Characteristics of *Phoma* Isolates from Nurseries of the Pacific Northwest Region

R. L. James
Plant Pathologist

Cooperative Forestry and Pest Management
USDA Forest Service
Northern Region
Missoula, Montana

April 1984

INTRODUCTION

The genus Phoma is defined by production of separate pycnidia with a single ostiole (occasionally confluent and multiostiolate) which are not aggregated on a stroma (Domsch et al. 1980). Pycnidial walls are thin, with a brown-black pigmented surface, and sometimes a sclerotioid base. An important characteristic of Phoma spp. is production of large numbers of slimy, mostly one-celled, ellipsoid to cylindrical hyaline conidia from pycnidia. Conidiogenous cells have a narrow phialidic opening and are hardly differentiated from the inner wall cells (Boerema and Bollen 1975). Pigmented chlamydospores are formed in some species either in simple chains or aggregated into dictyochlamydospores resembling the conidia of Alternaria or Stemphylium (Dorenbosch 1970; Luedemann 1959).

Identification of Phoma species is difficult because of the extensive synonymy that exists among taxa, and mycologists often disagree on which characters are of taxonomic value (Shear 1923; Wehmeyer 1946; Wollenweber and Hochnapfel 1936). Similar fungi often have several different names. Host plant or substratum has had important taxonomic significance in the past (Dennis 1946). However, most Phoma species have a wide host range (Wollenweber and Hochapfel 1936) and relating taxonomy to host species is not practical (Boerema 1969). Difficulty in assigning specific names to Phoma-like fungi has prompted some workers to describe these fungi using only the genus name (Jenkins 1943; Sumner 1974; Wallace and Dickinson 1978).

Identification of Phoma species requires well standardized techniques of culture and the simultaneous inspection of several characters since single characters are usually not specific (Boerema 1969; Domsch et al. 1980). Therefore, synoptic keys have been produced (Boerema 1976; Dorenbosch 1970) and are often more useful for identification than dichotomous keys.

Ten fungal isolates thought to be within or related to the genus Phoma and obtained from diseased nursery stock within the Pacific Northwest Region were submitted by S. J. Cooley for identification. Isolates were grown on oatmeal agar (OA)(Stevens 1974) and potato dextrose agar (PDA) within 90 mm (diameter) petri plates at 22° C in darkness for 7 days followed by 7 days of alternating 12 hour light and dark periods. OA was used to stimulate production of pycnidia and conidia; PDA was used to stimulate mycelial growth and production of chlamydospores and crystals (Dorenbosch 1970). Linear growth rates were measured after 7 and 14 days. Colony morphology and pigmentation, production of pycnidia, crystals, and chlamydospores, and color reactions (addition of NaOH)(Boerema and Howeler 1967) were described.

A description of the ten isolates and criteria used for identification follows. A discussion of the biological characteristics and pathology of these species is included after isolate descriptions.

ISOLATE CHARACTERISTICS

Isolate 19-35 (84-1)

1. Growth characteristics on PDA: slow growing initially (4.0 mm/day), then stops altogether without covering entire agar surface. Colony margin is uneven with cream-white appressed hyphae (figure 1). Colony becomes charcoal black with overlying olivaceous-grey aerial hyphae and whitish-grey hyphae near the margin. Colony is convoluted and semi-sclerotioid with tightly-packed networks of interwoven pigmented hyphae. Profuse sporulation occurs in older portions of the colony with pycnidia mostly immersed within the semi-sclerotioid hyphal network. No pigment is produced within the medium and the NaOH reaction is negative.

2. Growth characteristics on OA: slightly faster growing than on PDA at 4.1 mm/day and covering the entire agar surface in 11 days. Colony margin is mostly uniform with appressed, cream-colored hyphae. Colony has sparse aerial hyphae which is whitish-grey at first, then becoming olivaceous-grey. Profuse sporulation occurs throughout the entire colony with pycnidia mostly superficial on the agar surface. Spore exudate from pycnidia is whitish-cream colored. No pigment is produced within the medium and the NaOH reaction is negative.

3. Pycnidia and conidia: pycnidia are mostly separate, carbonaceous when mature, and globose to flask-shaped. They usually have a single ostiole (one locule), which is distinctly papillate and measure 137-186μ($\bar{x}$=165μ) in diameter. Pycnidia of this isolate and several other Phoma species develop from a single hyphal cell that divides transversely and diagonally to form a primordium (figure 2) (Kempton 1919). The primordium later develops into an ostiolate pycnidium. Conidia are mostly one-celled, hyaline, biguttalate, and ellipsoid to kidney-shaped. They measure 3.5-6.5μ ($\bar{x}$=4.8μ) in length and 1.5-3.0μ ($\bar{x}$=2.2μ)in width.

4. Hyphal characteristics: hyphal cells become dark brown with age and often aggregated to form distinct hyphal strands. Catenulate, circular chlamydospores (avg. diameter 11.5μ) form sparsely in older (>10 days) cultures. Hyphae become interwoven into a tight semi-sclerotioid network which also contains chlamydospores.

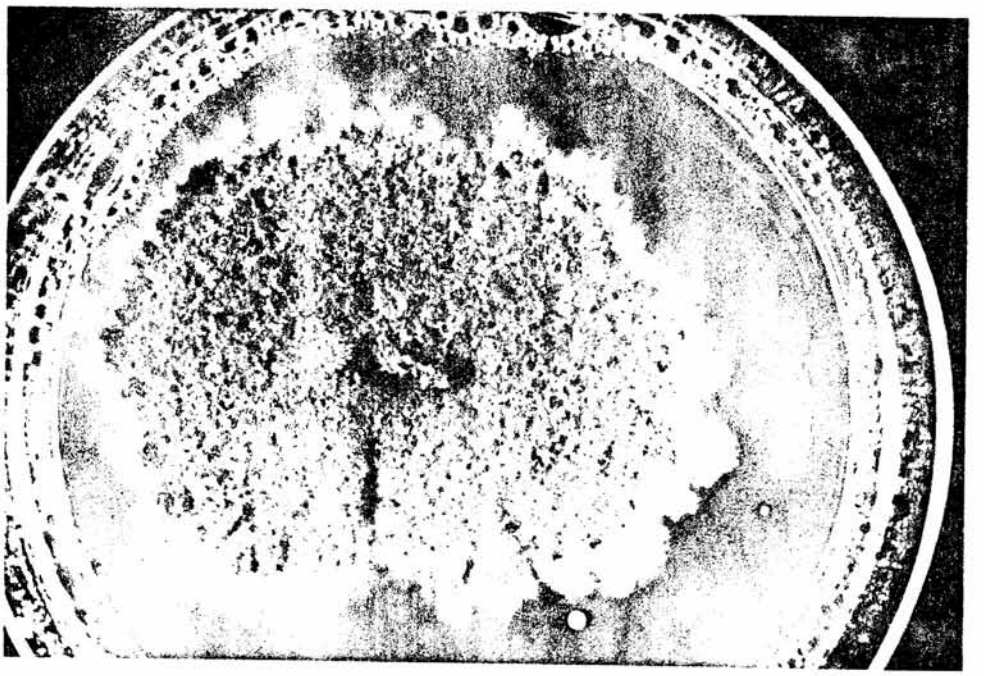

Figure 1. Isolate 19-35 on PDA after 14 days incubation at 22°C. Colony is olivaceous-black, convoluted, and with an irregular margin.

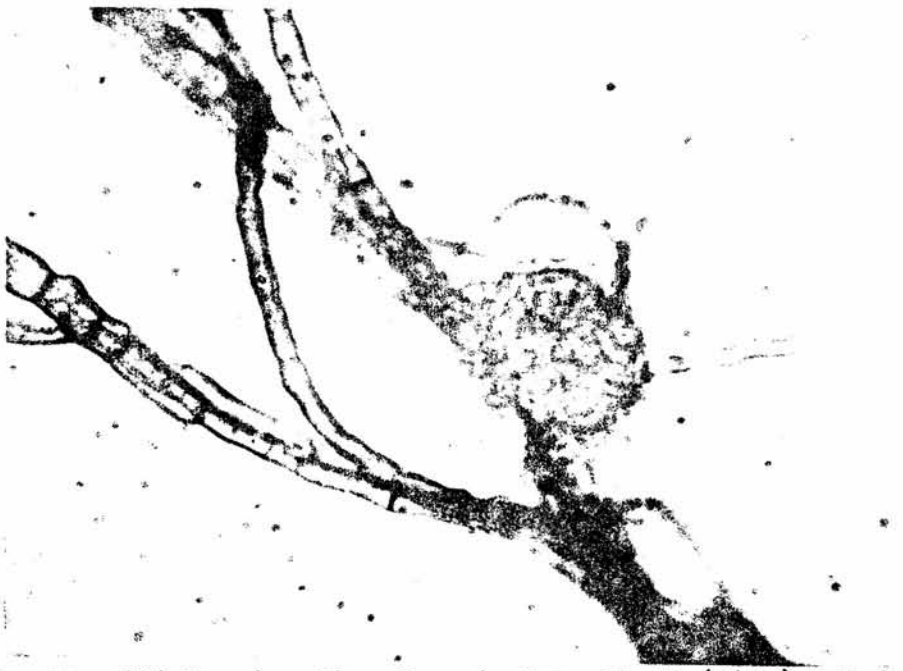

Figure 2. Pycnidial primordium from isolate 19-35 (X450). Primordia develop from a single hyphal cell that divides transversely and diagonally.

