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Comparison of the fungi *Pestalotiopsis funerea* (Desm.) Steyaert and *Truncatella hartigii* (Tubeuf) Steyaert isolated from some species of the genus *Pinus* L. in morphological characteristics of conidia and appendages

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ABSTRACT: The similarity of and differences between the fungi *Pestalotiopsis funerea* (Desmazières) Steyaert and *Truncatella hartigii* (Tubeuf) Steyaert that damage the needles of the host plants *Pinus ponderosa* Peter & Charles Lawson, *Pinus coulteri* D. Don and *Pinus nigra* Arnold planted in Mlyňany Arborétum, were evaluated with emphasis on comparison of the morphology of their conidia and appendages. Conidia of *P. funerea* were fusiform to ellipsoid, straight to slightly curved, 4-septate with 3 (4–6) apical sometimes branched appendages. The basal cell was short, hyaline, obtuse and thin-walled with basal, hyaline, filiform, straight, unbranched and single appendage. Conidia of *T. hartigii* were fusiform, 3-septate, without basal appendages. 2–3 apical appendages arising from the apical cell were attenuated, flexuous, sometimes dichotomously branched. The highest occurrence of the fungi *P. funerea* and *T. hartigii* indicates the influence of biotic stresses on properties of these pine trees.

Keywords: disease; *Pinus coulteri*; *Pinus nigra*; *Pinus ponderosa*; plant pathogens

Pestalotiopsis Steyaert is a species-rich genus occurring as pathogens, endophytes and saprobes (Jeewon et al. 2004; Kumar, Hyde 2004; Wei, Xu 2004) which include approximately 220 published names. Many of them were established based on slight morphological differences and host affiliation. *Pestalotiopsis* sp. is a fungus which plays several important roles in both protecting its host plant and in recycling nutrients in the ecosystems. Other fungal genera are *Truncatella* Steyaert, *Seiridium* Nees and *Monochaetia* (Saccardo) Allescher. Differentiation of these genera is primarily based on conidial septation and on the number of appendages on the conidium (Sutton 1980; Nag Raj 1993). Hu et al. (2007) found that conidial characters such as conidial length, median cell length, conidial width and colour of median cells were stable characters within *Pestalotiopsis*, however the length of the apical and basal appendages varied.

Pestalotiopsis species cause a variety of diseases in plants, including canker lesions, shoot dieback, leaf spots, leaf and stem blight, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and leaf spots (Espinoza et al. 2008; Zhang et al. 2012, 2013). *Truncatella* species are important and potential plant pathogens. They could be used for further investigation of their ability to reduce disease symptoms in healthy shrubs. In the genus *Pinus* Linnaeus *Truncatella* causes needle blight (Pitt, Hocking 1997).

The aim of this study was to isolate and identify the fungal species associated with the diseased pine species *Pinus ponderosa* Peter & Charles Lawson, *Pinus coulteri* D. Don and *Pinus nigra* Arnold dur-

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ing 2015 with emphasis on a comparison of the morphology of their conidia and appendages and to evaluate the relative tolerance of these species grown in many parks in Slovakia.

MATERIAL AND METHODS

From spring to autumn 2015 needles of *P. ponderosa*, *P. coulteri* and *P. nigra* with disease symptoms such as needle blight, tip blight and grey blight were collected at several locations from these trees growing in Mlyňany Arborétum. Altogether 10 trees were studied. The age of evaluated trees was between 25 and 40 years. An amount of 10 needles from each pine tree was used on our experiments. The samples of biological material were deposited in a herbarium at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plant Biology Nitra.

Cultivation on a nutritive 3% potato dextrose agar (PDA) medium in the test chamber with constant temperature and humidity (24 ± 1°C and 45% humidity in dark conditions in an MLR-351H versatile environmental test chamber (Sanyo Electric Co., Ltd., Osaca, Japan)) was used to isolate and obtain pure cultures. The needle parts cut from the diseased plants were surface-sterilized for 20 min. Study of fungal structures was performed with a light clinical microscope BX41 (Olympus, Tokyo, Japan) under 400× and 1,000× magnification. Measurements were done using the QuickPhoto Micro 2.2 programme (PROMICRA, s.r.o., Prague, Czech Republic) and the morphometric values were compared with previously published data for the taxa (Pitt, Hocking 1997; Lee et al. 2006; Hu et al. 2007). The fungi were identified based on their morphological characters (Steyaert 1949; Guba 1961; Nag Raj 1993) and according to keys (Dube, Bilgrami 1966; Sutton 1980).

