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THE PRESENCE OF FUNGUS *SPHAEROPSIS SAPINEA* ON PINES IN CROATIA

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Introduction

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. The fungus is wide spread in the temperate and tropical regions all over the world (Browne 1968, Punithalingam & Waterston 1970, Swart & al. 1985, Chou & MacKenzie 1988). Among different hosts *Pinus nigra* Arnold, *P. radiata* Don. and *P. sylvestris* L. have been found as the most susceptible to the fungus attack (Punithalingam & Waterston 1970).

First report of fungus presence in Croatia as causal agent of pines dieback was by Böhm (1959). He reported one-year-old diseased Austrian pines in nursery near Karlovac. Till than there haven't been reports on damaging impact of *S. sapinea*. In 1992 it turned out as causal agent of pines dieback in Istria (Diminić 1994). In the December 1991 the local foresters noticed dieback symptoms in pine crowns in plantation on the eastern coast of the peninsula. First observation in that plantation and neighbour one have been made in April 1992 (Figure 1, research area No. 6). The observed symptoms were dieback of young and older shoots, branches and hole crowns of pine trees, mainly of *P. nigra*. Analyses showed that *S. sapinea* was the most frequent fungus on collected samples.

Since 1992 health condition inventory of pine plantations have been carried out, and further on observations revealed pines dieback on other localities in Croatia. To investigate where and with what influence *S. sapinea* is present on pines in Croatia, not only locations with dieback symptoms were observed, but also sites with no reports of dieback.

Materials and Methods

Pine plantations and pine trees in urban areas were randomly selected. Research localities are shown in Figure 1. In each plantation 3 to 10 pine trees were evaluated according to health condition. Dieback needles, shoots, and branches from crowns, fallen needles and branches from the ground were collected. Cones from the crowns as well as on the ground were collected too. Pines in urban areas as ornamental trees were observed also, and collection of samples were done.

In the laboratory collected samples were analyses as follows. Needles, shoots, parts of branches and cone scales were kept in humid conditions in petri dishes for day or two. Analyses of prepared samples were done under the stereomicroscope (illuminations 4 – 16 x). Observed fungus fruit bodies and host tissue were used to make cross section slides, and analysed under the light microscope (illuminations 100 – 400 x). A few isolations from the necrotic tissues under the bark of dieback shoots and branches have been done. Malt Extract Agar (CM 59) "Oxoid" was used as media to obtain and purify fungus isolates.

Results

Field observations and laboratory analyses carried out from April 1992 to November 1998 revealed following data. On some localities pines were in relatively good condition. On the other hand on some localities dibeck symptoms on Austrian pine were noticed: Buje stanica, Kanegra, Fazana, Puntera, Marina, Prtlog, Krsan, Plomin, Rabac, Klenovica and Lopar (Figure 1). In the area of Zagreb city dieback was noticed on *Pinus heldreichii* var. *leucodermis* (Ant.) Markgraf and *P. nigra* as well. It was generally established that the crown dieback intensity vary form 5 to 100 %. On some localities (Kanegra, Marina and Prtlog, Figure 1, research area No.: 2 and 6) up to 30% of all observed trees were attacked.

Analyses of collected samples (needles, shoots, branches and cones) from pine trees with dieback symptoms showed that among different fungi found *S. sapinea* (Fr.) Dyko et Sutton was the most frequent. Other fungi found:

- on needles: *Cyclaneusma niveum* (Fr.) DiCosmo, Peredo et Minter, *Lophodermium seditiosum* Minter, Staley et Millar, *Lophodermium pinastri* (Schard. ex Hook.) Chev., *Leptostroma seditiosum* Fr., *Leptostroma pinastri* Desm., *Truncatella hartigii* (Tub.) Stay.;
- on shoots: *Truncatella hartigii* (Tub.) Stay.;
- on branches: *Cenangium ferruginosum* Fr.

Isolations from the necrotic tissues under the bark of dieback shoots and branches of *P. nigra* (Prtlog locality) and *P. heldreichii* var. *leucodermis* (Zagreb locality) confirmed the fungus presence.

