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CHLAMYDOSPORE-PRODUCING SPECIES OF PHOMA FROM CONIFER SEEDLINGS IN PACIFIC NORTHWEST FOREST TREE NURSERIES

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Abstract: Three species of Phoma that produce chlamydospores in culture are frequently isolated from diseased conifer seedlings from several Pacific Northwest nurseries. These include P. eupyrena, P. glomerata, and P. pomorum. Major differentiating characteristics of these species include abundance and morphology of chlamydospores, growth habit in culture, conidial exudate color, and conidial pigmentation, shape, septation and size. Cultural characteristics of these three Phoma species and a brief description of their habit in nature are discussed.

INTRODUCTION

The genus Phoma encompasses a wide range of fungi commonly isolated from plants and animals. Major taxonomic criteria of the genus include production of individual pycnidia (not aggregated within a stroma) which exude large numbers of mostly one-celled, ellipsoidal to cylindrical hyaline conidia produced in a slimy matrix (19,49,50). Because of the diversity of members of this genus, species classifications have been difficult and made often on the basis of slight morphological differences and host substrates (50). This has resulted in descriptions of more than 2,000 species of Phoma. However, these descriptions have often not reflected fundamental relationships among taxa nor are they of practical value to mycologists or pathologists. Therefore, G. H. Boerema and his coworkers developed a system for identification and naming species of Phoma based on in vitro behavior of isolates. Their system compared criteria such as colony habit, pigmentation, chlamydospore formation, crystal formation, features of the pycnidia, conidia and conidiogenous cells, and related these to described taxa (7,20). Unfortunately, gross cultural characteristics for many taxa may be extremely variable and dependent on the nature and carbon-nitrogen ratio of the medium and light regimes for growth (8,20). To help reduce variability, standard growing regimes have been adopted for taxonomic studies of Phoma-like fungi. Using standard techniques, key characters for differentiating

taxa are relatively stable (50).

In the course of investigation causes of above-ground damage to conifer seedlings in nurseries of the Pacific Northwest, species of Phoma were frequently isolated from necrotic tissues. Isolates originated from dead needles and tops of seedling as well as from dead branches or stem cankers. In many cases, Phoma spp. were the most consistently isolated group of fungi. Many isolates from diseased seedlings produced distinct chlamydospores and olivaceous to black mycelia in culture.

The purpose of this paper is to describe the three chlamydospore-producing species of Phoma most commonly isolated from diseased seedlings in Pacific Northwest nurseries. Stable criteria used for taxonomic differentiation of the species will be emphasized; a brief description of their habits in nature is included.

MATERIALS AND METHODS

Isolates of Phoma were obtained from surface-sterilized plant tissues planted on either 2 percent water agar or potato dextrose agar (PDA) containing 200mg/ml streptomycin; cultures were maintained for varying time periods on PDA at 20-22° C prior to growth tests. Standard conditions for determining growth rates included growth on oatmeal agar (OA) and malt agar (MA) or PDA at 20-22° C for 7 days in the dark. Colony diameter was then measured before growing cultures for another 7 days at the identical temperature under periods of alternating 12 hrs. near ultraviolet (black) light. This light regime stimulates pycnidial and pigment formation (50). OA promotes production of chlamydospores and crystals (20). Major criteria for differentiating chlamydospore-producing species of Phoma included abundance and morphology of chlamydospores, conidial dimensions (always obtained from OA cultures since those produced on MA or PDA are sometimes swollen and atypical) and pigmentation, and colony coloring, floccosity, and sectoring.

DESCRIPTION OF SPECIES

The following key, adapted from several workers (9,19,20), was used to differentiate species of chlamydospore-producing Phoma frequently isolated from diseased seedlings:

1. Chlamydospores only single-celled, often loosely catenulate, with longitudinal septa ---------- P. eupyrena

1. Chlamydospores both single-celled, and multi-celled; multi-celled spores with both longitudinal and traverse septa ----------2.

2. Catenulate dictyochlamydospores in chains of 2-20 elements that resemble conidial chains of Alternaria; fresh isolates with little aerial mycelia ---------P. glomerata

2. Dictyochlamydospores usually terminal on hyphal branches, resembling conidia of Stemphylium; also produces single-celled chlamydospores abundantly in long chains; colonies mostly with abundant olivaceous aerial mycelium ----------P. pomorum

Phoma eupyrena Sacc., Michelia I (5):525. 1879.

