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Coniochaeta prunicola – causal factor involved in health state decline of selected trees of the genus *Prunus*

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Abstract

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The record of *Coniochaeta prunicola* Damm & Crous (Coniochaetales, Sordariomycetes, Ascomycota) as a pathogen of host trees was described and illustrated from Nitra. This pathogen was isolated from symptomatic twigs and leaves of *Prunus laurocerasus* L. as well as from symptomatic leaves of *Prunus persica* Mill. and based on morphological attributes identified as a causative agent of this trees damage. *C. prunicola* is characterized by dark brown ascomata clothed with setae, the fasciculate, unitunicate, cylindrical asci and broadly almond-shaped, ellipsoidal ascospores with a longitudinal germ slit.

Keywords

Ascomycota, *Coniochaeta prunicola*, morphological attributes, *Prunus laurocerasus*, *Prunus persica*, Sordariomycetes

Introduction

Prunus laurocerasus L. (syn. *Laurocerasus officinalis* L.), evergreen shrub or a small tree in Rosaceae family, was frequently planted as an ornamental plant in temperate regions worldwide. It is often used as a mass landscape and ground cover plant in urban green areas.

Prunus persica L. (Batsch.) (syn. *Persica vulgaris* Mill.) is a deciduous tree, native to China, where it was first cultivated. It bears an edible juicy fruit called a peach. The species name of persica refers to its widespread cultivation in Persia, whence it was transplanted to Europe. It belongs to the family of Rosaceae.

These shrubs and trees are susceptible to various pathogens, which caused discoloration, brown spots, blight symptoms and necroses, affecting their aesthetic value. The symptoms of infection, which are observable from spring to autumn, increase when the plants are in bloom, resulting in dieback and leaf drop. The damage is caused by fungus *Coniochaeta prunicola* Damm & Crous.

The genus *Coniochaeta* (anamorph: *Lecythophora*) includes ascomycetous fungi known as pathogens of woody plants, but some species can also cause human infections. *Coniochaeta* contains more than 80 species occurring mostly on wood and bark, leaves and leaf litter of different trees, in dung of various animals, and in soil and water. Species of the genus *Coniochaeta* and their *Lecythophora* anamorphs occur on different plant material (on wood or bark of different trees, on leaves and leaf litter), in dung of various animals, in soil and in water with extremely low pH and high concentrations of heavy metals (Eriksson, 1992; Kamyia et al., 1995; López-Archilla et al., 2006; Asgari et al., 2007). Some *Coniochaeta* species have been found to exhibit useful biochemical properties. Species of *Coniochaeta* have been isolated from different plant parts of the representative genus *Prunus*.

During an investigation on mycoflora of cherry laurel trees and peach trees growing in urbanized area, besides the fungi of the classes Hyphomycetes and Coelomycetes isolated from affected cherry laurels (Bernadovičová and Ivanová, 2011), the ascomycetous fungus *Coniochaeta prunicola* (Coniochaetaceae, Coniochaetales) that affects leaves and twigs of the host trees was noticed. Although the incidence of disease was sporadic, the infected trees showed relatively severe damage.

This study aims for identification based on morphological attributes which the microscopic fungus iso-

lated from symptomatic cherry laurel and peach trees in connection with the new disease noticed recently, and to describe the distinctive morphological features for the isolated *Coniochaeta* species as a causal factor involved in health state decline and vitality weakening of *Prunus laurocerasus* and *Prunus persica*.

Material and methods

From spring to autumn 2009–2011, leaves and twigs of *Prunus laurocerasus* and leaves of *Prunus persica* (Redhaven) with blight symptoms were sampled from plants growing in private gardens and public greenery of the town of Nitra. The material was collected at several locations from the diseased *Prunus* trees in the areas of Nitra - Chrenová and Nitra - Zobor. Altogether 25 trees were studied (17 trees of *Prunus laurocerasus*, 8 trees of *Prunus persica*). The age of evaluated trees was between 15–35 years. The samples of biological material were deposed in herbarium at the Institute of Forest Ecology of the Slovak Academy of Sciences, Branch for Woody Plant Biology in Nitra.