5. Identification criteria: the keys of Dorenbosch (1970) and Domsch et al. (1980) indicate that this isolate is related to Phoma eupyrena Sacc. However, characteristics that differentiate this isolate from other P. eupyrena isolates studied include reduced growth rate on both PDA and OA, uneven convoluted growth on PDA, and delayed, sparse production of catenulate chlamydospores. Most isolates of P. eupyrena grow at about 5.5-7.0 mm/day and initiate chlamydospore production on PDA after about 7 days (see isolates P1-7 and P1-7-1 this report) (Dorenbosch 1970; James 1983b; James 1984). By 14 days, chlamydospore production is usually extensive. Colony pigmentation and pycnidial and conidial morphology of this isolate correspond to descriptions of P. eupyrena (Domsch et al. 1980; Dorenbosch 1970). Likewise, lack of pigment or crystal production in the medium and negative NaOH reaction is also indicative of P. eupyrena. Therefore, based on the characteristics found within the taxonomic frameworks used, it is concluded that this isolate is related to the species group of P. eupyrena.

Isolate 19-49 (84-2)

This isolate was initially thought to be within the species group Peyronellaea because of its characteristic production of dictyochlamydospores within the agar slant culture originally provided (Boerema et al. 1965). Growth characteristics and pigment production were similar to descriptions of Phoma sorghina (Sacc.) Boerema (Boerema et al. 1971; Boerema et al. 1977). However, cultures grown on both PDA and OA failed to produced either distinct Peyronellaea-like dictyochlamydospores or pycnidia characteristic of the genus Phoma. Instead, Fusarium macroconidia were consistently obtained. Subsequent attempts to obtain Phoma-like cultures from the original slant culture were unsuccessful. Therefore, Fusarium must have colonized the original culture. Cultural characteristics and macroconidial morphology indicate that the Fusarium is within the F. roseum (Lk.) Sacc. group (Toussoun and Nelson 1968).

The following is a description of hyphal characteristics of the original culture that may be associated with the genus Phoma: catenulate, oval chlamydospores (avg. diameter 11.0μ) were produced in the older portion of the original slant culture. Elongate, multi-celled, mostly intercalary dictyochlamydospores measuring 33-45μ ($\bar{x}$=38.0μ) long and 12-20μ ($\bar{x}$=16.5μ) wide were also formed.

Production of dictyochlamydospores by species of Phoma is characteristic of the sub-group Peyronellaea (Boerema et al. 1965). However, without pure culture characteristics, identification of this isolate within the sub-group cannot be made.

Isolate 19-10 (84-3)

1. Growth characteristics on PDA: moderately fast growing (5.2 mm/day), covering the entire agar surface in 9 days. Colony margin is even with appressed, cream-colored hyphae. Hyphae become dark brown to olivaceous with age and appressed or immersed within agar. Colony is covered with fluffy white to light grey aerial mycelium over the olivaceous hyphae. White aerial hyphae become grey with age. The olivaceous-brown hyphae become tightly interwoven to form a semi-sclerotioid network on and just below the agar surface. Pycnidia are not evident after 14 days and the colony produces a negative NaOH reaction.

2. Growth characteristics on OA: moderately fast growing (5.1 mm/day), covering the entire agar surface in 9 days. Colony has appressed, olivaceous hyphae overlain with fluffy white aerial hyphae (figure 3). A faint yellowish discoloration is evident in the agar medium. Pycnidia are slow to develop and are not evident until colony is more than 14 days old. Pycnidia are produced within isolated zones near the colony edge.

3. Pycnidia and conidia: pycnidia are mostly separate and globose to irregularly shaped. Papillae are not evident in most pyncidia. Pycnidia measure 115-210μ ($\bar{x}$=160.5μ) in diameter. Conidia are one-celled, oval to ellipsoid, not distinctly guttalate, and measure 3.0-5.0μ ($\bar{x}$=4.2μ) in length and 1.5-2.5μ ($\bar{x}$=2.0μ) in width. Conidia do not often exude through a definite papillate ostiole, but may be released through a perforation in the pycnidial wall.

4. Hyphal characteristics: hyphal cells become brown and rectanglar to barrel-shaped with age (figure 4). As the colony ages, these pigmented hyphae become vacuolate and tightly compacted into a semi-sclerotioid network on the agar. Chlamydospores or dictyochlamydospores are not formed. Hyphal strands are not prominent.

Figure 3. Isolate 19-10 on OA after 14 days at 22° C. Colony has distinct fluffy-white hyphae over appressed olivaceous-brown hyphae.

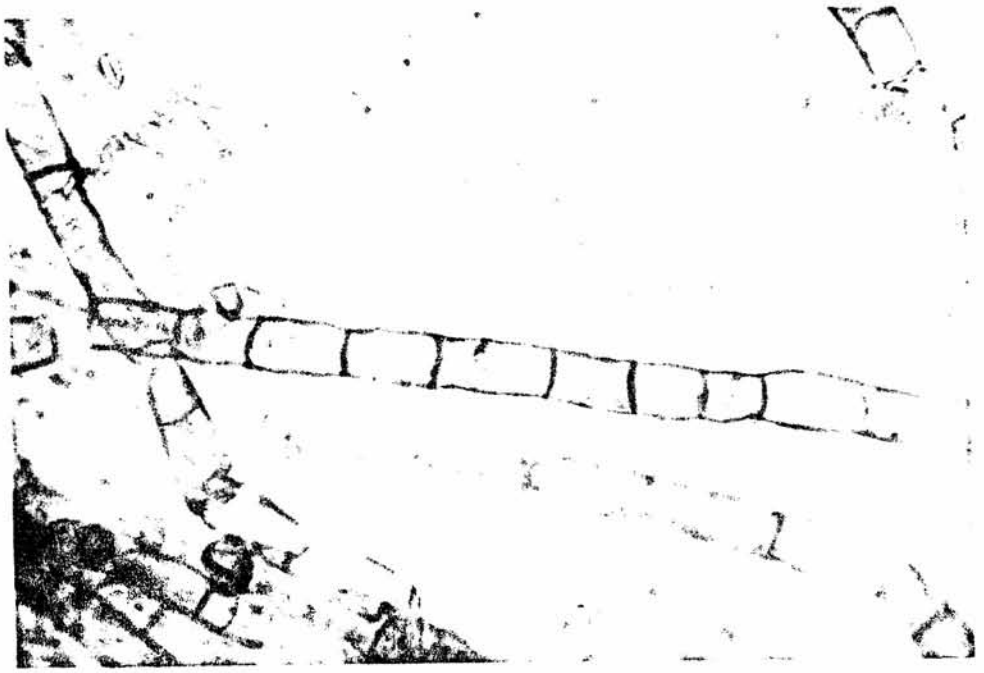

Figure 4. Rectangular hyphal cells from isolate 19-10 grown on PDA. These vacuolate cells become tightly compacted to form a semi-sclerotioid network on the agar surface. (x450)

5. Identification criteria: Identification of this isolate to a particular species is difficult because of its paucity of distinguishing characters. General colony morphology resembles some isolates of P. exigua Desm. (Boerema and Howeler 1967); Domsch et al. 1980) and P. lingam (Tode ex Fr.) Desm. (Boerema 1976; Dennis 1946; Grimes et al. 1932). However, it differs from P exigua because of its negative NaOH reaction and lack of irregularly lobed colony margin (Boerema and Howeler 1967). The isolate produces irregularly shaped pycnidia that could be described as pseudosclerenchymatous, characteristic of the species group Plenodomas of which P. lingam is a member (Boerema 1976; Boerema et al. 1981). However, an iodine staining test of pycnidia to place this isolate in the Plenodomas was non-definitive. Production of thick-walled hyaline cells (scleroplectenchyma) in the pycnidial peridium, another distinguishing character of the Plenodomas group (Boerema et al. 1981), could not be determined. On the other hand, several pycnidial characteristics of this isolate are similar to those described for P. lingam (Boerema and van Kesteren 1964). For example, pycnidia usually remain closed until they are almost mature. Most pycnidia also lack distinct papillate ostioles and release conidia through a pore in the pycnidial wall.