RESULTS AND DISSCUSION

Fungi were isolated from the needle segments of three species of the genus *Pinus* (*P. ponderosa*, *P. coulteri* and *P. nigra*) and the species compositions of the fungi were compared among the pines examined. The dominant observed fungi were *Pestalotiopsis funerea* (Desmazières) Steyaert (*P. ponderosa*, *P. nigra*) and *Truncatella hartigii* (Tubeuf) Steyaert (*P. ponderosa*, *Pinus strobus* Linnaeus and *P. nigra*). The presence of other fungi such as *Chaetomium* Kunze, *Stachybotrys* Corda, *Acremonium* Link and *Ulocladium* Preuss was described in another article (Ivanová 2016).

According to Vujanovic et al. (2000) *T. hartigii* was more frequently associated with necrotic lesions on cones/seeds of the pine (*Pinus* spp.) collection in comparison with *P. funerea*. Pirone (1978) considered that the *Pestalotiopsis* species are weak or opportunistic pathogens and may cause little damage to ornamental plants. However, Hopkins and McQuilken (2000) pointed out that some *Pestalotiopsis* species may cause serious damage to pot grown plants and the number of known infected plant species is generally increasing.

They initially make a contact with the host probably by means of the conidia and may cause primary infections (Espinoza et al. 2008). The sources of the inoculum for secondary infections which increase the severity of the disease can be wild plantation flowers, crop debris, disease stock plants, used growing media, soil and contaminated nursery tools, splashed water droplets and also spores in the air.

Pestalotiopsis is just one of a complex group of fungi. This fungus is also considered as a weak pathogen (Madar et al. 1991), which penetrates the host through natural openings such as stomata, lenticels and hydathodes (Agrios 2005). Wright et al. (1998), Hopkins and McQuilken (2000) stated that *Pestalotiopsis* species infect only wounded or stressed plants which may be stressed due to insects, pesticides or sun damage. High rainfall, high temperature and human activities may lead to disease development (Tuset et al. 1999; Hopkins, McQuilken 2000; Elliott et al. 2004). Differentiation of these genera is primarily based on conidial septation and on the number of appendages on the conidium (Nag Raj 1993).

According to Hu et al. (2007) most species are divided into different groups based on the size of the

Table 1. Comparison of our results for the conidium size of the observed fungi with results of other authors

Pestalotiopsis funerea (Desmazières) Steyaert		
Our experiment	Pitt and Hocking (1997)	Hu et al. (2007)
16.8–20 (23)–(30) × 5 (10) µm	20–28 × 6–9 µm	18–26 × 5 (7)–8 (9.5) µm
Truncatella hartigii (Tubeuf) Steyaert		
Our experiment	Pitt and Hocking (1997)	Lee et al. (2006)
(20)–25 × (2.5)–3–(5)–(7.5) µm	15–20 × 6–8 µm	(16)–17–18–(20) × (6)–7–(8) µm

Table 2. Comparison of our results for apical and basal appendage occurrence in the observed fungi with results of other authors

Pestalotiopsis funerea (Desmazières) Steyaert							
Our experiment		Steyaert (1959)		Pitt and Hocking (1997)		Sousa et al. (2009)	
AA	BA	AA	BA	AA	BA	AA	BA
3 (4–6) simple or branched, (4–6) 15–20 (24)–30 µm long	1 single, 2–4 µm, unbranched	2–3 branched or unbranched	usually present	2 or more simple or branched	4 single, unbranched	3–4	1
Truncatella hartigii (Tubeuf) Steyaert							
Our experiment		Steyaert (1959)		Pitt and Hocking (1997)		Lee et al. (2006)	
AA	BA	AA	BA	AA	BA	AA	BA
2–3, branched, 5–10 (15)–21–30 × 1 µm	–	1–4, branched, unbranched	–	variable in number, branched	–	2–4 (5), often dichotomously branched, 26–31 × 1 µm	–

AA – apical appendages, BA – basal appendages

conidia. Conidial morphology is the most widely used taxonomic character for the genus *Pestalotiopsis.* The length and the number of the apical appendages are also widely used characters for species identification. The apical appendages often divided into branches can arise from the top, middle, bottom or different positions in the apical hyaline cells. In some species presence or absence of the basal appendages is another character for species diagnosis. In our experiment the size of the conidia (Table 1) and the length, branching and number of the apical and basal appendages (Table 2) were compared with results obtained by other authors.

Conidiomata of the genus are variable, ranging from acervuli to pycnidia, they can often be immersed (Nag Raj 1993). Conidiophores develop partly or entirely inside the conidiomata, often reduced to conidiogenous cells which are discrete or integrated, cylindrical, smooth, colourless and invested in mucus. Pycnidia can mostly be seen as black or brown spore masses with copious conidia (Nag Raj 1993). *Pestalotiopsis* may produce large numbers of dry spores easily dispersed in the air or by water splash.

