Sphaeropsis tip blight disease of Austrian pine in urban greenery

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ABSTRACT: An extent of the damage of Austrian pine trees was evaluated in urban greenery in selected sites in Slovak Republic during the years 2004–2005. Fungi *Sphaeropsis sapinea* (Fr.: Fr.) Dyko & B. Sutton (syn. *Diplodia pinea* (Desm.) J. Kickx fil) and *Pestalotia* sp. were diagnosed on all observed trees. Symptoms of the disease were recorded. *S. sapinea* was isolated successfully from needles and from cone scales. The growth rate of hyphae of mycelium and the mean daily growth were evaluated on three types of cultivated media at different temperatures. The highest mean daily growth of mycelium was recorded on maltose agar at 25°C after 24 hours of cultivation (31.7 mm). Conidia of *S. sapinea* and *Pestalotia* sp. on water agar began to germinate after 3 and 2 hours, respectively. After 6 hours the mean germination rate of conidia of *S. sapinea* was 81.75% (from cone) and 89.3% (from needles); for *Pestalotia* it was 88.5%.

Keywords: *Sphaeropsis sapinea*; *Pinus nigra*; *Pestalotia*; Slovak Republic

Austrian pine (*Pinus nigra* Arnold) is the most cultivated introduced woody plant in Slovakia after poplar and acacia tree. The health problems of this tree have been constantly developing in the last years (Kunca et al. 2005).

Parasitic fungi *Dothistroma pini*, *Sphaeropsis sapinea* and *Cenangium ferruginosum* were noted on Austrian pine in forests of Slovakia (Kunca, Foffová 2000; Leontovyč 1962). These fungi were detected not only in our forests, but also in the neighbouring countries (Jankovský et al. 2002; Koltay 2001).

The aim of our work was to determine the cause of drying of Austrian pine in urban greenery in Slovakia and to study the biological questions of the appointed pathogen (*S. sapinea*) in the field and in laboratory conditions.

MATERIALS AND METHODS

Randomly selected damaged Austrian pine trees were examined in urban greenery in selected sites in Slovakia. Trees were examined during the years 2004–2005. Symptoms of the disease were evaluated and samples were taken from the affected parts of trees (cones, needles, shoots).

After the surface sterilization (0.15% NaClO) the pieces of infected host tissue were placed on cultivating media.

Three types of media were used for isolation of fungus: 3% maltose agar, 3% Czapek Dox agar, and 3% potato dextrose agar. The isolates were cultivated in dark, at three different temperatures and in constant pH and relative air humidity. Growth rate of hyphae of mycelium was measured after each 24 hours.

The size of spores was determined by 300 measurements of pycnospores under a binocular light microscope.

Germination of conidia of the fungi isolated from the host tissue was evaluated on 1% water agar at the temperature of 24°C and at constant air humidity. The material placed on agar disks on glass slide was examined using a light microscope Carl-Zeiss Jena. Dissolved cultivation media were poured on slides. After the hardening of the medium several drops of distilled water with fungal conidia were added onto the slide surface. The slides were placed in desiccators and evaluated regularly.

RESULTS AND DISCUSSION

Fungi *Sphaeropsis sapinea* (Fr.: Fr.) Dyko & B. Sutton and *Pestalotia* sp. were determined on all damaged Austrian pine trees that were observed. Fungus *S. sapinea* (syn. *Diplodia pinea* [Desm.] J. Kickx fil) caused fungal disease Sphaeropsis tip blight. The most visible symptoms of Sphaeropsis tip blight are stunted new

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Fig. 1. Trees that were repeatedly infected had dead older shoots and older needles. After several years of infection there were large dead branches in the crown

Fig. 2. Small black fruiting bodies (pycnidia) of the fungus *Sphaeropsis sapinea* were formed on the scales of second-year seed cones

shoots with short, tan needles. Tip blight primarily causes the death of new shoots growth in the spring, but subsequently entire new shoots die rapidly.

The damage was confined to the new shoots of trees that were infected for the first time. Trees infected repeatedly had also dead older shoots and older needles. Initially, dead shoots were evident in the lower part of the tree crown. After several years of infection large dead branches were throughout the whole crown (Fig. 1).