Since there were no other distinguishing characteristics, such as chlamydospores, it is difficult to assign this isolate to a specific taxon. Although the pigmented vacuolate rectangular hyphal cells were distinct, they are not dealt with in taxonomic treatments (Boerema 1976; Domsch et al. 1980; Dorenbosch 1970). Therefore, affinity of this isolate to a species of Phoma cannot be made at this time, although it may belong within the species group Plenodomas and be related to P. lingam.

Isolate 19-36A (84-4)

1. Growth characteristics on PDA: moderate growth rate (4.4 mm/day), covering entire agar surface in 10 days. Colony margin has cream-white colored appressed hyphae (figure 5). Hyphae become olivaceous brown to black with age. Aerial hyphae are sparse at first, but may form a greyish covering in the center of older cultures. Colony is generally olivaceous-black with abundant sporulation throughout. Pycnidial exudate is creamy-white. No pigment or crystals are formed in the medium; reaction to NaOH is negative.

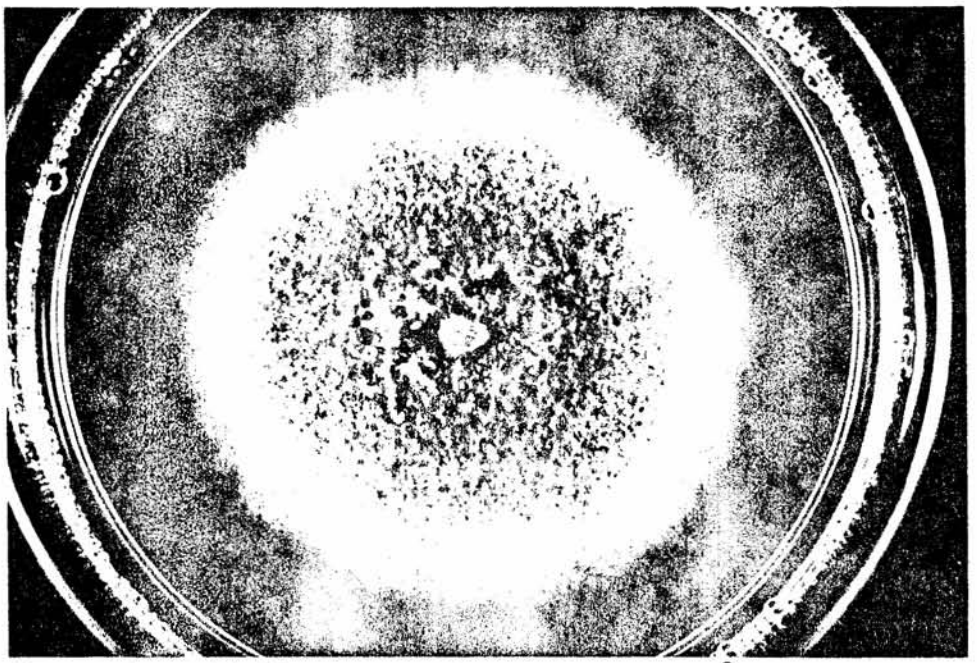

Figure 5. Isolate 19-36A on PDA after 7 days at 22°C. Colony has white appressed margin hyphae which become olivaceous-brown with age.

2. Growth characteristics on OA: moderately fast growing (5.2 mm/day), covering entire agar surface in 9 days. Aerial hyphae are sparse and limited to tufts of greyish-white hyphae. Colony has appressed or immersed olivaceous-brown hyphae and abundant sporulation throughout. No pigment or crystals are formed within the medium; reaction to NaOH is negative.

3. Pycnidia and conidia: pycnidia are mostly separate, globose to flask-shaped, and initially light brown but becoming carbonaceous with age. They measure 127-325μ ($\bar{x}$=230μ) in diameter. Conidia are hyaline, mostly one-celled, bacilliform, and measure 4-8μ ($\bar{x}$=6.1μ) in length and 1.5-3.0μ($\bar{x}$=2.0μ) in width. Conidial shape is generally elongated rather than oval.

4. Hyphal characteristics: hyphae become pigmented brown in young cultures (7 days), then become rectangular and vacuolate. Catenulate chlamydospores (figure 6) form sparsely after 10-14 days. These chlamydospores are oval, terminal or intercalary, and measure 10-13μ ($\bar{x}$=11μ) in width. Hyphal strands composed of aggregated pigmented hyphae form abundantly.

5. Identification criteria: colony morphology and production of chlamydospores indicate that this isolate is related to P. eupyrena (Domsch et al. 1980; Dorenbosch 1970). However, the appressed nature of hyphal growth and sparse production of chlamydospores are two characteristics which differentiate this isolate from other P. eupyrena isolates studied (James 1983b; James 1984). There were no other characteristics which would place this isolate in another described taxon were not found.

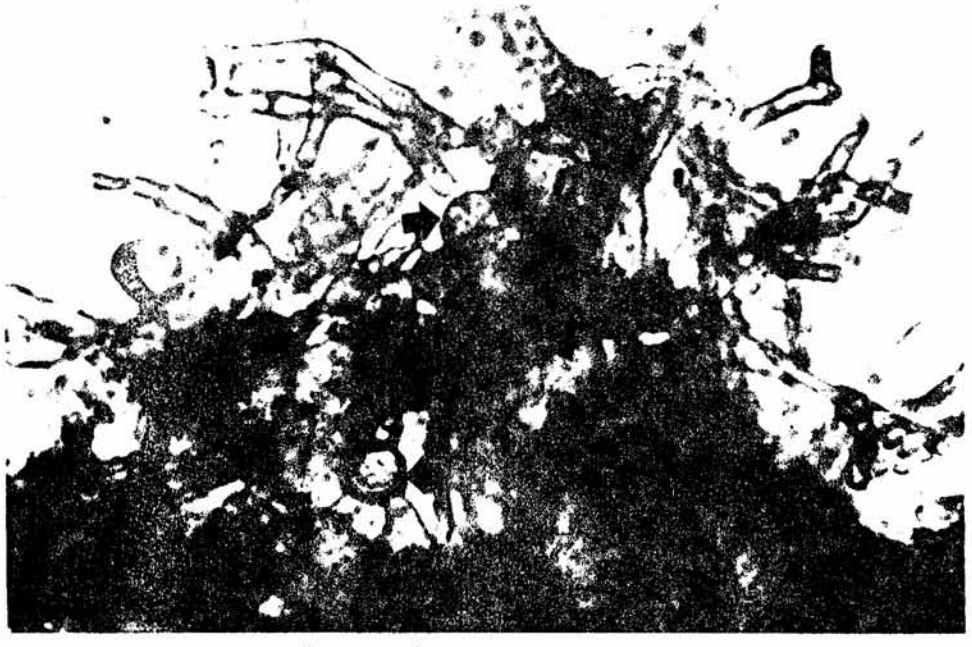

Figure 6. Chlamydospores (arrows) of isolate 19-36A grown on PDA for 14 days at 22°C (X450). Chlamydospores are not as abundant as other isolates of P. eupyrena studied.

Isolate 20-57 (84-5)

1. Growth characteristics on PDA: moderately fast growing (5.5 mm/day), covering the entire agar surface in 10 days. Colonies are uniform with appressed cream colored margin hyphae. Colonies become olivaceous-grey in color with olivaceous aerial hyphae in the center and sometimes a ring of aerial whitish-grey hyphae (figure 7). Abundant sporulation occurs throughout the colony. No pigment or crystals form within the medium; reaction to NaOH is negative.

2. Growth characteristics on OA: slower growing than on PDA (4.6 mm/day), covering the entire agar surface in 10 days. Colony margins have appressed cream colored hyphae which become olivaceous-grey with age. Aerial hyphae are more abundant than on PDA and occurs in concentric rings throughout the colony. Aerial hyphae are white to olivaceous-grey. Sporulation is abundant throughout the colony. No pigment or crystals are formed within the medium; reaction to NaOH is negative.

3. Pycnidia and conidia: pycnidia are light brown, becoming carbonaceous with age, globose to obpyriform, generally with a ridged or furrowed surface, and usually with one ostiole. Size of pycnidia is variable, but they usually measure 100-210μ ($\bar{x}$=160μ) in diameter. Pycnidia are produced superficially on or immersed within the agar; smaller pycnidia may also form within the aerial mycelium. Pycnidia often coalesce to form irregular, large fructifications with many ostioles (furcate). Conidia are hyaline, mostly one-celled, guttalate, ovoid to ellipsoid, and measure 4.5-8μ ($\bar{x}$=6.5μ) in length and 1.5-2.5μ ($\bar{x}$2.3μ) in width.