S.sapinea was also found on samples of *P. brutia* Ten., *P. halepensis* Mill., and *P. sylvestris* L. trees, but with no significant dieback symptoms.

Discussion

Research revealed the differences in *S. sapinea* impact on different pine species and in its occurrence on research localities in Croatia. On some of them it has been established just a presence of the fungus (Figure 1, research area No.: 1, 3, 4, 9, 11, 12) and on the other localities its outbreak (Figure 1, research area No.: 2, 5, 6, 7, 8, 10).

The symptoms observed in the field were dieback of young and older shoots, branches and hole crowns of pine trees. The same symptoms were notified in parks and gardens. *P. nigra* turned out as the most susceptible pine species in plantations, parks and gardens, although dieback was also strongly observed on *P. heldreicii* var. *leucodermis* in parks and gardens in the city of Zagreb.

According to research on fungus isolates (Diminić 1997), obtained from pycnidia on necrotic needles from different localities, it was established that all belong to the group of virulent morphotype A described by Palmer et al. (1987). Isolates significantly differ among each other in growth rate, but in inoculation experiment they showed, with no significant difference, capability to attack pine seedlings through wounds on the bark, and cause the tissue necrosis, which led to seedlings dieback.

Based on aforementioned results on *S. sapinea* occurrence and the presence of only virulent morphotype A on pines in Croatia, it has been concluded that the reason for fungus outbreak on some localities should be targeted to predisposing factors. Drought, poor site conditions, increased air born nitrogen deposition, wounds caused by hail, frost, insects and pruning, are considered to be the pines predisposing factors to fungus attack by many authors

(Haddow & Newman 1942, Punithalingam & Waterston 1970, Torres-Juan 1971, Chou 1984, Bachi & Peterson 1985, De Kam & al. 1991, Nicholls & Ostry 1990, Stanosz 1994).

The following factors have been analysed according to available data.

Drought. During the period March – September 1991 approximately 30% precipitation deficit occurred in Istria region (Figure 1, research area No.: 1 to 6). In the December 1991 the local foresters noticed first dieback symptoms in pine crowns on locality No. 6. Research area No. 6 (with pines dieback) had 559 mm and research area No. 1 (with no dieback symptoms) had 871 mm of total precipitation in that period.

Pure site conditions. Pine plantations on research area No. 3 were generally planted on sites with better soil conditions comparing to sites in area No. 2 and 6. The similar situation was on sites in area No. 7 and 8.

Aeolic salinisation. Mayer (1979) found out, according to his research in area No. 6, that sites near sea had increased content of natrium and magnesium in the soil adsorption complex and in forest litter, which revealed intensive processes of aeolic salinisation, especially alkalisation.

Insects. On some samples injuries were found under the bark and in the heart of dieback shoots. Found insects belong to families Anobiiadae and Scolytidae. It hasn't been investigated the possible relationship between fungus and insects occurrence. The research carried out in 1993 in the area No. 7 revealed that one group of the dieback shoots were attacked by *S. sapinea*, second group by mentioned insects, and third group by fungus and insects (Diminić & al. 1995).

SO_2 concentrations. Increased SO_2 concentration above certain level could damage the plant leaf surface and have influence on leaves physiology (Komlenović & Pezdirc 1987). According to Komlenović (1989) it could lead to increased transpiration, and together with precipitation deficit consequence will be drought stress. Results of research on sulphur concentration in Austrian pine needles on some localities in Istria and in Zagreb city (Komlenović 1990, Komlenović & al. 1990) were compared to results of *S. sapine* presence. Below of the critical concentrations were found on areas No. 1 and 3. Critical concentrations were found on areas No. 2, 4 and 5. Above the critical concentrations of sulphur in needles were found on areas No. 6 and 10 (Figure 1). Pines dieback were observed on localities No. 2, 5, 6 and 10.

Based on research results of the presence of only fungus virulent morphotype A on pines in Croatia, and analyses of aforementioned factors, it is supposed that one, more often two or more factors predisposed pine trees to *S. sapinea* outbreak on some localities.