The newly developing shoots are most susceptible to the infection when buds open. The first symptoms are well visible in late May or early in June. Small, discoloured lesions appear on needles. As they grow, needles turn yellow and later tan. The infected needles are stunted and die prematurely.

Kunca et al. (2005) observed similar symptoms on Austrian pines in Slovakia. Tissues of infected shoots were permeated with resin and it formed small drops in the tissue surface.

The fungus also entered through the wounds in the bark of older shoots and branches, where it caused canker. The cankers were discoloured. Where the bark died, sunken and swollen areas appeared.

S. sapinea can infect young trees, but the disease is most severe on mature trees (30 years or older). The low amount of infection on young trees is attributed to the sparsity of their cones, which limits spore inoculum.

Trees that are not stressed are relatively resistant to the infection. In this case the fungus causes the death of first-year shoots and buds and second-year cones. The older branches are affected only if trees are predisposed to stress (lack of soil water, affected roots, excessive shade or extreme heat) (Kunca et al. 2005).

Small black fruiting bodies (pycnidia) were formed at the base of attacked needles from late summer. Pycnidia were formed also on the scales of second-year cones (Fig. 2) and they were much easier to find than those on needles. Pycnidia were globose, separate or grouped whereas conidia (Fig. 3) were dark, ovoid, elongate, 1-celled, mature 2-celled.

As a result of multiple measurements the size of conidia was following 33.2–41.2 × 16.6 µm. In Slovak

Fig. 3. Conidia of fungus *Sphaeropsis sapinea* are dark, ovoid, elongate, 1 – celled, mature 2 – celled

Fig. 4. Conidia of *Pestalotia* are dark, several-celled. End of cells are hyaline and pointed with two or more hyaline apical appendages

Table 1. Growth rate of mycelium of *Sphaeropsis sapinea* on different media within 96 hours at the temperature of 25°C

Medium	Minimum and maximum range of growth of mycelium (mm)			
	after 24 h	after 48 h	after 72 h	after 96 h
Maltose agar	30–37	60–66	75–85	85–85
Czapek Dox agar	21–27	32–40	42–63	45–72
Potato-dextrose agar	17–23	35–46	45–64	65–73

Table 2. Growth rate of mycelium of *Sphaeropsis sapinea* on different media within 96 hours at the temperature of 20°C

Medium	Minimum and maximum range of growth of mycelium (mm)			
	after 24 h	after 48 h	after 72 h	after 96 h
Maltose agar	21–28	47–55	70–75	77–85
Czapek Dox agar	19–23	35–45	50–60	55–67
Potato-dextrose agar	16–20	34–40	47–62	55–75

literature, no specific data on size of conidia were found; in foreign mycological and phytopathological literature the data are different. In Czech sources (Jankovský, Palovčíková 2003) the size of conidia is 25–40 × 15 µm; in Hungary 35–40 × 15–20 µm (Koltay 2001).

Beside *S. sapinea* another fungus from genus *Pestalotia* was detected on damaged needles. Acervuli were dark, discoid or cushion-shaped, subepidermal. Conidia (Fig. 4) are dark, several-celled. End of cells are hyaline and pointed with two or more hyaline apical appendages.

S. sapinea was isolated successfully from needles and from cone's scales. Growth rate of hyphae of mycelium and average daily growth were evaluated on three types of cultivated media: 3% maltose agar, 3% Czapek Dox agar, and 3% potato dextrose agar. They were cultivated at 25°C, 20°C and 10°C. The fastest growth rate of fungal mycelium was observed on maltose agar at the temperature of 25°C (Table 1, Fig. 5). The mycelial growth rate on Czapek Dox agar was slower, but symmetric (Fig. 5). The mycelial growth rate at the temperature of 20°C was slower than at 25°C on all three types of media (Table 2). At the beginning (after 24 h) the mean growth rate was the same on all media (Fig. 6). Later the hyphae grew faster on maltose agar; on Czapek Dox and potato-dextrose agar the growth was similar. Non-mycelial growth rate was observed at 10°C on all three types of media after 96 hours.