Classical phytopathological methods – cultivation on nutritive medium in test chamber with constant temperature and humidity were used to isolate and obtain pure cultures. The leaf and twig parts cut from the diseased plants were surface-sterilized by immersion in sodium hypochlorite solution (1% available chlorine) for 20 minutes, rinsed twice or three times in sterile distilled water and then dried carefully with filter paper. After that, the plant samples were cut to fragments of 3–5 mm which were placed on 3% potato-dextrose agar (PDA) in Petri dishes. This was followed by cultivation at 24 ± 1 °C and 45% humidity in dark conditions in a versatile environmental test chamber MLR-351H (Sanyo) and subsequent isolation on the 3% PDA medium. Pure fungal cultures were obtained by using multiple purifications. The obtained isolates were transferred on 3% PDA medium to induce sporulation. Study of fungal structures was performed with a clinical microscope BX41 (Olympus) under a 400× and 1,000× magnification.

The isolated fungus was identified by microscopic analyses based on morphological characteristics of the fruiting bodies (perithecia), spore bearing organs (asci) and reproduction organs (conidia and ascospores). The identification was performed using morphological keys assembled by Hawksworth and Yip (1981), Ellis and Ellis (1987), Checa et al. (1988), Romero et al. (1999), Asgari et al. (2007) and morphological studies in Mahoney and LaFavre (1981), Hanlin (1990), Weber (2002) and Damm et al. (2010).

Results and discussion

Concerning all morphological characteristics and determined differences, the fungus under investigation in our study isolated from branches showing necrosis symptoms and blighted leaves of cherry laurel trees and from blighted leaves of peach trees was identified as *Coniochaeta prunicola*.

Anatomical-morphologically characteristics of fungus *Coniochaeta prunicola* Damm & Crous on *P. laurocerasus* and *P. persica* are in Table 1.

Review of the literature shows that although the characteristics of asci and ascospores are very important, setae are still the prominent feature of the most *Coniochaeta* species. Most of the described setae are dark brown to black rigid hairs, straight or bent, unbranched with a sharp apex. They may be scattered over the perithecial wall or concentrated in its upper portion (Mahoney and LaFavre, 1981). Some species are described as lacking setae (Romero et al., 1999). According to Damm et al. (2010) fungus *C. prunicola* isolated from branches of stone fruit (*Prunus* sp.) produced subglobose to pyriform ascomata, 200–250 µm in diameter, neck 50–60 µm long. Peridium was pseudoparenchymatous, 20–25 µm (5–8 layers), outer wall consists of dark brown textura angularis, with setae. Setae were brown (or hyaline), straight, cylindrical, tapering to a round tip, smooth-walled or granulate, 2–3.5 µm wide, up to 80 µm long. Results of our study are in Table 1.

The key provided in Asgari et al. (2007) leads our results to *Coniochaeta velutina*, except that the ascospores of this species have guttules, and isolates of *Coniochaeta prunicola* produce larger ascospores compared to *Coniochaeta velutina*. These ascospore features correspond to those provided by Munk (1957), where isolates from *Prunus* sp. produced ascospores 6–8 × 4–6 × 3–4 µm or 9–10.5 (12.5) × 5 (7.5) µm in size (Ivanová, not published yet) and by description in Damm et al. (2010) and another authors. The other species (*Coniochaetidium* sp., *Ephemeroascus* sp. and *Poroconiochaeta* sp.) transferred into *Coniochaeta* by García et al. (2006) differed from *Coniochaeta prunicola* by displaying ornamental ascospore walls, or by lacking *Lecythophora* anamorphs. Most of the *Coniochaeta* species exhibit different ascospore sizes: *Coniochaeta leucoplaca* (Berk. & Ravenel) 7–10 × 5–9 × 4–8 µm and *Coniolariella ershadii* (Zare, Asgari & W. Gams) Zare, Asgari & W. Gams (basionym *Coniochaeta ershadii* Zare, Asgari & W. Gams) 16 × 18 × 9.5–10 µm isolated from twigs of *Pistacia vera* L. (Asgari et al., 2007; Zare et al., 2010), *Coniolariella gamsii* (Asgari & Zare) Dania García, Stchigel & Guarro (basionym *Coniochaeta gamsii* Asgari & Zare) 16–19 × 6–11 µm isolated from leaves of *Hordeum vulgare* L. (Zare et al., 2010; Asgari and Zare, 2006), *Coniochaeta ligniaria* (Grev.) Massee 9–20 × 8–15 × 4–8 µm (Mahoney and LaFavre, 1981), *Coniochaeta rhapalochaeta* sp. nov. (Romero & Carmarán) 10–14 × 7.5–9 × 5–6 µm isolated from wood of *Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb. (Romero et al., 1999), *Coniochaeta prunicola* Damm & Crous 9–10.5 (12.5)

Table 1. Comparison of morphological characteristics of *Coniochaeta prunicola* Damm & Crous identified in genus *Prunus*