4. Hyphal characteristics: hyphae become darkly pigmented and form catenulate oval chlamydospores after 10-14 days (figure 8). After 14 days, multi-septate dictyochlamydospores form mostly as single terminal spores on hyphal branches (figure 8). Dictyochlamydospores may sometimes be intercalary, especially when adjacent to catenulate chlamydospores. Dictyoschlamydospores are ovoid to ellipsoid, sometimes clavate to oblong, and with 3-5 transverse walls and several longitudinal walls. They measure 18-40μ ($\bar{x}$=25μ) in length and 10-30μ ($\bar{x}$=8μ) in width. Strands of aggregated pigmented hyphae are also common.

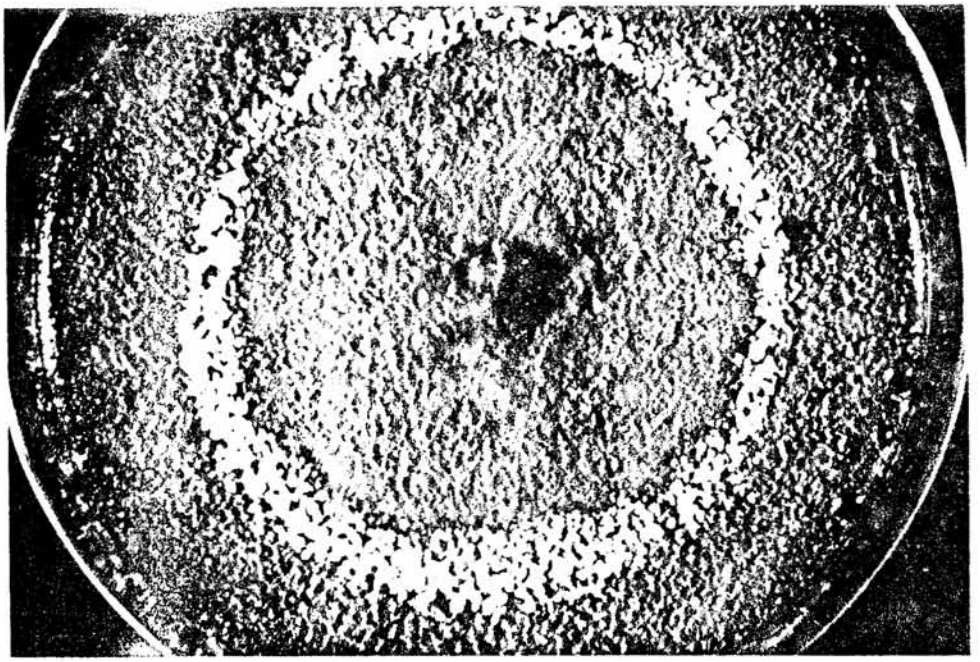

Figure 7. Isolate 20-57 grown on PDA for 14 days at 22 C. Colony is mostly olivaceous-grey with a ring of whitish-grey hyphae.

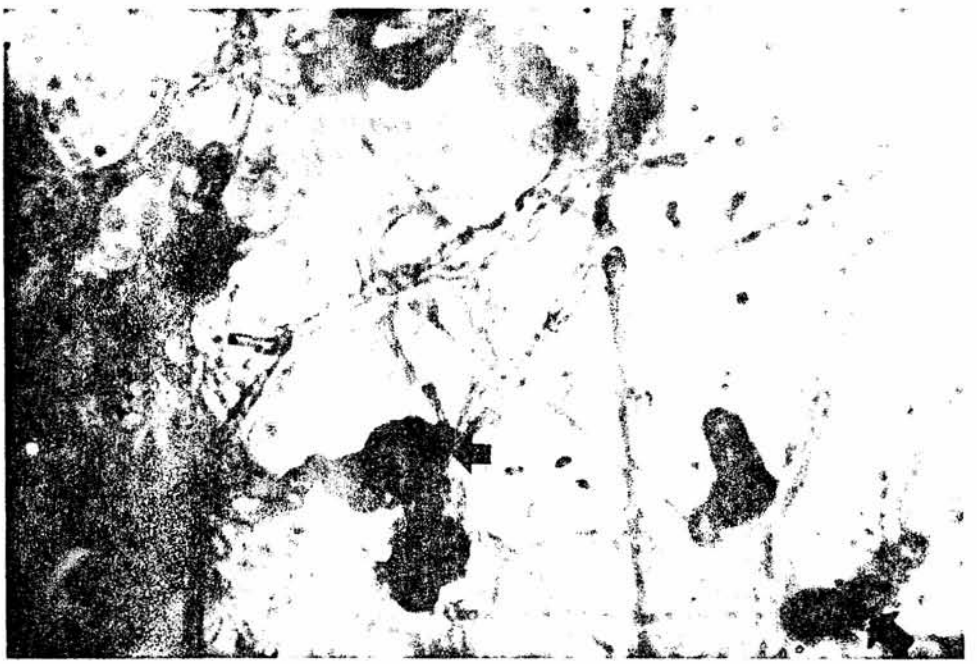

Figure 8. Dictyochlamydospores (black arrow) and catenulate single-celled chlamydospores (red arrow) from isolate 20-57 grown on PDA for 14 days at 22 C (x200). These multi-celled dictyochlamydospores are produced mostly terminally on hyphal branches.

5. Identification criteria: Production of dictyochlamydospores by species of Phoma is indicative of the group Peyronellaea (Boerema et al. 1965). Within this sub-group, differentiation is based on dictyochlamydospore morphology, habit on hyphal branches, and relative abundance of single-celled catenulate chlamydospores. This isolate is best described by the species P. pomorum Thum. (Boerema et al. 1971), previously named P. prunicola (Opiz) Wollenw. & Hochapf. (Boerema et al. 1965; Dorenbosch 1980). This species is differentiated from others that produce dictyochlamydospores by its production of these multi-celled spores singly (rather than in chains), usually terminally on hyphal branches and in association with abundant production of single-celled catenulate chlamydospores (Boerema et. al 1965; Boerema et al. 1971).

Isolate 19-15 (84-6)

Growth characteristics could not be determined because this isolate did not grow on either PDA or OA. The agar slant culture provided was apparently not viable.

1. Pycnidia and conidia: pycnidia from the agar slant culture are immersed in agar, globose, without distinct papillae, and measure 100-210μ ($\bar{x}$=185μ) in diameter. Conidia are mostly one-celled, ellipsoid to bacilliform, and measure 5-10μ ($\bar{x}$=8.2μ) in length and 1.5-3.0μ ($\bar{x}$=2.0μ) in width.

2. Hyphal characteristics: hyphae are pigmented from light to dark brown and often aggregate into hyphal strands. Hyphal cells are irregularly shaped from rectangular to nearly oval and highly vacuolate. Chlamydospores and dictyochlamydospores are not present.

3. Identification criteria: without cultural growth characteristics, insufficient diagnostic information is available for identification. Pycnidial and conidial characteristics place this isolate in the genus Phoma, but affinity to a described species cannot be made.

Isolate 19-55 (84-7)

1. Growth characteristics on PDA: moderate growth rate (5.3 mm/day), covering the entire agar surface in 10 days. Colony margins are even with appressed, cream colored hyphae. Colony has olivaceous-grey and some whitish-grey aerial hyphae. Very slight yellow-orange discoloration occurs within the agar. Sporulation is profuse, especially on the edge of the colony. No crystals are formed within the agar; reaction to NaOH is negative.

2. Growth characteristics on OA: moderately fast growing (5.7 mm/day), covering the entire agar surface in 10 days. Colony margins are even with appressed, cream colored hyphae. Hyphae become olivaceous-grey with age (figure 9). Aerial hyphae are sparse, usually occurring as tufts of greyish-white hyphae. Sporulation is abundant over the entire colony. Pycnidial exudate is cream-colored. No crystals or pigment are produced in culture; reaction to NaOH is negative.

3. Pycnidia and conidia: pycnidia are superficial on the surface or immersed within agar, mostly globose to obpyriform, papillate, and usually with a single ostiole. Pycnidia measure 110-150μ ($\bar{x}$=140μ) in diameter and sometimes coalesce to form irregular-shaped fructifications with several ostioles. Conidia are ellipsoid to bacilliform, hyaline, and usually one-celled, although 1-septate conidia do occur (figure 10). Conidia measure 4-9μ ($\bar{x}$=7μ) in length and 1.5-2.5μ ($\bar{x}$=2.1μ) in width.

4. Hyphal characteristics: hyphal strands of aggregated pigmented hyphae are common. Single-celled catenulate chlamydospores are usually produced on PDA cultures after 14 days. These chlamydospores are brown and measure 8-10μ in diameter. Multi-celled dictyochlamydospores are also produced (figure 11), usually as single terminal spores on hyphal branches. They may also be intercalary, particulary when adjacent to single-celled catenulate chlamydospores. Dictyochlamydospores are ovoid to ellipsoid, sometimes clavate to oblong, with several transverse and longitudinal walls, and measure 20-45μ ($\bar{x}$=35.0μ) in length and 15-25μ ($\bar{x}$=18μ) in width.