According to our experiments with infected plants of *P. ponderosa* and *P. nigra* with lesions 2–3 mm in diameter, we obtained pure cultures of *P. funerea* on PDA medium which exhibit the pinkish mycelium containing conidiophores produced within compact fruiting structures. Spore masses contained conidia, which are fusiform to ellipsoid, straight to slightly curved, 16.8–20 (23–30) × 5 (10) µm, 4-septate, slightly constricted at septa, with the three central cells dark brown and with a short basal cell which is obtuse, hyaline, thin-walled and verruculose, 3–4 (5) µm long. Three median cells are thick-walled, dark and

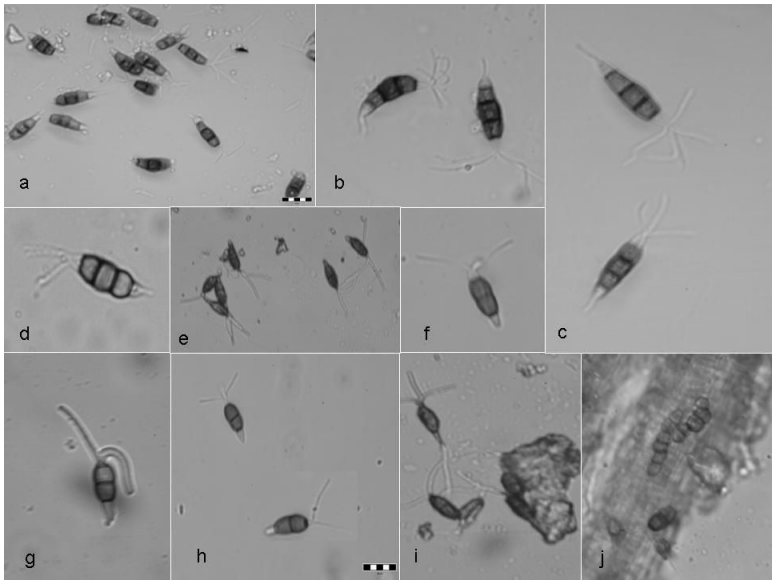

Fig. 1. Comparison of different conidium size and shape of the observed fungi: *Pestalotiopsis funerea* (Desmazières) Steyaert (a–d), *Truncatella hartigii* (Tubeuf) Steyaert (e–j), scale bars: 20 µm (a, h)

sometimes roughened, subcylindrical, olivaceous brown, with septa and periclinal walls darker than the rest of the cell, together 10.8–20 (25) µm long. The second cell from the base is 2.6–2.8–4 (5) µm; third cell 5–8 (10) µm; fourth cell 4.8–5 (6) µm; apical cell hyaline, conic, 2.7–2.8 (4) µm long, with 3 (4–6) apical sometimes branched appendages arising from the apex of the apical cell collecting in a wet mass outside the acervulus, (4–6) 15–20 (24)–30 µm long. Basal appendage is hyaline, straight, filiform, unbranched and single, 2–4 µm long (Fig. 1).

The identification of *Pestalotiopsis* species has mainly been based on the degree of pigmentation of median cells, apical appendage tip morphology (presence or absence of spatula), apical appendage length and spore length and width and basal appendage length (Jeewon et al. 2003). According to Hu et al. (2007) median pigmented cells are concolourous, olivaceous and pale brown with apical appendages that are not knobbed. According to Pitt and Hocking (1997) colonies of this fungus were growing rapidly, the mycelium was usually white, sometimes off-white to pale brown. Conidia produced in flat, black acervuli, opening irregularly at maturity, filled with a dense layer of conidia, which are fusiform, five celled. The central 3 cells brown, 15–20 µm long, the apical and basal cells hyaline, the basal one with a single usually unbranched spike-like appendage and the apical one with two or more simple or branched spiky appendages. According to Steyaert (1949) conidia are usually fusiform, straight or slightly curved, and 3–4 septate. The 3 median cells are pigmented (either concolourous or versicolorous). Apical appendages are mostly filiform, occasionally knobbed, one to many (mostly 2–3), branched or unbranched, and arise from the apical cell. Basal appendages are usually present, and arise from the basal cell. Sousa et al. (2004) from infected plants of *Hakea sericea* Schrader & J.C. Wendland exhibiting reddish leaves bearing black circular lesions of 1–3 mm in diameter obtained pure cultures of *P. funerea* on PDA medium which exhibit the pinkish mycelium bearing compact acervuli containing black and slimy spore masses. The fungus has 5-celled spores (3 colourless median and 2 hyaline end cells) with 3–4 apical and 1 basal appendage.

