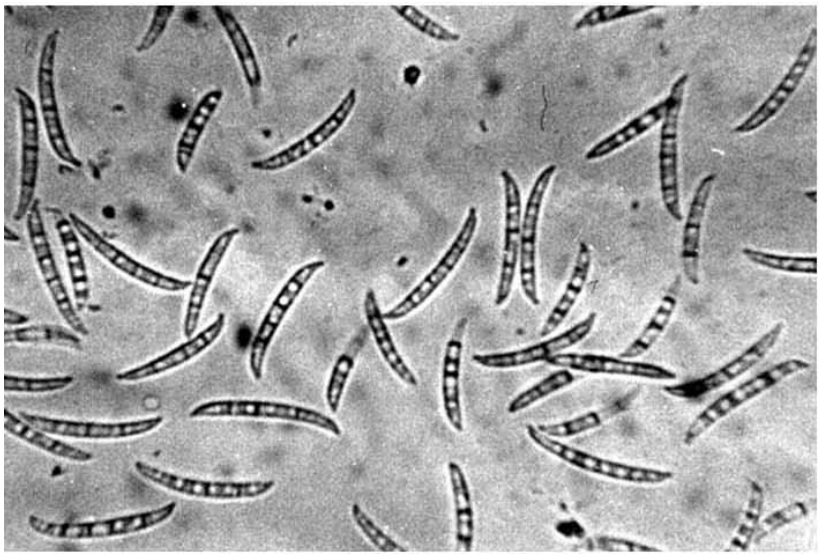

Figure 3. The macroconidia of *Fusarium lateritium*

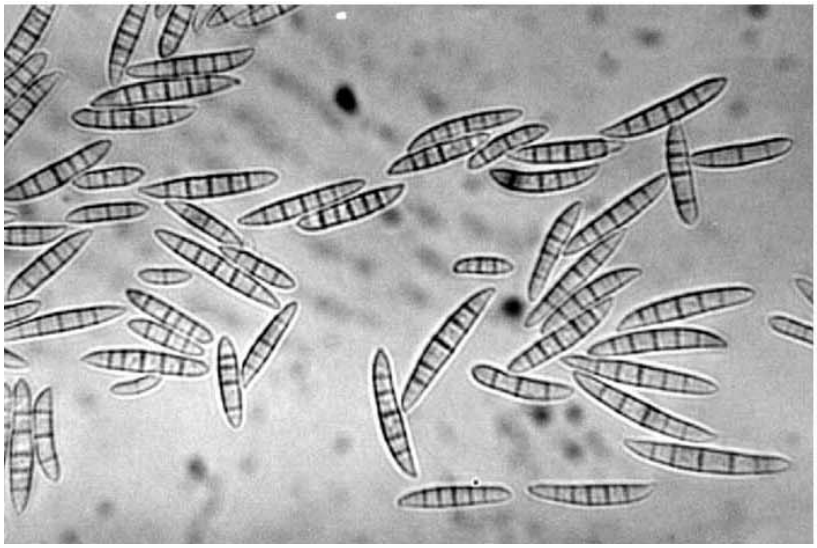

Figure 4. The macroconidia of *Fusarium semitectum*

Fusarium lateritium Nees.

I isolated this fungus only two times. On a dead branch attacked by the *Xyleborus dispar* the orange sporodochia were near the gallery, so I suppose the beetle has a vector role in the spreading of the fungus. In this case the fungus did not cause the sinking and discolouration of the bark. In the other case *Fusarium lateritium* was isolated from a lens shaped necrosis caused by the infection of *Phomopsis oncostoma*.

The culture is slow-growing, reaching only 14–15 mm diameter in 4 days. Pigmentation is beige or pale salmon coloured, but in older cultures also blue pigmentation can occur. The sporodochia develop after two weeks. The macroconidia are very uniform in shape and size, almost straight in the central part, with a hooked apical and a distinct pedicellate basal cell, mainly 3–5-septate and 20–50 × 2.5–4.5 μm.

Fusarium semitectum Berk. & Rav.

This species was isolated from bases of the stems of withered, older black locust trees in all cases. On the surface of the infected bark pinkish-orange coloured fabric of mycelia can often be seen. The diseased phloem had a dark-brown discolouration. The fungus infects the trees probably from soil. It is likely that the wood-boring insects (chiefly long-horn beetle larvae in the wood and moth larvae in the bark) occurring on some of the investigated stem played a central role in the infection.

The culture of the fungus is fast-growing, reaching 52–66 mm in 4 days. The pigmentation is whitish or peach coloured, in older cultures finally buff-brown. The macroconidia are rather thick-walled, straight and spindle-shaped, sometimes slightly curved with 3–5 septa, up to 18–36 × 3–5 μm.

Fusarium solani (Mart.) Sacc.

Fusarium solani was isolated from older trees and seedlings attacked by other fungus species (*Fusarium avenaceum, Fusarium semitectum*). The fungus infects the trees from the soil, so often colonizes frost-damaged trees. (Sudden considerable cooling can cause the dying of the bark at the base of the black locust stem.)