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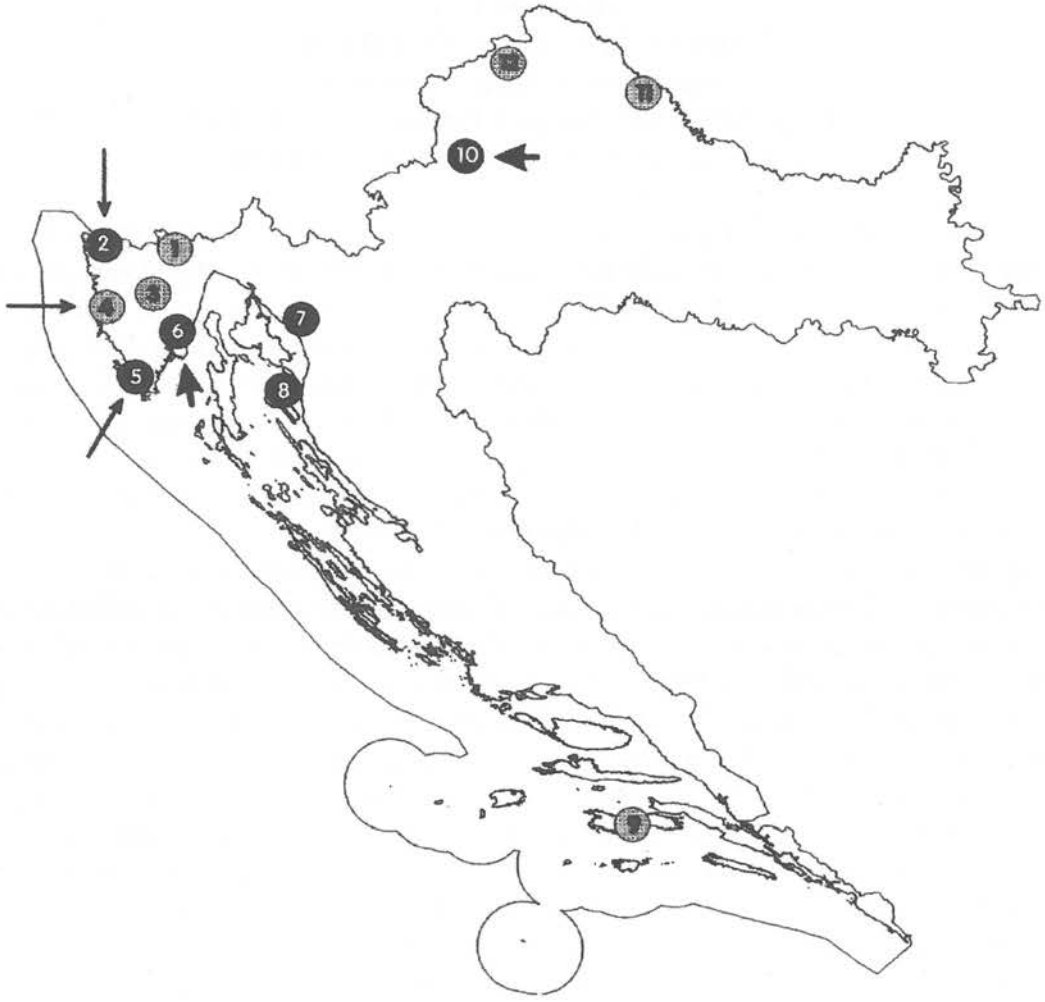
Fig. 1. Research localities in Croatia on which *Sphaeropsis sapinea* presence was established.



Legend:

Pine research area with no significant dieback symptoms

Pine research area with significant dieback symptoms observed on *P. nigra* and *P. heldreichii* var. *leucodermis* (area No. 10)

Pine species on research areas:
P. nigra - areas No.: 1, 2, 3, 7, 8
P. nigra, *P. halepensis* - area No. 5
P. nigra, *P. halepensis*, *P. brutia* - areas No.: 4, 6
P. nigra ssp. *dalmatica*, *P. halepensis* - area No. 9
P. nigra, *P. sylvestris* - area No. 11
P. nigra, *P. sylvestris*, *P. heldreichii* var. *leucodermis* - area No. 10
P. sylvestris - area No. 12

Critical sulphur concentration in Austrian pine needles

Above critical sulphur concentration in Austrian pine needles