Truncatella hartigii (syn. *Pestalotia hartigii* Tubeuf) has been associated with damage to many coniferous species (*Abies* spp., *Picea* spp., *Pinus* spp.) where it shows dieback symptoms (Vujanovic et al. 2000). The fungus was introduced by Steyaert (1949) to accommodate five former *Pestalotia* species having 3-septate conidia with 1–4-branched or unbranched apical appendages. In the study of Jeewon et al. (2002) a comparison of rDNA sequence data revealed that isolates with 3-septate conidia cluster in the *Truncatella* clade, distant from those of the *Pestalotiopsis* clade with 4-septate conidia. Results of Lee et al. (2006) support this opinion, and agree with Steyaert's original concept of the genus, that *Truncatella* should be restricted to fungi with 3-septate conidia. More than 80% of the currently known *Pestalotiopsis* species have 4-septate conidia, whereas only around 34 species (15%) have 3-septate conidia, and thus belong to *Truncatella*. During research on pine needles Kattai (2012) confirmed the occurrence of *T. hartigii* as a new and quarantine disease – brown spot needle blight in Estonia. Karadzič and Milijaševič (2008) included the fungus *T. hartigii* which occurs on old needles among the most important parasitic and saprophytic fungi in Austrian pine and Scots pine plantations in Serbia. *T. hartigii* has been reported on *Pinus massoniana* Lambert in China (Farr, Rossman 2009).

Scattered or gregarious pycnidial conidiomata in our experiments are subepidermal, often remaining immersed, 130 × (80)–150 µm in size. Conidiophores and conidiogenous cells are hyaline, smooth, cylindrical, 20 × 2 µm. Conidia fusiform, (20)–25 × (2.5)–3–(5)–(7.5) µm, 3-septate; apical cells conical to trapezoid, hyaline, 5–6.5 (7.5)–(10) × 3–5 µm, smooth, thin-walled; median cells brown, 13–15 × 7.5 µm, thick-walled; basal cells hyaline, obconical, 5 × 2.5 µm wide, without basal appendages. 2–3 apical appendages are inserted in the topmost part of the apical cell and arise at the same point. The appendages are attenuated, flexuous, 5–10 (15)–21–30 × 1 µm, sometimes dichotomously branched (Fig. 1e–j). Based on conidial dimensions and the branching pattern of apical appendages, our collections are best accommodated in *T. hartigii*.

According to Lee et al. (2006) *T. hartigii* isolated on *Abies alba* Miller has conidiomata pycnidioid, visible at the surface by dark exuding conidial masses; 106–156 × (73)–124–177 µm. Conidiophores branched at the base are cylindrical, 0–4-septate, 11–25 × 2–3 µm. Conidiogenous cells hyaline, smooth, cylindrical, 6–19 × 2 µm. Conidia fusiform, 3-septate; conical apical cells 2.5–3 × 2.5–4 µm, echinulate brown median cells, 13–14 × 7 µm, basal cells hyaline, 2–3 × 2–3 µm wide. Apical appendages inserted in the topmost part of the apical cell are flexuous and attenuated. Obvious difference between different taxa is in the branching

patterns of their apical appendages (Guba 1961; Nag Raj 1993). *Truncatella hartigii* has more than one apical appendage, often irregularly or dichotomously branched, in most cases it has two equal branches that branch dichotomously again (Lee et al. 2006).

In the case of *Truncatella* and *Pestalotiopsis* the fructification is acervular or pycnidial and the conidia are produced in subcutaneous receptacles. Ostiolation of the epidermis occurs only when the conidia are released. In *Pestalotiopsis* the number of coloured cells is three and the endogenous appendage is simple or branched, whereas, in the case of *Truncatella* the number of coloured cells is two and the endogenous appendage is unbranched (Steyaert 1955).

The fungus *Truncatella* is characterized by the formation of black acervuli containing relatively large fusiform conidia with three or four transverse septa and spiky appendages from one or both ends. The fungus produces colonies similar to those of *Pestalotiopsis* and produces similar conidiomata. Conidia are fusiform with tree septa. The two median cells are brown and 11–14 μm long. Apical and basal cells are hyaline (Pitt, Hocking 1997).

Some endophytic fungi are actually latent pathogens or saprotrophs that become active and reproduce only under specific environmental conditions or when their host plants are stressed or begin to senesce (Petrini 1991; Nagabhyru et al. 2013). The greatest occurrence of the fungi *P. funerea* and *T. hartigii* in our experiments indicates the potential influence of biotic stresses on properties of evaluated pine trees.

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