Mean daily growth of mycelium was calculated from their daily increase after each 24 hours. Daily growth of mycelium on maltose agar and Czapek Dox agar was highest at the beginning of cultivation and it decreased regularly after each 24 hours; on potato-dextrose agar it was highest after 48 hours and later it decreased regularly (Fig. 7).

The germination rate of the conidia of *S. sapinea* and *Pestalotia* sp. was evaluated. The germination of conidia of *S. sapinea* taken from scales of cone and from needles was 0% after 2 hours. After 3 hours the germination of conidia from cone was higher

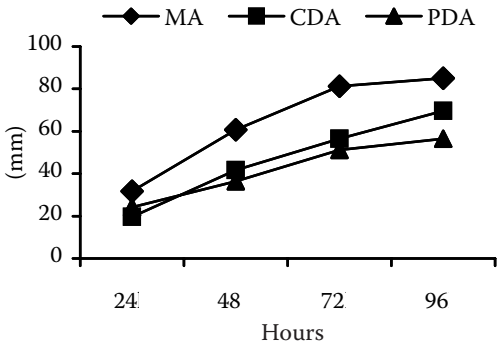

Fig. 5. Growth rates of mycelium of the fungus *Sphaeropsis sapinea* on different media at the temperature of 25°C (MA – maltose agar, CDA – Czapek Dox agar, PDA – potato-dextrose agar)

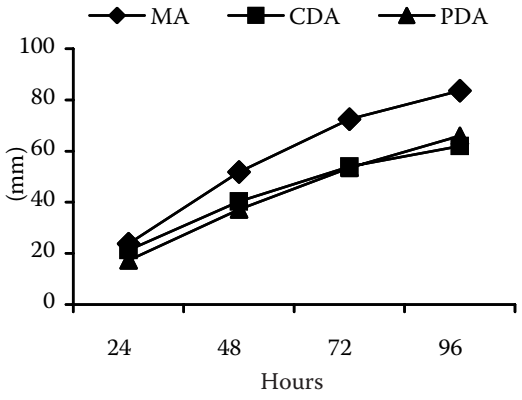

Fig. 6. Growth rates of mycelium of the fungus *Sphaeropsis sapinea* on different media at the temperature of 20°C (MA – maltose agar, CDA – Czapek Dox agar, PDA – potato-dextrose agar)

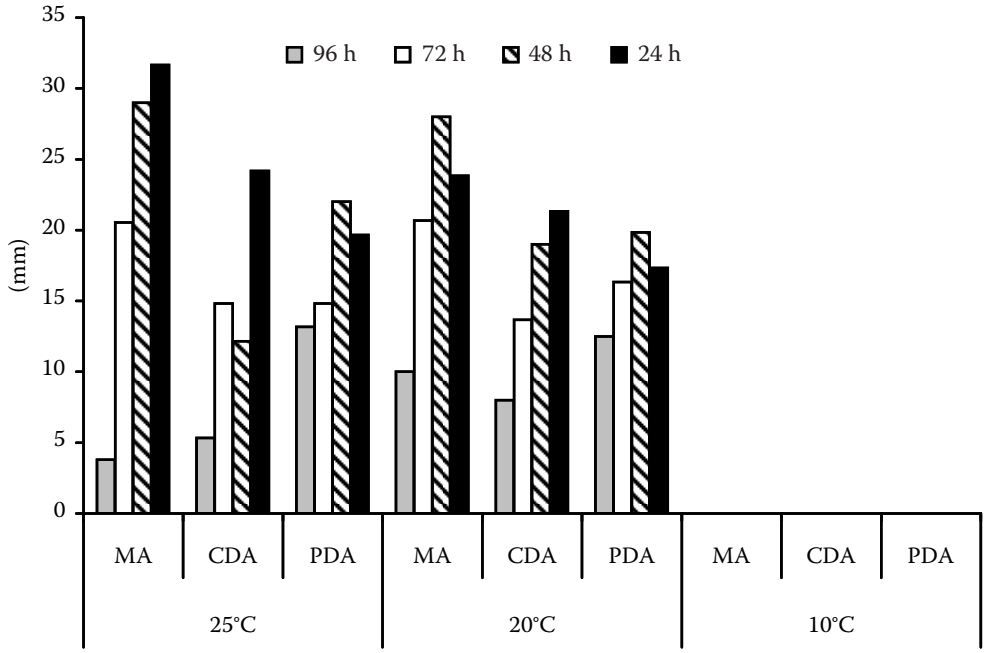

Fig. 7. Mean daily growth of mycelium of *Sphaeropsis sapinea* on different media at the temperature of 25°C, 20°C and 10°C (MA – maltose agar, CDA – Czapek Dox agar, PDA – potato-dextrose agar)