Colonies moderately to fast growing, reaching 5.5-5.9 cm in diameter in 7 days. Colonies with cream-colored to white floccose mycelium that becomes olivaceous-grey and eventually black with age. Mature cultures appear black at the center, becoming olivaceous-grey towards the margin. Pycnidia readily formed on both OA and PDA by some isolates; others did not produce pycnidia on PDA. Pycnidia either separate or aggregated to form multi-ostiolar fructifications; produced either superficially on or immersed within agar. Small pycnidia produced within the aerial mycelium of some isolates, especially concentrated in colony centers. Conidial exudate creamy white in color. Individual conidia ellipsoidal to bacilliform, hyaline, non-septate, and biguttulate. Conidia measuring 3.5-6.0 x 1.5-3.5 um. Catenulate single-celled chlamydospores produced by all isolates. Chlamydospores globose-spherical or slightly elongate, measuring 8-12 x 5-10 um. Abundance of chlamydospores varied among isolates; often not formed untill cultures were 14 or more days old.

MATERIAL EXAMINED: Idaho: James, R. L., #80-42, isolated from Picea engelmanni needles; Montana: James, R. L., #82-56a, isolated from Eleagnus angustifolia stem cankers; California: Kliejunas, J. T., "Bayleton Bed" (James, R.L., #83-12), isolated from Abies magnifica seedlings; Idaho: James, R. L., #83-24B, isolated from Pseudotsuga menziesii stem tips; Idaho: James, R. L., #83-27-C1, isolated from Pinus ponderosa needles; Idaho: James, R. L., #83-56, isolated from Picea engelmanni stem tips; Idaho: James, R. L., #83-72, isolated from Pinus mugo needles; Idaho: James, R. L., #83-79, isolated from Pinus ponderosa stem

tips; Oregon: Hamm, P. B., #19-35 (James, R. L., #84-1), isolated from Pseudotsuga menziesii midstem canker; Oregon: Hamm, P. B., #19-36A (James, R.L., #84-4), isolated from Pseudotsuga menziesii midstem canker; Oregon: Cooley, S. J., #P1-7 (James, R. L., #84-8), isolated from Pseudotsuga menziesii midstem canker; Washington: Cooley, S. J., #P1-7-1 (James, R. L., #84-10), isolated from Pseudotsuga menziesii midstem canker; Washington: Hamm, P. B., #5-PT (James, R. L., #84-96), isolated from Pseudotsuga menziesii midstem canker; Oregon: Hamm, P. B., #24-15 (James, R. L., #84-98), isolated from Pseudotsuga menziesii midstem canker; Washington: Hamm, P. B., #23-11 (James, R. L., #84-103), isolated from Pseudotsuga menziesii stem tip.

NOTES: Taxonomic schemes of Boerema and coworkers (8,9,20,50) differentiate Phoma species producing chlamydospores on the basis of chlamydospore size and morphology, conidial size and septation, and production of crystals in culture. Isolates that produce occasional dictyochlamydospores as well as abundant catenulate chlamydospores would be within the sub-group Peyronellaea and possibly the species P. pomorum, depending on dictyochlamydospore morphology and production habit. However, production of dictyochlamydospores may be inconsistent in certain isolates (9,12), and isolates of P. eupyrena may sometimes be confused with P. pomorum. Species of Phoma with chains of single chlamydospores might also be classified as P. medicaginis Malb. & Roum., especially if the chlamydospores are globose, and occasional two-celled conidia and crystals (on MA) are produced (19,20). However, none of the isolates classified here as P. eupyrena produced crystals or two-celled conidia.

Phoma glomerata (Cda.) Wollenw. & Hochapf., Z. ParasitKde 8:592. 1936.

= Alternaria polymorpha Planchon, Annls. Sci. nat. (Bot.), ser. 8, 11:48-89. 1900.

= Coniothyrium glomeratum Cda., Ic. Fung. 4:39. 1840.

= Peyronellaea glomerata (Cda.) Goid., Rc. Accad. Lincei I:455,658. 1946.

= Phoma alternariceum Brooks & Searle, Trans. Brit. mycol. Soc. 7:193. 1921.

= Phoma conidiogena Schnegg, Zenlbl. Bakt. ParasitKde (Abt. 2) 43:326-364. 1915.

= Phoma hominis Agnostini & Tredici, Atti Ist. bot. Univ. Lab. crittog. Pavia, ser. 4, 6:154. 1935.

= Phoma radicis-vaccinii Ternetz, Jb. wiss. Bot. 46:366-367. 1907.

= Phoma saprophytica Eveleigh, Trans. Brit. mycol. Soc. 44:582-583. 1961.