Host plant	*Prunus persica*	*Prunus laurocerasus*
Plant part	Leaves	Twigs, leaves
Causal agent	*C. prunicola*	*C. prunicola*
Ascomata	Perithecial, solitary, subglobose to pyriform, 125–173 (265) × 95–145 (229) µm, with a central ostiole, neck 31–42 µm	Perithecial, solitary, 162–221 × 119–159 µm, subglobose to pyriform, with a central ostiole, neck 38–42 µm
Setae	Hyaline or brown setae, smooth walled, 3–4.5 × 21–29 µm	Hyaline or brown setae, smooth walled, 3–4.5 × 35–51 µm
Paraphyses	Hyaline, septate, 63 × 3–4 µm	Hyaline, septate, 74–78 × 3–4 µm
Asci	Fasciculate, unitunicate, cylindrical with truncate apex, obtuse end, small apical ring 4–5 µm long, 8 ascospores/ ascus, 58–68 (94) × 8–10 µm	Cylindrical, unitunicate with obtuse end, with a small apical rings 4–5 µm, 8 ascospores/ ascus, 68–81 × 8–10 µm
Ascospores	Uniseriate, 1-celled, ellipsoidal, smooth-walled without ornamentation wall, green to brown with granular contents, 9 (10)–12 × 5 (6) µm, longitudinal germ slit 5 × 8 µm	Uniseriate, 1-celled, ellipsoidal to almond shaped, brown, smooth-walled with granular content, 9(10–)13 × (5–)6–7(–8) µm, without ornamentation of the ascospore wall, longitudinal germ slit 7 × 6 µm
Guttules	Absent	Absent
Hyphae	–	Hyaline, 2–3 µm wide
Conidia	Hyaline, 1-celled, smooth walled, cylindrical to ovoid, (2–)3–6(–7) × 1–2 µm	Hyaline, 1-celled, smooth walled, cylindrical to ovoid, sometimes allantoid (2–)3–4(–7) × 1–2 µm formed on hyphal coil
Conidiophores	Directly on hyphae	Directly on hyphae
Colarette	Distinct, cylindrical, 2–3 µm long	Inconspicuous
Colonies on PDA	Pale saffron, pale buff to white, flat, with sparse aerial mycelium	Pale buff to white, flat, with sparse aerial mycelium
Chlamydosp.	Lacking	Lacking

× 5 (7.5) µm isolated from leaves of *Prunus domestica* (Ivanová, not published yet).

Causal organism was systematically isolated from leaf and twig tissue showing rusty to brown coloured blight symptoms. Growth on PDA was slow. Colonies appeared white at first, than turned on pale buff to white or pale saffron. Conidia were produced in great numbers in culture media. Perithecia developed on PDA after about 4–5 (*P. laurocerasus*) or 8–10 (*P. persica*) weeks. Cultures of *Coniochaeta prunicola* do not turn dark as *Coniochaeta velutina* cultures (Weber, 2002; Damm et al., 2010). This fact was also confirmed in our study with isolates of fungus *C. prunicola* from peach trees (Ivanová and Bernadovičová, 2012) and cherry laurel shrubs (Ivanová and Bernadovičová, 2013), (Table 1).

In anamorph stage of *Coniochaeta velutina* described from various tree and shrub hosts in *Lecythophora* genus, sizes of conidia obtained from pure cultures varied: 3–6 × 2–4 µm (Taylor, 1970), 2.5–3.5 × 1.5–2 µm (Udagawa and Horie, 1982), 2–4 × 1–2.5 µm (Hutchinson and Reid, 1988), and 3–8 µm long (Kirschner, 1998). According to Damm et al. (2010), the anamorph of *Coniochaeta prunicola* is also similar to that of *Coniochaeta velutina*, but the collarettes in the latter are shorter, up to 1 µm in length, and the conidia are wider and not regularly allantoid. This fact has also been confirmed in our study (Table 1).

The fungus *Coniochaeta prunicola* was found in the examined samples relatively uncommonly. Our studies and morphological identification have shown that *Coniochaeta prunicola* was a new pathogenic fungus associated with affected branches and leaves of *P. persica* and *P. laurocerasus* in Slovakia. This preliminary identification, however, needs using methods of molecular biology for confirmation, since the morphological characteristics alone may not be fully reliable for this purpose. Further studies are required for determination of pathogenicity and relevance of *Coniochaeta* infection in connection with peach trees and cherry laurel damage. The planned molecular analysis based on large subunit nuclear ribosomal DNA sequences is required for detailed study of the discussed pathogens.

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