CHARACTERISTICS OF *PHOMA HERBARUM* ISOLATES FROM DISEASED FOREST TREE SEEDLINGS

by

R. L. James, Plant Pathologist

Cooperative Forestry and Pest Management

USDA Forest Service

Northern Region

Missoula, Montana

April 1985

Nursery Disease Notes No. 22

ABSTRACT

Phoma herbarum is frequently isolated from forest tree seedlings displaying tip dieback or stem canker symptoms from nurseries in the northern Rocky Mountains. *In vitro* growth characteristics of fungal colonies are used to differentiate this species from others in the genus *Phoma*. Descriptions of several isolates and notes on nomenclature and habits in nature are discussed.

INTRODUCTION

During the course of investigating diseases of forest tree seedlings at two private nurseries in northern Idaho (Clifty View and Nishek Nurseries, Bonners Ferry), the Montana State Nursery in Missoula, Montana (James 1983), and a nursery in Oregon (James 1984), several isolates of *Phoma herbarum* Westend. were consistently isolated from diseased seedlings. In most cases, this fungus was the most common organism obtained from necrotic tissues. Although pathogenicity tests were not conducted, it is suspected that *P. herbarum* was important in the etiology of most diseases. This report summarizes characteristics of several *P. herbarum* isolates obtained from diseased forest tree seedlings.

MATERIALS AND METHODS

Taxonomic studies of fungi within the genus Phoma are difficult because of the wide host range and great diversity within individual species. Because of this diversity, species classifications are difficult and often made on the basis of slight morphological differences and host substrates (Sutton 1980). 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, reaction to certain reagents, features of the pycnidia, conidia, and conidiogenous cells, and related these to described taxa (Boerema 1969; Dorenbosch 1970). To help reduce variablility, standard growing regimes have been adopted for taxonomic studies of Phoma-like fungi.

Standard conditions for determining growth rates included growth on oatmeal agar (OA) and malt agar (MA) or potato dextrose agar (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 temperatures under periods of alternating 12 hrs. darkness and 12 hrs. near ultraviolet (black) light. This light regime stimulates pycnidial and pigment formation (Sutton 1980). Oatmeal agar promotes pycnidial and conidial production and MA or PDA stimulates mycelial growth and production of chlamydospores and crystals (Dorenbosch 1970).

DESCRIPTION OF SPECIES

Phoma herbarum Westend., Bull. Acad. Belg. 19(3):118. 1852.

=Aposphaeria violacea Bertel, Ost. bot Z. 54:205, 233, 288. 1904.

=Phoma chartícola Speg., An. Soc. cient. argent. 10:153, 154. 1880.

=Phoma exigua var. minor Desm., Annls Sci. nat. (Bot.) III, 11:283. 1889.

=Phoma exigua var. ranunculorum Desm. ex Sacc., Sylloge Fung. 3:134. 1884.

=Phoma herbarum var. chenopodii-albi Roum., Revue mycol. 5:28. 1883.

=Phoma herbarum var. erysimi Roum., Revue mycol. 3/No. 9:30. 1881.

=Phoma herbarum f. humuli Gonz.-Frag., Trab. Mus. nac. cienc. nat., Madr., Ser. bot. 12:30. 1917.

=Phoma herbarum var. lacteria Sutton, Trans. Br. mycol. Soc. 47:501. 1964.

=Phoma herbarum f. minor Unamuno, An. Jard. bot. Madr. 2:56. 1942.

=Phoma herbarum var. sambuci Roum., Revue mycol. 3/No. 9:30. 1881.

=Phoma herbarum var. tetragoniae Sacc. & Berl., Revue mycol. 8:35. 1886.

=Phoma hibernica Grimes, O'Conner & Cummins, Trans. Br. mycol. Soc. 17:99-101. 1932.

=Phoma lignicola Rennerfelt, Svenska SkogsvFor. Tidskr. 35:60. 1937.

=Phoma oleracea Sacc., Michelia 2(1):91. 1880.

=Phoma oleracea f. bryoniae Sacc., Annls mycol. 7:435. 1909.

=Phoma pigmentivora Massee, Bull. misc. Inf. R. bot. Gdns. Kew 8:326. 1911.

=Phoma urticae Schulzer & Sacc., Hedwigia 23:91. 1884.

=Phoma violaceae (Bertel) Eveleigh, Trans. Brit. mycol. Soc. 44:577. 1961.

=Phyllosticta ruscigena Sacc., Nuovo G. bot. ital. II, 22:45. 1915.

Most colonies moderate to slow growing, reaching 2.5-3.5 cm in diameter in 7 days. Aerial mycelium generally sparse and becomes more appressed with colony age. Mycelial coloration extremely variable with isolates producing shades of olivaceous, yellow, pink, black, and white mycelia. All isolates produce distinct pink to pink-orange pigment below the colony (figure 1). All isolates turn deep violet with the addition of NaOH (positive reaction)(figure 2). Isolates generally do not sporulate (produce pycnidia) on MA of PDA; most sporulate profusely on OA, although sporulation may be sectored. Pycnidia simple or compound; simple pycnidia mostly globular, sometimes lenticular or flask-shaped, 50-500 u in diameter. Pycnidial ostioles distinct. Spore mass usually salmon pink to cream colored. Conidia 4.5-5.0 X 2.0-2.5 u, often guttulate. No chlamydospores or sclerotial bodies found.