The culture of the fungus is moderately fast-growing, reaching 28–38 mm in 4 days. The aerial mycelium is usually rather sparse, felt-like, sometimes zonate. The pigmentation is predominantly cream or buff coloured, often with bluish-brown tinges. The macroconidia are rather thick-walled, subcylindric, only slightly curved, with a short and blunt apical and an indistinctly pedicellate basal cell. The conidia are often indistinctly septate, predominantly 2–5-septate, 10–48 × 4–6 μm.

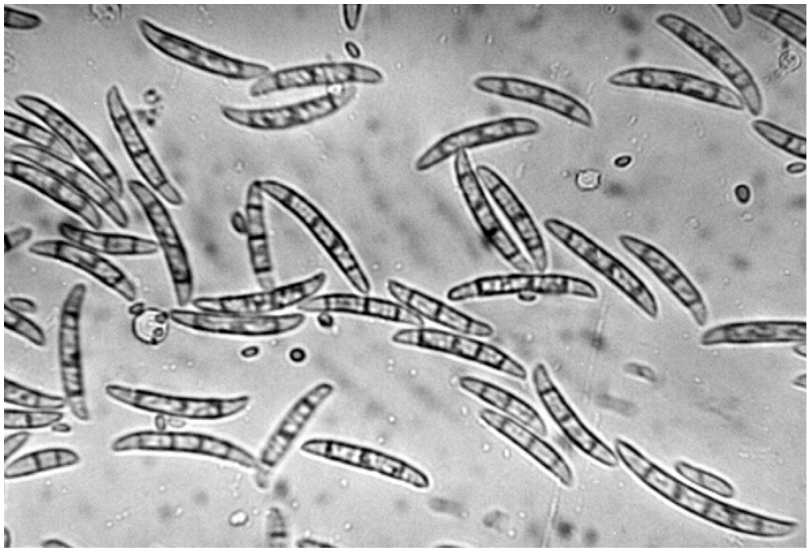

Figure 5. The macroconidia of *Fusarium solani*

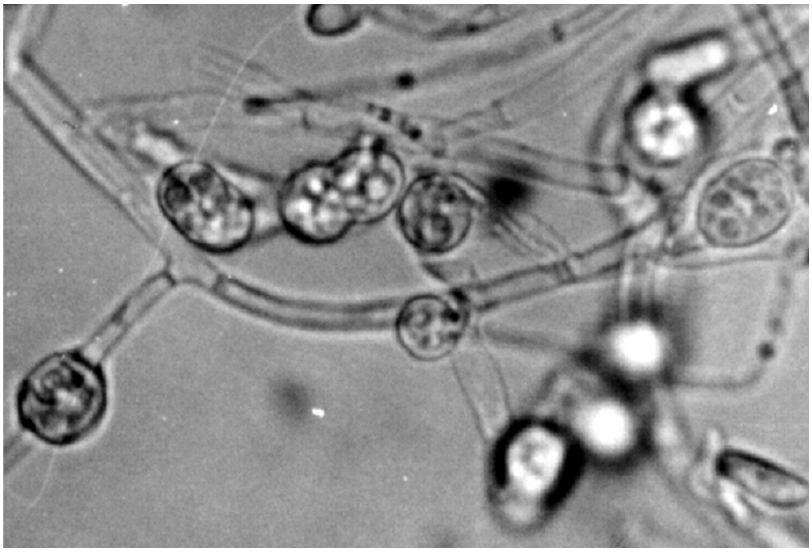

Figure 6. The clamydospores of *Fusarium solani*

The pathogenecity of the fungi

The results of the provocation infection of one-year-old black locust seedlings are shown in Table I.

Table I

Results of provocation infection carried out on one-year-old black locust (*Robinia pseudoacacia*) seedlings

Taxa	Infection through the incision of the bark and phloem (n=15) (Evaluation after 3 months)					Penetration infection (n=5) (Evaluation after 2 weeks)		
	Number of seedlings showing the following mentioned symptoms (pc)			Average length of the necrosis (cm)		Number of successful infection		
	Dieback of the apical shoots above the point of infections	Withering and dieback of the lateral shoots and lateral branches	Canker	On the bark	In the phloem	On green shoots	On brownish green shoots	On brown bark
Control	0	0	0	0.6	0.6	–	–	–
F. avenaceum 14	0	0	15	2.9	3.3	4	3	3
F. lateritium 1	0	0	4	1.7	4.3	0	0	0
F. semitectum 2	0	0	15	4.7	7.7	2	1	0
F. solani 1	0	0	0	0.8	1.1	0	0	0
F. sulphureum 4	2	13	13	10.1	36.2	5	5	5

In the case of *Fusarium sulphureum* this necrotic process was very fast. The part of the shoot situated above the point of inoculation died in 2–4 weeks in the case of 30% *of seedlings*, but the withering of lateral shoots was observed on each seedlings in 2 weeks. Very often only the apical shoot survived, but the growth rate of this shoots decreased considerably. In the beginning the infected bark was orange brown-coloured, but later a very dark discolouration was observed on the edge of the necrosis on significant areas spreading upwards from the point of infection. Mean vascular necrosis were the largest of all the inoculation treatments. The dimensions of these lesions were equivalent to 70–80% of the mean plant high. The fungus attacks the xylem as well and it spreads very fast upwards. In the infected xylem a long, drop shaped black discolouration can be seen. Two months after the infection a lot of orange coloured sporodochia appeared on the surface of infected bark near the point of infection.