5. Identification criteria: most characteristics of this isolate are similar to those described for isolate 20-57. Both isolates have catenulate single-celled chlamydospores and multi-celled dictyochlamydospores of similar morphology. However, there are differences in colony morphology on PDA and OA. Also, this isolate (19-55) produces a slight but noticeable orange pigment not found in isolate 20-57. Presence of two-celled conidia was also more noticeable in this isolate than isolate 20-57. Nevertheless, characteristics of major taxonomic value, i.e presence and morphology of single-celled chlamydospores and dictyochlamydospores, indicate that this isolate is described by the species P. pomorum, within the species group Peyronellaea (Boerema et al. 1965; Boerema et al. 1971). The intra-specific variation seen between this isolate and isolate 20-57 may be normal for most Phoma species.

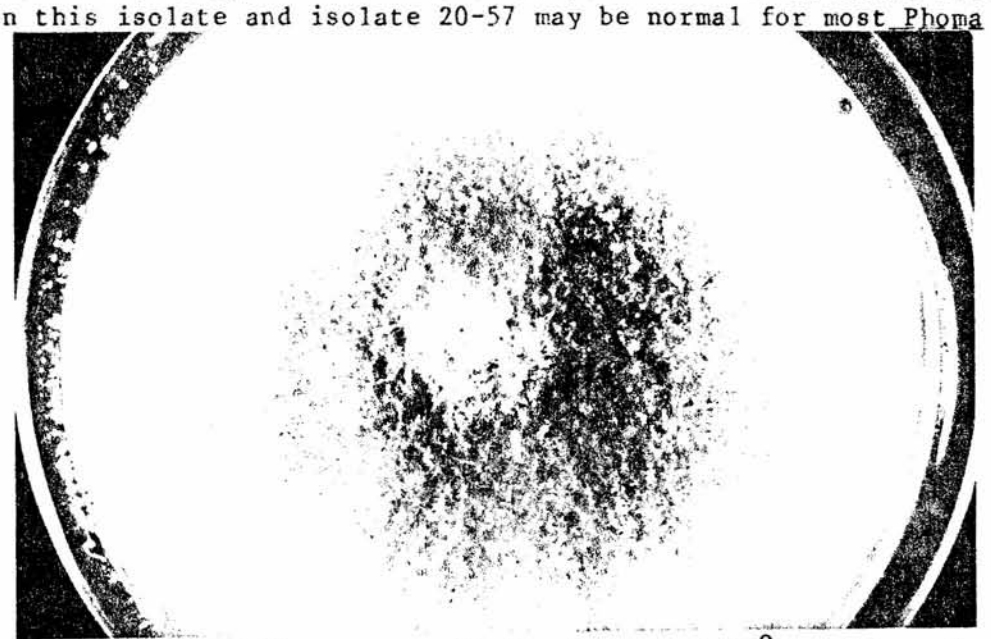

Figure 9. Isolate 19-55 grown on OA for 7 days at 22°C. Colony has whitish cream-colored margin hyphae which become olivaceous-grey with age.

Figure 10. One-and two-celled (arrow) conidia from isolate 19-55 grown on OA for 14 days at 22 C. Conidia are hyaline and ellipsoid to bacilliform in shape. (x450)

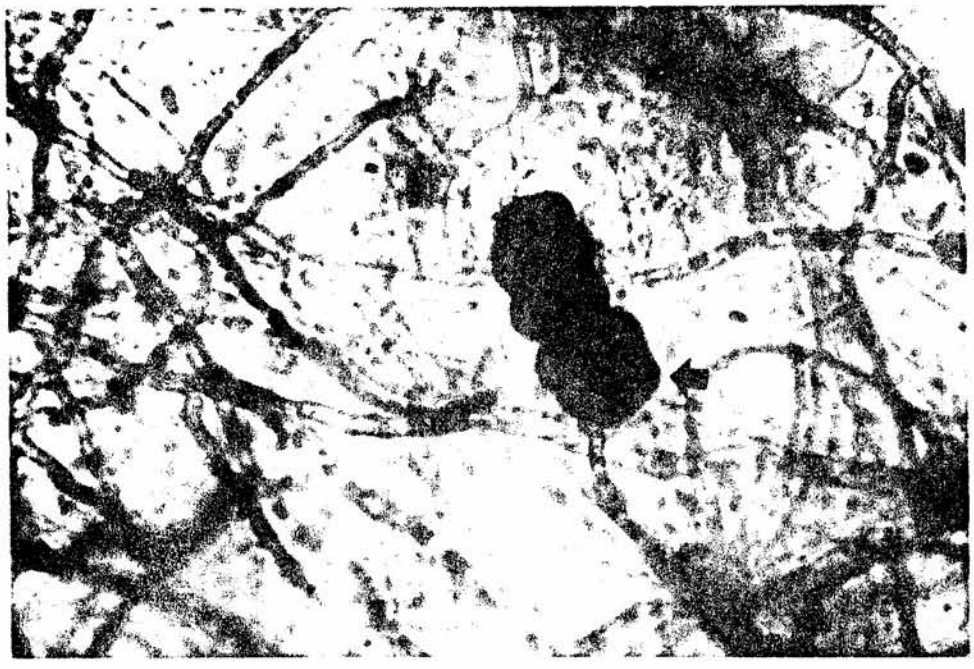

Figure 11. Dictyochlamydospore (arrow) from isolate 19-55 grown on PDA for 14 days at 22 C. These multi-celled spores have several transverse and longitudinal walls. (x450)

Isolate P1-7 (84-8)

1. Growth characteristics on PDA: rapid growth (7.0 mm/day), covering the entire agar surface in 7 days. Colony margin is even with appressed white hyphae. Colony is initially covered with fluffy white hyphae which start to turn olivaceous-grey after 7 days (figure 12). By 14 days, the colony is composed mostly of olivaceous-grey to black aerial hyphae. Slight yellow-orange pigment is produced in older cultures. Sporulation is not evident after 7 days, but is common by 14 days. No crystals are formed within the medium; reaction to NaOH is negative.

2. Growth characteristics on OA: moderately fast growth (5.8 mm/day), covering entire agar surface in 8 days. Colony margin is even with appressed cream colored hyphae. Appressed hyphae become olivaceous-grey and covered with white fluffy aerial hyphae which eventually turn grey. Sporulation does not occur until 10-14 days and then becomes distributed throughout the colony. Pycnidial exudate is cream colored. No pigment or crystals are formed in the medium; reaction to NaOH is negative.

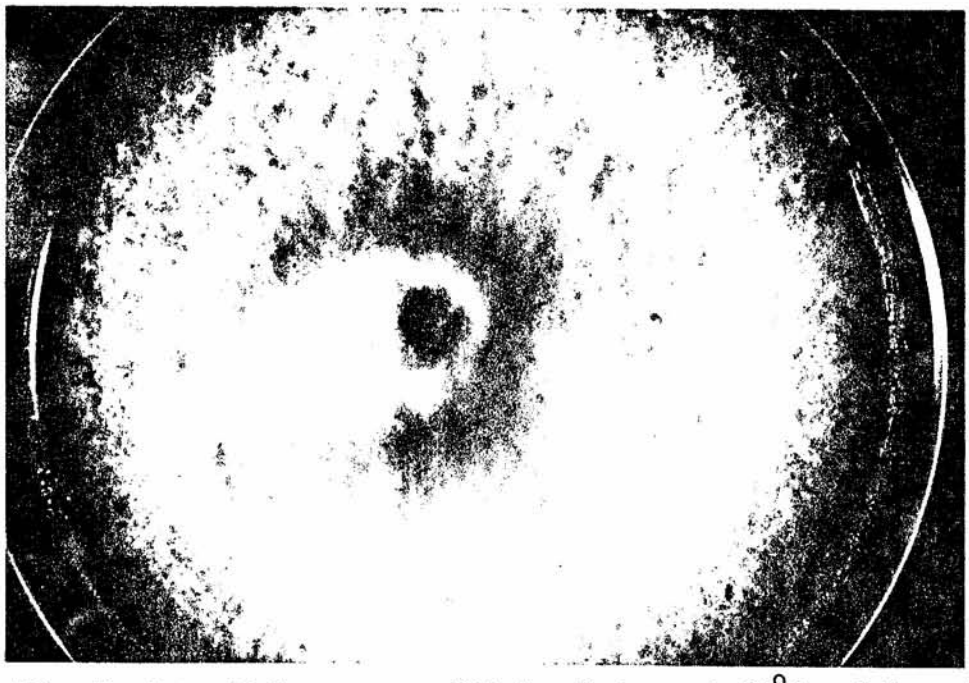

Figure 12. Isolate P1-7 grown on PDA for 7 days at 22°C. Colony has abundant fluffy white hyphae beginning to turn olivaceous-grey.