Most colonies moderately to fast growing, reaching 5.2-7.7 cm in diameter in 7 days. Some colonies very slow growing, reaching only 2.1-3.6 cm in diameter. Little aerial mycelium produced; that which was produced consisted of tufts of olivaceous or white hyphae often sectored within PDA colonies. Appressed mycelia slimy and olivaceous to cream colored. Pycnidia abundant on both PDA and OA, usually produced superficially on agar surface and often sectored within colony. Pycnidia either solitary or aggregated to form multi-ostiolar fructifications. Conidial exudates orange-pink to greyish-brown. Individual conidia hyaline when young, but some with slight greyish pigments with age, ellipsoid to bacilliform, mostly one-celled, and with multiple guttules. Conidia measuring 4-15 x 1.5-5.0 um. Most isolates produced catenulate, dark brown to black, multi-celled dictyochlamydospores, often originating from the walls of pycnidia in chains of from 2-20 elements resembling conidial chains of Alternaria. Single-celled chlamydospores and intermediate stages alternating between chlamydospores and dictyochlamydospores also produced. Ictyochlamydospores obclavate-ovoid to obpyriform with 3-6 transverse septa and some longitudinal or oblique septa, measuring 20-70 x 15-25 um.

MATERIAL EXAMINED: Idaho: James, R. L., #83-23B, isolated from Pinus contorta stem tips; Idaho: James, R. L., #83-23D, isolated form Pinus contorta stem tips; Idaho: James, R. L., #83-23E, isolated from Pinus contorta stem tips; Idaho: James, R. L., #83-24A, Isolated from Pseudotsuga menziesii stem tips; Idaho: James, R. L., #83-27A, Isolated from Pinus ponderosa needles; Idaho: James, R. L., #83-27A-2, isolated from Pinus ponderosa needles; Idaho: James, R. L., #83-40A, isolated from Pseudotsuga menziesii stem tips and needles; Idaho: James, R. L., #83-40B, isolated from Pseudotsuga menziesii stem tips and needles.

NOTES: The sub-group Peyronellaea within the genus Phoma was established for fungi which commonly produce pigmented multi-celled dictyochlamydospores (9). The most common species of this group, P. glomerata, produces catenulate dictyochlamydospores resembling conidia chains of Alternaria. However, two isolates thought to be P. glomerata failed to produce dictyochlamydospores within 4 weeks. Other isolates usually produced these spores within 1-2 weeks. Previous work (9,12) indicated and this work confirms that fungi classified as P. glomerata may vary in the number, size, and shape of dictyochlamydospores produced. When dictyochlamydospore production is variable, other characteristics

such as colony morphology, conidial exudate color, and conidial dimensions must be used for classification (4).

Phoma pomorum Thum., Fungi Pomicoli:105. 1879.

= Peyronellaea nicotiae Leduc, Revue gen. Bot. 65:545. 1958.

= Peyronellaea prunicola (Opiz) Goid., Rc. Accad. Lancei I:455. 1946.

= Phoma prunicola (Opiz) Wollenw. & Hochpf., Z. ParasitKde 8:595. 1936.

= Phyllosticta cydoniicola Allesch., Hedwigia 36:158. 1897.

= Phyllosticta prunicola (Opiz) Sacc., Michelia I:157. 1878.

= Phyllosticta pyrina Sacc., Michelia I:134. 1878.

Colonies moderate to fast growing, reaching 6.4-9.8 cm in 7 days. Growth rate on PDA and OA similar. Colonies on PDA with abundant floccose, olivaceous-grey mycelium turning darker with age. Most colonies on OA with appressed mycelium, although sectors of floccose olivaceous to white hyphae sometimes produced. Abundant pycnidia usually produced on OA and PDA; two isolates produced much fewer pycnidia on PDA. Pycnidia produced superficially on or immersed within agar; either single or aggregated to form multi-ostiolar fructifications. Conidial exudate mostly cream to light grey. Individual conidia globose to ellipsoid, hyaline (sometimes becoming light grey with age), non-septate, and usually with some guttules. Conidia measure 3.0-10.0 x 1.5-4 um. Pigmented, multi-celled dictyochlamydospores as well as abundant catenulate single-celled chlamydospores produced by all isolates. Dictyochlamydospores usually arising as single terminal spores on mycelial branches; rarely formed in chains of 2-3 elements. Dictyochlamydosproes usually ovoid to ellipsoid with 3-7 transverse septa and some longitudinal or oblique septa, measuring 25-50 x 15-24 um, and resemble conidia of Stemphylium.

