Phyton (Austria) Special issue: "Plant Physiology"	Vol. 39	Fasc. 3	(231)-(234)	30. 11. 1999

Some Aspects of *Sphaeropsis sapinea* Presence on Austrian Pine in Croatia and Slovenia

By

Danko DIMINIĆ[1)] & Maja JURC[2)]

Key words: *Sphaeropsis sapinea*, *Pinus nigra*, endophyte, dieback, pathogenicity.

Summary

DIMINIĆ D. & JURC M. 1999. Some aspects of *Sphaeropsis sapinea* presence on Austrian pine in Croatia and Slovenia. - Phyton (Horn, Austria) 39 (3): (231) - (234).

The fungus *Sphaeropsis sapinea* (Fr.) Dyko et Sutton causes different types of diseases on conifers. In 1992 it turned out as the major cause of Austrian pine dieback in Istria (Croatia). Up-to-date pine dieback symptoms have been observed in the North Adriatic area and in the region of Zagreb city. In Slovenia fungus was isolated as an endophyte from green needles of Austrian pine collected on few localities in 1993-95. Inoculation experiment confirmed the fungus pathogenicity and revealed no difference between Croatian and Slovenian isolates.

Introduction

Wide spread in the temperate and tropical regions all over the world the fungus *Sphaeropsis sapinea* (Fr.) Dyko et Sutton causes different types of diseases on conifers: stunting of a new growth, browning of needles, shoot blight, twig and branch dieback, crown wilt, bark cankers, root disease, damping-off, root rot, and as saprophyte blue stain of sapwood of fallen or freshly cut timbers (BROWNE 1968, PUNITHALINGAM & WATERSTON 1970). Among different hosts *Pinus* species (specially *P. nigra* Arnold, *P. radiata* Don. and *P. sylvestris* L.) have been found as the most susceptible to the fungus attack (PUNITHALINGAM & WATERSTON 1970).

Research on the health condition of pine plantations in Istria (Croatia) in 1992 revealed significant Austrian pine (*P. nigra*) dieback on some localities. The symptoms observed in the field were dieback of young and older shoots, branches

[1)] Faculty of Forestry University of Zagreb, Svetošimunska 25, P.O.Box 178, 10000 Zagreb, HR.

[2)] Slovenian Forestry Institute, Večna pot 2, P.O.Box 523-X, 1001 Ljubljana, SLO.

and hole crowns of pine trees. *S. sapinea* turned out as the major cause of pine dieback on these localities (DIMINIĆ 1994).

The study on endophytic fungi in the needles of Austrian pine have been done in Slovenia by JURC & JURC 1995. Authors reported of the presence of different fungi among which *S. sapinea* was also found.

The aim of this study is to find out where and under what conditions the fungus *S. sapinea* is present on Austrian pine trees in Croatia and Slovenia, and to reveal by performed inoculation experiment if there are any differences among Croatian and Slovenian isolates.

Materials and Methods

Field observations of randomly selected pine trees and plantations have been done. Needles from pines with no dieback symptoms, and needles, shoots, branches and cones from pines with dieback symptoms were collected.

To isolate *S. sapinea*, from the pine trees with dieback symptoms, necrotic needles up to 2 year-old with developed mature pycnidia collected from diseased shoots were used. The pure cultures were obtained from pycnidia. Malt Extract Agar (CM 59) "Oxoid" (MEA) has been used as media. To isolate the fungus, from the pine trees with no dieback symptoms, collected green needles (1-8 year-old) were prepared using the methods described by JURC & JURC 1995.

Inoculation experiment was carried out with *S. sapinea* isolates DP 04-1 (needles collected in 1992) and DP 06-3 (needles collected in 1995) from Austrian pine tree with dieback symptoms, and VIPAVA from healthy-looking Austrian pine tree (needles collected in 1994).

2.5 year-old Austrian pine seedlings were used in inoculation experiment. 30 seedlings were inoculated by fungus isolates, and 10 were used for control. The seedling's bark in diameter of 3 mm was removed with a special tool. Disc of 3 mm, contained mycelium and agar (MEA), was than placed in the fresh wound. The inoculation place was covered with cotton wool moisten in distilled water, and wrapped with parafilm. Control seedlings were inoculated only with agar discs, and the rest procedure was the same. After 2 days parafilm and cotton wool were removed. Analyses of the inoculated seedlings were done after 20 days. The bark was removed with scalpel starting at the inoculation place. Length of the developed necrosis were measured. Obtained data were analysed under the software program STATISTICA StatSoft© 1993. An analysis of variance (at $p < 0.05$) was performed.

Results

S. sapinea was found in Slovenia as an endophyte in green Austrian pine needles on a few localities (Fig. 1). In Croatia the fungus was found on dead needles, dieback shoots and branches, and on cones. The presence of *S. sapinea* on some localities was with no damaging influence on Austrian pine trees, found in crowns and on fallen dead needles and branches on the ground. On other investigated localities the fungus was found as causal agent of shoots, branches and crowns dieback (Fig. 1).

Morphological characteristics of the obtained fungus isolates were similar to characteristics of virulent morphotype A, described by PALMER & al. 1987.

Analysis of inoculated pine seedlings with fungus isolates after 20 days revealed tissue necrosis in different lengths under the bark of all inoculated plants.

Each necrosis girdled the seedling stem. On all inoculated seedlings, except one seedling inoculated with VIPAVA isolate, developed pycnidia were noticed on the bark. Approximately 1/3 necrosis length was developed below the inoculation place, and 2/3 necrosis length was developed above the inoculation place in all inoculated seedlings. In the Table 1 measured necrosis length are given.

Analysis of control plants after the experiment period showed no needle chlorosis and under the bark no signs of necrotic tissues.