3. Pycnidia and conidia: pycnidia are mostly superficial on or immersed within the agar. A few small pycnidia are also produced within the aerial whitish-grey mycelium. Pycnidia are either separate or aggregated into irregularly shaped fructifications with one or more ostioles. They are initially sub-hyaline, later becoming dark brown and then carbonaceous. Short

but distinct papillae are present on most pycnidia. Pycnidia are globose to pyriform and measure 105-230μ($\bar{x}$=159μ) in diameter. Conidia are ellipsoid to bacilliform, mostly one-celled (some two-celled conidia are produced), biguttalate, and measure 4-9μ ($\bar{x}$=6.9μ) in length and 1.5-3.5μ ($\bar{x}$=2.1μ) in width.

4. Hyphal characteristics: pigmented hyphae commonly aggregate to form hyphal strands. Catenulate, single-celled chlamydospores form in PDA colonies after 10-14 days (figure 13). Chlamydospores are initially sub-hyaline, but become darkly pigmented with age and measure 8-15μ ($\bar{x}$=11.2μ) in diameter. As colonies on PDA age, pigmented hyphae with chlamydospores form a tight semi-sclerotioid network.

5. Identification criteria: colony morphology, pigmentation, and production of catenulate chlamydospores indicate that this isolate is probably related to P. eupyrena (Boerema 1976; Domsch et al. 1980; Dorenbosch 1970). However, this isolate has several characteristics that differentiate it from isolates 19-35 or 19-36A described above. Its growth pattern in culture is different, particularly the extensive fluffy white mycelium produced during early growth stages. This isolate also produces many more chlamydospores at an earlier age. However, based on published taxonomic treatments, this isolate as well as isolates 19-35 and 19-36A are all best described as P. eupyrena.

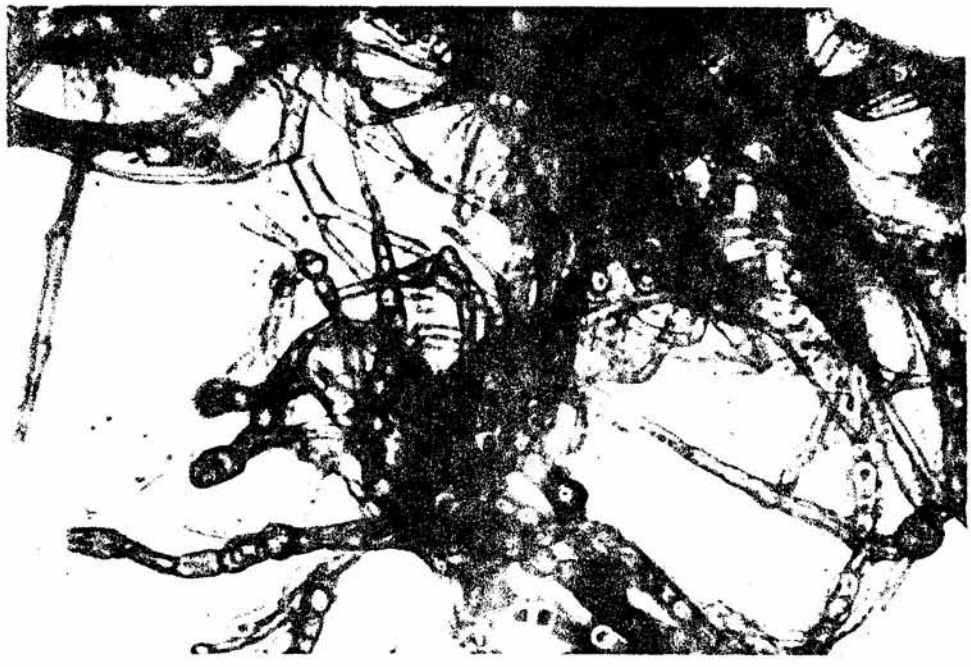

Figure 13. Globose single-celled catenulate chlamydospores of isolate P1-7 formed on PDA after 10-14 days at 22°C (X200).

Isolate P2-8 (84-9)

1. Growth characteristics on PDA: slow growing (2.5 mm/day), covering the entire agar surface in 18 days. Colony margin even with appressed cream colored hyphae. Colony has dark red appressed hyphae covered by a salmon-pink aerial mycelium (figure 14). Between 20-25 days, the entire colony becomes appressed with a slimy appearance. The colony becomes deep red with convolutions in the center. No sporulation occurs during the growth cycle. A yellow-orange pigment is produced in the medium. A positive reaction to NaOH occurs, resulting in a deep violet discoloration (figure 15).

2. Growth characteristics on OA: slow growing (2.5 mm/day), covering the entire colony in 18 days. Colony margin is even with appressed cream colored hyphae. Colony has mostly appressed, orange-red hyphae with some zones or tufts of aerial white hyphae (figure 16). Center of the colony usually darkens to reddish-brown with age. No sporulation occurs during the growth cycle. A slightly yellow pigment is produced in the medium; reaction to NaOH is positive with a deep violet discoloration resulting.

3. Pycnidia and conidia: none produced.

4. Hyphal characteristics: pigmented hyphae aggregate to form hyphal strands. Many individual hyphal cells are highly vacuolate. No chlamydospores are produced.

5. Identification criteria: This isolate cannot be identified because of its non-production of pycnidia or other distinguishing characters in culture. Its appressed, slimy colony appearance and positive (deep violet) reaction to NaOH is characteristic of P. herbarum Westend. (Boerema 1964; Boerema 1970; Dorenbosch 1970). However, P. herbarum isolates previously studied (Boerema 1964; James 1983a) produced abundant pycnidia on both PDA and OA. If this isolate is a species of Phoma, it is unknown why it will not sporulate, particularly since sporulation of other Phoma isolates studied can be induced by growing on OA in alternating light/dark regimes. Without sporulation, this isolate cannot be categorized at this time.

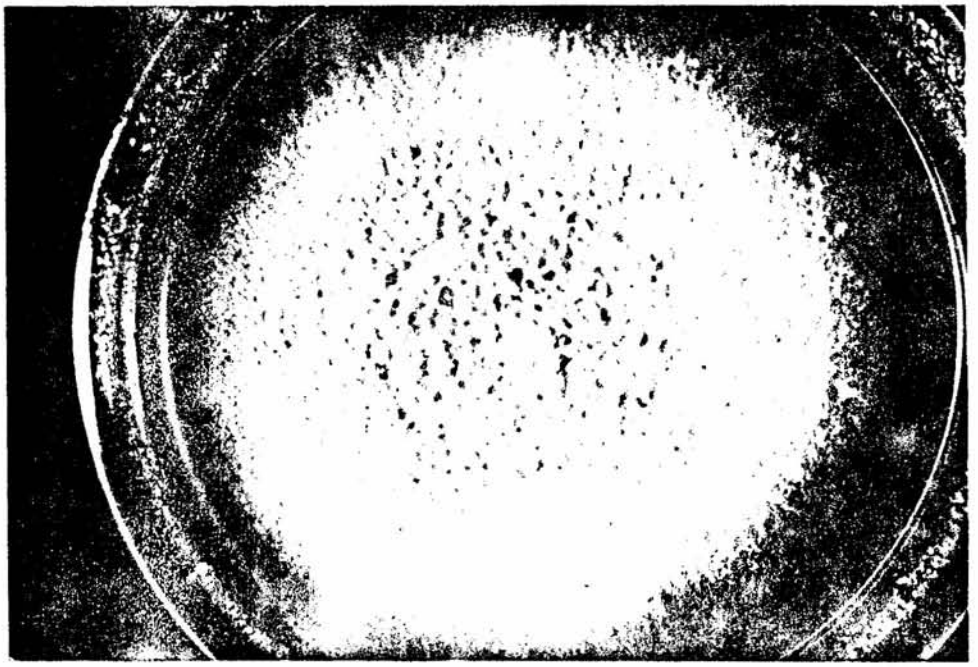

Figure 14. Isolate P2-8 on PDA after 14 days at 22°C. Red appressed hyphae are covered with a salmon-pink aerial mycelium.

Figure 15. Isolate P2-8 on PDA with positive reaction to NaOH resulting in deep violet discoloration.