MATERIAL EXAMINED: Idaho: James, R. L., #83-81, isolated from Pinus ponderosa roots; Washington: Hamm, P. B., #20-57 (James, R. L., #84-5), isolated from Pseudotsuga menziesii midstem canker; Washington: Hamm, P. B., #19-55 (James, R. L., #84-7), isolated from Pseudotsuga menziesii midstem canker; Washington: Hamm, P. B., #32-30 (James, R. L., #84-97), isolated from Pseudotsuga menziesii midstem canker; Washington: Hamm, P. B., #27-6 (James, R. L., #84-102), isolated from Pseudotsuga

menziesii asymptomatic seedling; Washington: Hamm, P. B., #27-2 (James, R. L., #84-105), isolated from Pseudotsuga menziesii asymptomatic seedling; Oregon: Hamm, P. B., #32-36 (James R. L., #84-106), isolated from Pseudotsuga menziesii asymptomatic seedling.

NOTES: As a member of the subgroup Peyronellaea within the genus Phoma, P. pomorum is differentiated from other members of this group by production of single (non-catenulate) dictyochlamydospores on the ends of hyphal branches and abundant single-celled chlamydospores (9,50). Colonies also produce more abundant floccose olivaceous mycelium and smaller conidia than P. glomerata (19,50). However, as indicated before, dictyochlamydospore production may be irregular (9,12), resulting in confusion with P.eupyrena.

ADDITIONAL NOTES ON HABITS IN NATURE

All three of these chlamydospore-producing species of Phoma are considered ubiquitous fungi occurring on many different substrates, including parts of dead or diseased plants (19,20,50). They often colonize the rhizosphere and roots of plants (20,23,33).

Phoma eupyrena has frequently been reported in forest tree nursery soils (25,43,51), but its activity as a pathogen on seedlings remains unclear. The fungus has previously been isolated from diseased lodgepole pine (28), Mugo pine (31), and Engelmann spruce (29) seedlings from Idaho and Russian-olive seedlings (30) from Montana. It has also been associated with extensive mortality of red fir in California (36,37) and Douglas-fir in Oregon and Washington (16). Pathogenicity of the fungus on Douglas-fir was confirmed with stem inoculation tests (Hamm, unpublished) and on Douglas-fir and red fir with infested soil tests (Kliejunas. unpublished). However, Pathogenicity on other conifer hosts has not been confirmed. Therefore, the role of P. eupyrena as a potential soil-borne pathogen of conifer seedlings remains unclear.

First reports of P. eupyrena associated with plant diseases involved potato tubers. Symptoms produced included lesions (8,18), dry rot (13,38), and gangrene-decay (39). Some workers (32,38,53,) considered p. eupyrena a pathogen of potato tissues, whereas others (18,20,41) thought the fungus was a secondary invader and not pathogenic. Other plant diseases associated with P. eupyrena include rotting of cucumber and apple (18), lesions on grasses (2), decay of birch (46), maple stain (55), and root diseases of several plants (19,20). Unfortunately, pathogenicity of the fungus in many of these diseases has not been confirmed.

Several workers (18,20,40) consider P. glomerata a secondary invader of necrotic plant tissues while others report that the fungus causes important plant diseases. For example, the fungus causes foliage blight of triticale and wheat (27,34), blight of mango (48), pears (15), grapes (47), and potatoes (38), various leaf spots (1,45), fruit rots (14), and pitting of apples (22,24). P. glomerata has been reported from several genera of trees including Pinus, Cedrus, Juglans, Malus, and Salix (10,44). The fungus was frequently isolated from dead bark and wood (11) and shown to incite wood decay (21,46). Although the role of P. glomerata as the incitant of several diseases has been supported by pathogenicity tests, the ability of the fungus to parasitize conifer seedlings remains unclear. Pathogenicity tests will be required to properly assess the role of the fungus on seedlings.

Phoma pomorum has frequently been associated with decay of strawberry roots and may be the primary cause of this important disease (5,10,52). The fungus has also long been considered an important leaf spot pathogen, particularly of fruit trees within the genera Malus, Prunus, and Pyrus (3,9,54). It has frequently been associated with blister and canker diseases of woody plants (6,42) and diseases of fruits such as peanuts (26) and pecans(35). Although several workers (12,17,20) consider the fungus a secondary invader of plant tissues, it has been shown to be pathogenic in several above-listed diseases. Stem inoculations of P. pomorum on Douglas-fir failed to cause damage (Hamm, unpublished).

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