Performed analysis of variance (at $p < 0.05$) revealed no significant differences between height of inoculated seedlings and necrosis length.

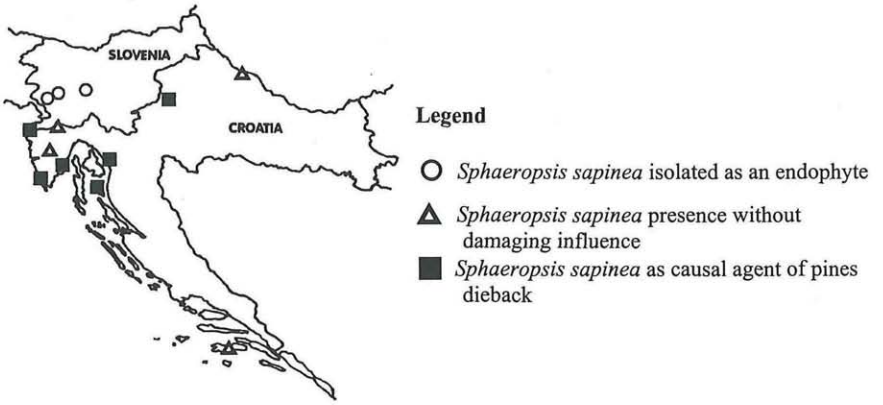

Fig. 1. *Pinus nigra* research localities on which *Sphaeropsis sapinea* presence was established.

Table 1. Height of inoculated *Pinus nigra* seedlings and length of measured necrosis under the bark per each isolate.

Isolate DP 04-1		Isolate DP 06-3		Isolate VIPAVA	
Seedling's height (cm)	Necrosis length (mm)	Seedling's height (cm)	Necrosis length (mm)	Seedling's height (cm)	Necrosis length (mm)
15.0	54	16.0	167	11.5	116
14.5	103	11.5	146	14.0	109
11.0	105	12.5	119	13.0	122
13.0	82	12.0	106	13.0	130
13.0	92	10.0	127	11.0	67
11.0	110	12.0	132	10.5	138
11.5	100	11.5	115	12.0	52
10.5	108	9.5	99	9.5	77
10.5	105	8.5	68	11.5	136
12.0	129	10.0	128	11.0	115

Underlined number = necrosis which couldn't be measured precisely, because it was longer than seedling's height (above the ground).

Discussion

Results of the study revealed that *S. sapinea* could live as an endophyte in green needles, causing no damage, and it could also be a causal agent of shoots and branches dieback.

In inoculation experiment isolates DP 04-1, DP 06-3 and VIPAVA showed capability to attack pine seedlings through wounds on the bark, and cause the tissue necrosis, which led to seedlings dieback.

According to CARROLL 1986 endophytic fungus lives in the tissue of an health plant, causing not seeable, nonsymptomatic infection. Some parasitic fungi spend a part of their life cycle in latent condition, without causing visible damage, but in stress situations or in ageing tissues of the host plant they produce symptoms of disease.

Stress as pines predisposing factor to *S. sapinea* attack has been considered by different authors (PUNITHALINGAM & WATERSTON 1970, BACHI & PETERSON 1985, NICHOLLS & OSTRY 1990, DE KAM & al. 1991, STANOSZ 1994). Drought and poor site conditions have been assumed as main stress factors on some localities in Croatia in 1992 (DIMINIĆ 1994).

Based on these preliminary study it could be supposed that *S. sapinea* has the latent part of its life cycle, living in the health pine tissues as an endophyte, and that it could turn to serious pathogen, causing shoots and branches dieback, under the specific stress conditions.

References

BACHI P.R. & PETERSON J.L. 1985. Enhancement of *Sphaeropsis sapinea* stem invasion of pines by water deficits. - Plant Disease 69 (9): 798-799.

BROWNE F.G. 1968. Pests and diseases of forest plantation trees. - Clarendon Press, Oxford, 1330 pp.

CARROLL G. C. 1986. The biology of endophytism in plants with particular reference to woody perennials. - In: FOKKEMA N. J. & VAN DEN HEUVEL J. (Eds.), Microbiology of the *Phyllosphere*, pp. 205-222. - Cambridge University Press.

DE KAM M., VERSTEEGEN C.M., VAN DEN BURG J. & VAN DER WERF D.C. 1991. Effects of fertilisation with ammonium sulphate and potassium sulphate on the development of *Sphaeropsis sapinea* in Corsican pine. - Neth. J. Pl. Path. 97: 265-274.

DIMINIĆ D. 1994. Prilog poznavanju mikoza borovih kultura u Istri. - Glas. šum. pokuse 30: 21-60.

JURC M. & JURC D. 1995. Endophytic fungi in the needles of healthy-looking Austrian pine (*Pinus nigra* Arn.). - Acta Pharm. 45 (2) suppl. I: 341-345.

NICHOLLS T.H. & OSTRY M.E. 1990. *Sphaeropsis sapinea* cankers on stressed red and jack pines in Minnesota and Wisconsin. - Plant Disease 74 (1): 54-56.

PALMER M.A, STEWART E.L. & WINGFIELD M.J. 1987. Variation among isolates of *Sphaeropsis sapinea* in the north central United States. - Phytopathology 77 (6): 944-948.

PUNITHALINGAM E. & WATERSTON J.M. 1970. *Diplodia pinea*. - C.M.I. Descriptions of pathogenic fungi and bacteria No. 273, p. 2.

STANOSZ G.R. 1994. Factors influencing *Sphaeropsis* shoot blight and canker epidemics in central Wisconsin, USA. - In: CAPRETTI P., HEINIGER U. & STEPHAN R. (Eds.), Shoot and foliage diseases in forest trees, pp. 254-262.- Instituto di Patologia e Zoologia Forestale e Agraria, Firenze.