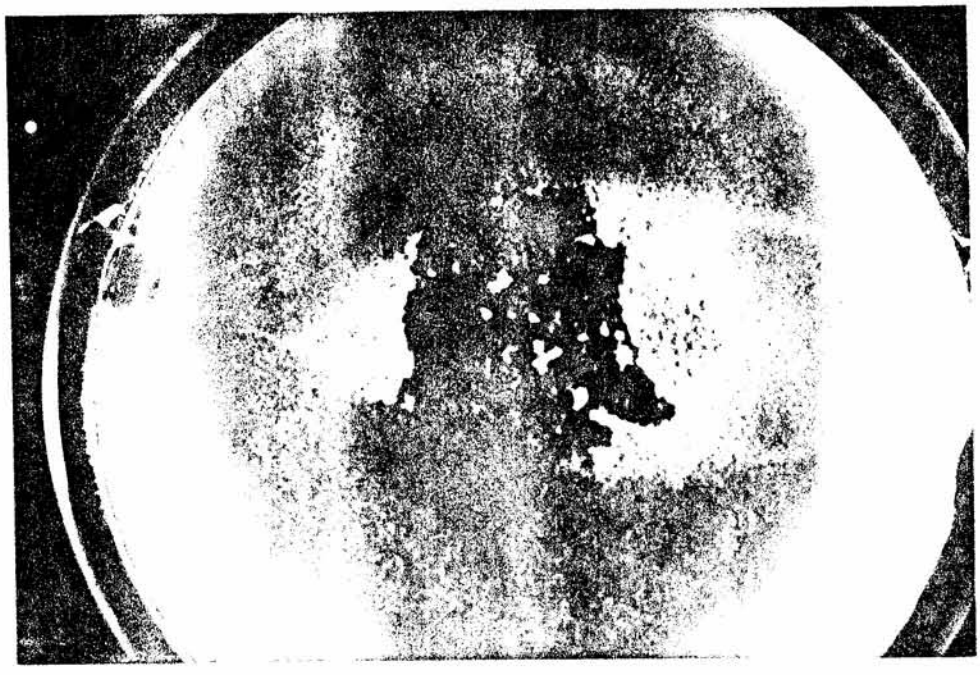

Figure 16. Isolate P2-8 on OA after 14 days at 22°C. Colony composed of appressed orange-red hyphae with zones and tufts of aerial white hyphae.

Isolate P1-7-1 (84-10)

1. Growth characteristics on PDA: moderately fast growing (6.5mm/day), covering entire agar surface in 7 days. Colony margin even with appressed cream-colored hyphae. Young colonies have extensive fluffy white aerial mycelium, portions of which become olivaceous-grey with age. Some aerial hyphae become grey and appressed hyphae become dark brown to black as colonies age. Pycnidia are produced after 7 days; by 14 days, profuse sporulation occurs beneath the whitish-grey aerial mycelium. A faintly yellow pigment is produced within the agar medium. No crystals are formed and reaction to NaOH is negative.

2. Growth characteristics on OA: moderate growth rate (6.2 mm/day), covering entire agar surface in 7 days. Colony margin is even with appressed white hyphae. Colony initially has fluffy white aerial mycelium which turns olivaceous-grey after 10 days. By 14 days most of the colony is olivaceous-grey with only portions of the white mycelium remaining (figure 17). Profuse sporulation occurs after 7 days throughout the colony. Pycnidial exudate is cream-yellow. No pigment or crystals are formed within the medium; reation to NaOH is negative.

3. Pycnidia and conidia: pycnidia are globose to oblong, subhyaline at first, then becoming carbonaceous. They are either separate or aggregated to form irregular-shaped fructifications with several ostioles. They are produced superficially on or immersed within the agar and measure 135-240μ ($\bar{x}$=189μ) in diameter. Conidia are hyaline, mostly one-celled (some two-celled spores occur), bacilliform to kidney-shaped, and measure 3.0-8.5μ ($\bar{x}$=5.5μ) in length and 1.5-3.0μ ($\bar{x}$=2.2μ) in width.

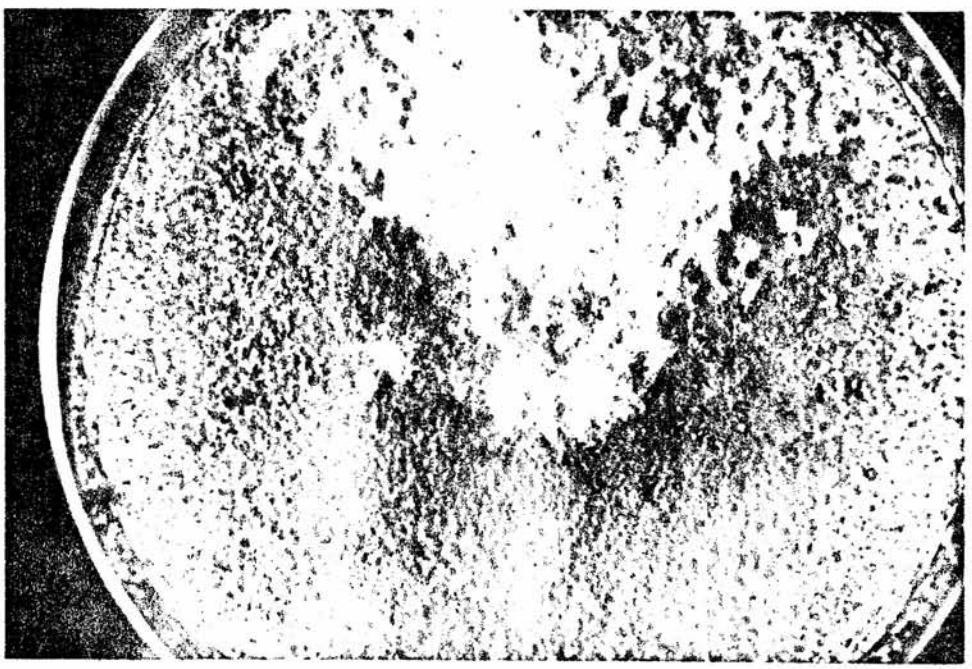

Figure 17. Isolate P1-7-1 on PDA after 14 days at 22°C. Colony is composed of mostly olivaceous-grey hyphae with some zones of aerial white hyphae.

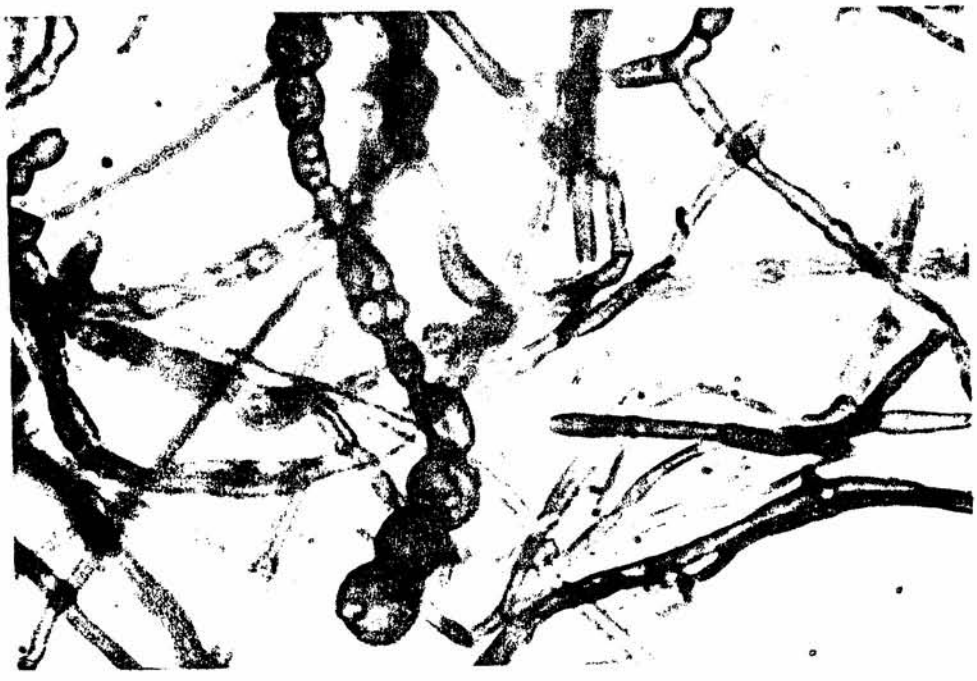

Figure 18. Catenulate chlamydospores from isolate P1-7-1 grown on PDA for 14 days at 22°C. (x450)

4. Hyphal characteristics: pigmented catenulate chlamydospores are abundant in PDA cultures 10-14 days old (figure 18). Chlamydospores measure from 8-18μ ($\bar{x}$=12.0u) in diameter and arise from individual hyphal cells. Hyphae with chlamydospores become aggregated into a tight, semi-sclerotioid network in older cultures.

5. Identification criteria: this isolate is very similar to isolate P1-7 and is related to P. eupyrena (Boerema 1976; Domsch et al. 1980; Dorenbosch 1970). Like isolate P1-7, it differs from the other two isolates of this collection (19-35, 19-36A) thought to be related to P. eupyrena. This isolate closely resembles some other P. eupyrena isolates studied previously (James 1983a; James 1983b; James 1984).

DISCUSSION

Phoma eupyrena is one of the most common soil-inhabiting members of Phoma (Diener et al. 1976; Domsch et al. 1980; Dorenbosch 1970). The fungus prefers upper soil layers (Warcup 1951) and has been reported in forest nurseries, particularly within sandy soils (Mollison 1953; Warcup 1951). Presence of chlamydospores favor its isolation from soil (Domsch et al. 1980).