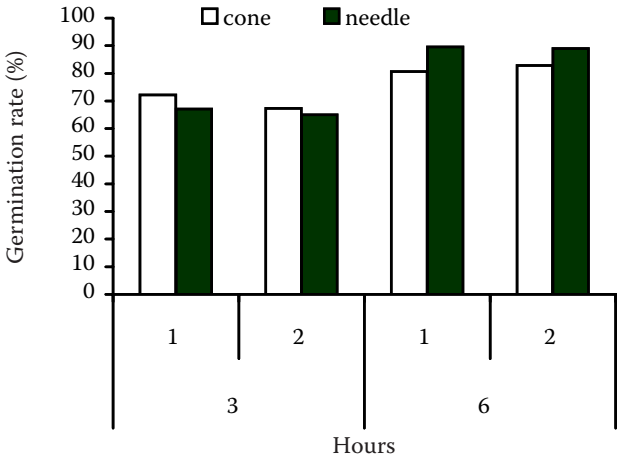

Fig. 8. Germination rate of conidia of *Sphaeropsis sapinea* taken from cone and needle on water agar

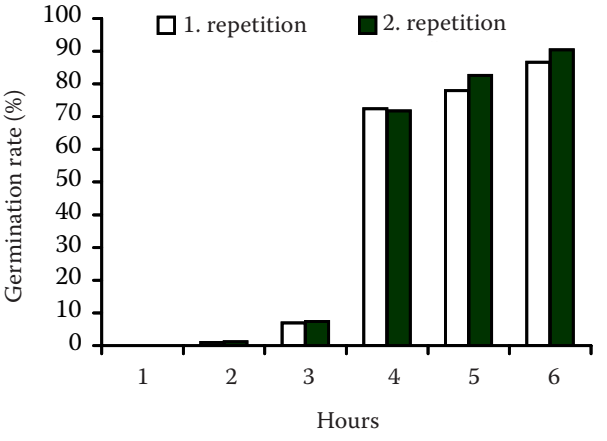

Fig. 9. Germination rate of conidia of *Pestalotia* sp. on water agar

by 3.7%; after 6 hours the germination of conidia taken from needle was higher by 7.55% (Fig. 8). The conidia of *Pestalotia* germinated after 2 hours but the germination rate was low. It increased markedly after 4 hours and then rose evenly (Fig. 9).

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Poškodenie borovice čiernej hubou *Sphaeropsis sapinea* v mestskom prostredí

ABSTRAKT: V rokoch 2004–2005 sme hodnotili poškodenie borovice čiernej v mestskom prostredí na vybraných lokalitách na Slovensku. Na všetkých sledovaných stromoch sme zaevidovali huby *Sphaeropsis sapinea* a *Pestalotia* sp. Zaznamenali sme symptómy ochorenia. *S. sapinea* sme úspešne izolovali z ihlíc aj zo šupín šišiek. Rýchlosť rastu hýf mycélia a priemerný denný prírastok sme hodnotili na troch druhoch kultivačných médií pri rôznej teplote. Najvyšší priemerný denný prírastok mycélia sme zaznamenali na sladinovom agare pri teplote 25 °C po 24 hodinách kultivácie (31,7 mm). Konídie húb na vodnom agare začali klíčiť po dvoch hodinách (*Pestalotia* sp.) a po troch hodinách (*S. sapinea*). Priemerná klíčivosť konídií huby *Sphaeropsis sapinea* po šiestich hodinách bola 81,75 % (zo šišiek), 89,3 % (z ihlíc) a u huby *Pestalotia* 88,5 %.

Kľúčové slová: *Sphaeropsis sapinea*; *Pinus nigra*; *Pestalotia;* Slovenská republika

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