MATERIAL EXAMINED: Idaho: James, R. L., #84-76A, isolated from Pinus sylvestris (Guadalajara) stem tips; Idaho: James, R. L., #84-76B, isolated from Pinus sylvestris (Gualdalajara) stem tips; Idaho: James, R. L. #84-77, isolated from Pinus monticola stem tips; Idaho: James, R. L. #84-78B, isolated from Pinus sylvestris (French Blue) stem tips; James, R. L., #84-80A, isolated from Pinus nigra stem tips; Montana: James, R. L. #82-56C, isolated from Eleagnus angustifolia stem cankers; Oregon: Cooley S. J. #P2-8 (James, R. L., #84-9), isolated from Pseudotsuga menziesii midstem canker.

NOTES: Phoma herbarum is the type-species of the form-genus Phoma (Boerema 1964). Isolates classified as P. herbarum are variable in colony morphology, rate of growth, and pycnidial production. Rate of growth is mostly influenced by the composition and acidity of the nutrient substrate (Eveleigh 1961). Pycnidial size and number often varies within different sectors of colonies (Boerema 1964). A major distinguishing characteristic of P. herbarum in culture is production of pink to orange-red pigment which varies in intensity among different strains and growth media. Media pH influences the pigmentation; in an acid medium the color darkens as the pH is raised. In culture, some sectors may show striking differences in pigmentation.

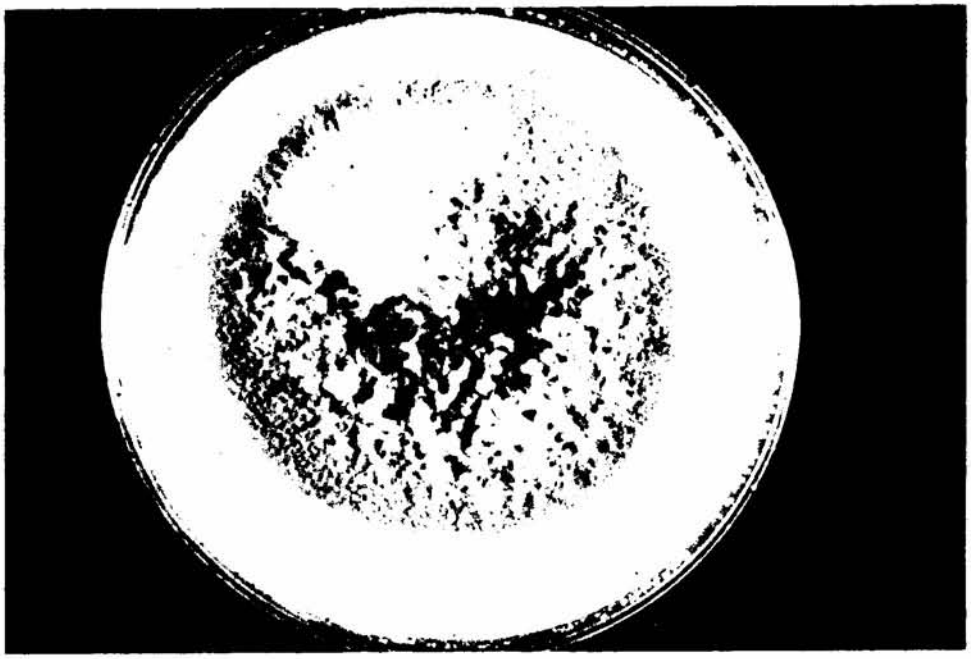

Figure 1. Phoma herbarum (isolate 84-76B) after 14 days on OA. Distinct pink pigmentation and colony sectoring is noticeable.

Figure 2. Deep violet discoloration of Phoma herbarum (isolate 84-9) upon addition of NaOH.

For example, strong pigmentation is often associated with aerial mycelium (Boerema 1964). Another major distinguishing characteristic of P. herbarum is production of a deep violet discoloration upon addition of NaOH to colonies (Boerema 1970; Dorenbosch 1970); isolates with a strong pigmentation usually produce a rapid and very noticeable violet discoloration.

HABIT IN NATURE: Phoma herbarum has world-wide distribution and occurs on very diverse substrates such as dead or dying herbaceous and woody plants, soil, water, paints and dairy products (Boerema 1964). Although sometimes associated with plant diseases, the species is generally considered to be saprophytic (Dorenbosch 1970). The fungus also frequently occurs on seedcoats of many different plants (Byford and Gambogi 1985; Dorenbosch 1970) and together with other seed-borne fungi may initiate seed or seedling diseases.

Role of P. herbarum as a pathogen of conifer seedlings needs investigation. The fungus is probably a common resident of nursery soils. It is possible that P. herbarum colonizes necrotic seedling tissues following damage by other biotic agents or abiotic stresses. On the other hand, it is also possible that the fungus may initiate infection of relatively healthy tissues. Tests to evaluate pathogenicity of this species would help our understanding of its potential as an important pathogen of forest tree seedlings.

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