Phoma eupyrena has been reported associated with several different plant diseases. It was first described growing in association with potatoes (Malcolmson 1958; Wollenweber 1920) and causes superficial necrosis on and dryrot of tubers (Boyd 1972; Kranz 1962). The fungus may cause damping-off symptoms of several plants (Hampel 1970) and has commonly been isolated from the rhizosphere of crop plants including wheat and some grasses (Domsch et al. 1980). P. eupyrena has also been associated with diseases of tree seedlings, including cankers of Russian-olive seedlings in Montana (James 1983a), mortality of red fir seedlings in California (James 1983b), and mortality, stunting and chlorosis of Mugo pine seedlings in Idaho (James 1984). Although P. eupyrena has been associated with several plant diseases, its role as a pathogen remains unclear. Although pathogenicity tests indicate that the fungus can attack plant tissues (Hampel 1970; Malcolmson 1958), it is often considered only a secondary invader (Boerema and van Kesteren 1961; Dennis 1946). In several cases, P. eupyrena is associated with diseased tree seedlings in the absence of other known pathogens (James 1983b; James 1984). This would indicate that the fungus is likely involved in disease processes. However, pathogencity tests have not been conducted to help elucidate the role of P. eupyrena in causing diseases of forest tree seedlings.

Phoma pomorum is a soil-borne fungus found on a wide variety of dead and diseased plant material (Boerema et al. 1965; Boerema et al. 1968; Dorenbosch 1970). Thirty-one plant families have been listed as hosts of the fungus (Boerema et al. 1971). Host genera which include forest trees are Acer, Alnus, Betula, Chamaecyparis, Juniperus, Pinus, Populus, Prunus, and Robinia. P. pomorum is perhaps best known as the causal agent of leaf spots on Prunus species in Europe (Boerema et al. 1965; Domsch et al. 1980; Dorenbosch 1970). The fungus has previously been reported in the United States (Huang and Schmitt 1975), but not on forest tree seedlings.

Other possible Phoma species in this group include P herbarum, P. exigua, and P. lingam. P. herbarum has a worldwide distribution on several different substrates including herbaceous and woody plants, soil and water (Boerema 1964; Boerema 1970). P. exigua is a common soil inhabitant, implicated in several soil-borne diseases and leaf spots (Boerema and Howeler 1967; Domsch et al. 1980). P. lingam is a soil-borne fungus often associated with leaf spots, particularly of Brassica spp. (Boerema 1976; Boerema and van Kesteren 1964). None of these three fungi have previously been reported on forest tree seedlings.

LITERATURE CITED

Boerema, G. H.
1964. Phoma herbarum Westend., the type-species of the form-genus Phoma Sacc. Persoonia 3:9-16.

Boerema, G. H.
1969. The use of the term forma specialis for Phoma-like fungi. Trans. Br. Mycol. Soc. 52:509-513.

Boerema, G. H.
1970. Additional notes on Phoma herbarum. Persoonia 6:15-48.

Boerema, G. H.
1976. The Phoma species studied in culture by Dr. R. W. G. Dennis. Trans. Br. Mycol. Soc. 67:289-319.

Boerema, G. H. and G. J. Bollen
1975. Conidiogenesis and conidial septation as differentiating criteria between Phoma and Ascochyta. Persoonia 8:111-144.

Boerema, G. H. and L. H. Howeler
1967. Phoma exigua Desm. and its varieties. Persoonia 5:15-28.

Boerema, G. H. and H. A. van Kesteren.
1961. Phoma-achtige Schimmels Bij Aardappel. Meded. Plziektenk. Dienst Wageningen 136 (Jaarb. 1961), 201-209.

Boerema, G. H. and H. A. van Kesteren.
1964. The nomenclature of two fungi parasitizing Brassica. Persoonia 3:17-28.

Boerema, G. H., M. M. J. Dorenbosch and H. A. van Kesteren.
1965. Remarks on species of Phoma referred to Peyronellaea. Persoonia 4:47-68.

Boerema, G. H., M. M. J. Dorenbosch and H. A. van Kesteren.
1968. Remarks on species of Phoma referred to Peyronellaea-II. Persoonia 5:201-205.

Boerema, G. H., M. M. J. Dorenbosch and H. A. van Kesteren.
1971. Remarks on species of Phoma referred to Peyronellaea-III. Persoonia 6:171-177.

Boerema, G. H., M. M. J. Dorenbosch and H. A. van Kesteren.
1977. Remarks on species of Phoma referred to Peyronellaea-V. Kew Bull. 31:533-544.

Boerema, G. H., H. A. van Kesteren and W. M. Loerakker.
1981. Notes on Phoma. Trans. Br. Mycol. Soc. 77:61-74.

Boyd, A. E. W.
1972. Potato storage diseases. Rev. Plant Pathol. 51:279-321.

Dennis, R. W. G.
1946. Notes on some British fungi ascribed to Phoma and related genera. Trans. Br. Mycol. Soc. 29:11-41.

Diener, U. L., G. Morgan-Jones, W. M. Hagler and W. D. Davis.
1976. Mycoflora of activated sewage sludge. Mycopathologia 58:115-116.

Domsch, K. H., W. Gams and T. H. Anderson.
1980. Compendium of soil fungi. Academic Press, New York. 859p.

Dorenbosch, M. M. J.
1970. Key to nine ubiquitous soil-borne Phoma-like fungi. Persoonia 6:1-14.

Grimes, M., M. O'Connor and H. A. Cummins.
1932. A study of some Phoma species. Trans. Br. Mycol. Soc. 17:97-111.

Hampel, M.
1970. Phoma eupyrena Sacc. ss Wr. und Plectosphaerella cucumeris Kleb., zwenwenig bekannte Keimlingspathogene. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz. 77:225-227.

Huang, L. H. and J. A. Schmitt.
1975. Soil microfungi of central and southern Ohio. Mycotaxon 3:55-80.

James, R. L.
1983a. Cankers of Russian-olive seedlings at the Montana State Forest Tree Nursery, Missoula, Montana. USDA For. Ser., Northern Region. Rept. 83-6. 6p.

James, R. L.
1983b. Characterization of Phoma species on red fir seedlings from the Humboldt Nursery, California. USDA For. Ser., Northern Region. 6p.

James, R. L.
1984. Mortality of Mugo pine seedlings at the Fantasy Farms Nursery, Peck, Idaho. USDA For. Ser., Northern Region. 7p.

Jenkins, A. E.
1943. Leaf spot on Terminalia arjuna. Phytopathology 33:404-405.

Kempton, F. E.
1919. Origin and development of the pycnidium. Bot. Gaz. 68:233-261.

Kranz, J.
1962. Vergleichende Untersuchungen as Phoma-isolierungen von der Kartoffel. Sydowia 16:1-40.

Luedemann, G. M.
1959. The dictyochlamydospore of Peyronellaea glomerata (Corda) Gordanich ex Togliani contrasted with the dictyoporospore of Alternaria tenuis Auct. Mycologia 51;772-775.

Malcolmson, J. F.
1958. A consideration of the species of Phoma which parasitize potatoes. Trans. Br. Mycol. Soc. 41:413-418.

Mollison, J. E.
1953. Effect of partial sterilization and acidification of soil on the fungal population. Trans. Br. Mycol. Soc. 41:413-418.

Shear, C. L.
1923. Phoma: a sample of mycological nomenclature and classification. Mycologia 15:174-182.

Stevens, R. B.
1974. Mycology guidebook. Univ. of Washington Press. Seattle. 703 p.

Sumner, D. R.
1974. Ecology and control of seedling diseases on crucifers. Phytopathology 64: 692-697.

Toussoun, T. A. and P. E. Nelson.
1968. A pictorial guide to the identification of Fusarium species. Pennsylvania St. Univ. Press, University Park. 51 p.

Wallace, B. and C. H. Dickinson.
1978. Peat microfungi in three habitats in the Florida Everglades. Mycologia 70:1151-1163.

Warcup, J. H.
1951. Effect of partial sterilization by steam or formalin on the fungus flora of an old forest nursery soil. Trans. Br. Mycol. Soc. 34:520-532.

Wehmeyer, L. E.
1946. Studies on some fungi from northwestern Wyoming. II. Fungi imperfecti. Mycologia 38:306-330.

Wollenweber, H. W.
1920. Der Kartoffelschorf. Arb. Forschinst. KartBau, Berl. 2:1-102.

Wollenweber, H. W. and H. Hochapfel.
1936. Beitrage zur Kenntriis parasitarer und saprophytischer Pilze. I. Phomopsis, Dendrophoma, Phoma und Ascochyta und ihre Beziehung zur Fruchtfaule. Zeitschur. f. Parasitenk. 8:561-605.