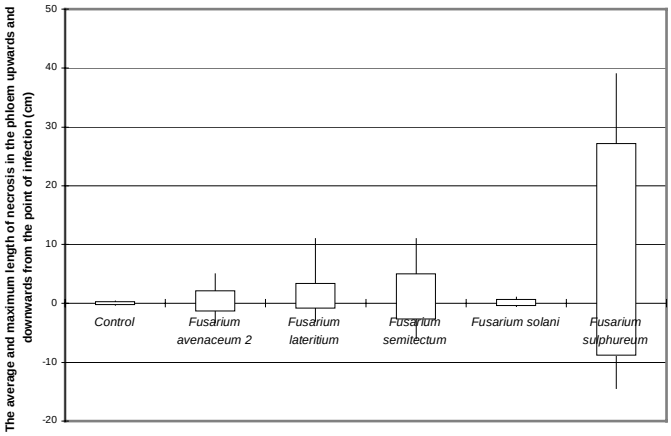

Figure 7. Results of provocation infection of 1-year-old black locust (*Robinia pseudoacacia*) seedlings after 3 months

Fusarium semitectum also caused necrosis on significant bark area. The diseased bark was flattened and very dark-coloured. The fungus did not cause the withering of shoots, but the growth rate of the seedlings decreased.

The cankers caused by *Fusarium avenaceum* were strongly apparent, but not so large as in the case of the two aforementioned species. The infected bark was reddish-brown coloured, bordered with a dark, narrow line. The growth rate of the infected shoots did not decrease significantly.

In the case of *Fusarium lateritium* usually little external discolouration of the bark was observed. In some cases a 1–2 cm long longitudinal split appeared on the bark above the point of inoculation. In spite of this fact sometimes there was a significant necrosis (14 cm) in the phloem.

In comparison with the other fungi *Fusarium solani* produced small bark canker limited to 1–2 mm around the point of inoculation and the relatively minor lesions soon healed up.

In all cases of the fungi tested in the provocation infection the lateral shoots growing below the place of infection and the shoots growing after the cutting back of the seedlings were healthy.

In the late growing season penetration infections were initiated on fresh green shoots and on older bark surfaces without opening an incision into the stem. *Fusarium sulphureum* and *Fusarium avenaceum* were the most successful fungus species among the tested ones to penetrate healthy bark tissues. The young green shoots infected by

Fusarium sulphureum broke in two after one week due to the intensive attack of the fungus. On the older bark surface the infections happened through the lenticels.

These results proved that *Fusarium avenaceum* and *Fusarium sulphureum* are able to cause cankers on the stem of black locust without frost effect. The spreading of these species in the young black locust stands probably are also promoted by the green pruning of the lateral branches.

Discussion and practical recommendation

The *Fusarium* canker is probably not a new disease in black locust stands. *Fusarium sambucinum, Fusarium avenaceum* and *Fusarium lateritium* were reported from England as pathogenes causing stem canker on two-year old *Robinia pseudoacacia* seedlings [7]. *Fusarium oxysporum* and *Fusarium solani* were also found as pathogenic fungi of canker of black locust in China [8]. *Fusarium solani f. robiniae* was shown as a casual agent of twig blight of *Robinia pseudoacacia* in Japan [9]. The occurrence of *Fusarium lateritium,* and *Fusarium sulphureum* on black locust is documented in Hungary [10]), but without mentioning its pathological role. Precedent Hungarian investigations [2, 3] showed that *Fusarium avenaceum* and *Fusarium lateritium* proved to be wound pathogenes infecting the host at the lesions caused by frost and their presence and activity resulted the perennial canker deformations of the black locust stems. No data were found about *Fusarium semitectum* causing the withering symptom of black locust.

In forestry conditions the preventive control has the most important role against these fungus species. We have to avoid the establishment of black locust stands on unsuitable sites (mainly on frost hollow, and dry sites of the crests of sand hills). After planting we have to cut back the seedlings. Otherwise the trees will have a lower resistance to drought due to the disadvantageous foliage-root rate. By means of the appropriate control of the game stock and the wood-boring insects we can decrease the number of bark injury. The young 1–4 year old stands are the most susceptible to frost, because the fast growing green shoots have a longer growing season. So the stands of this age growing on suitable sites accidentally can suffer serious frost damage. (In the Nyírség the last serious frost damage happened in the winter of 1999–2000, when the temperature drop reached –20 °C. The analysis of the annual rings in the stem section showed that the bark injury had happened in most cases in this time as well.) In this case we have to cut back the damaged stand and we have to carry out root ripping in the given area. The fast growing root-shoots compensate soon the lag and if they manage to avoid serious frost in the future these stands will be more healthy and give better stem quality than the untreated stands suffering from several pathogens.

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