

CHARACTERIZATION OF PHOMA SPECIES ON RED FIR SEEDLINGS
FROM THE HUMBOLDT NURSERY, CALIFORNIA

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Nursery Disease Notes No. 1

Isolate Designation

83-12 (Bayleton Bed)
83-13 (Keith Wallin's Phoma)

In vitro Characteristics

A. Isolate 83-12

1. Rapid growth in the dark at 24° C on PDA (4.5 mm/day)
2. Young colonies with white, appressed margins; growth uniform. Some aerial white mycelium interspersed with darkened olivaceous hyphae in the center of young colonies (figure 1).
3. Colonies become dark green to black with age (figure 2). This is mostly due to chlamyospore formation.
4. Chlamyospores consist of elongate cells about 4-7 μ in diameter and are produced singly or in chains (figure 3). Chlamyospores are initially hyaline, but later become pigmented. Initial formation of chlamyospores occurs after 1 week in culture; they are quite common after 14-20 days. Chlamyospores more readily form on PDA or malt agar.
5. Pycnidia are typical of Phoma: separate (not aggregated on a stroma), usually with a single ostiole (sometimes multi-ostiolate), with a thin wall and brown-black surface. Pycnidia more readily form on oatmeal agar (OMA); they will form sparsely on PDA cultures after 4-5 weeks.
6. Conidia are ellipsoidal, 3-5 x 1.5-2.5 μ , hyaline, 1-celled, and usually biguttulate.

B. Isolate 83-13

1. Rapid growth on PDA (4.5-5 mm/day).
2. Colony has distinct white margin during early growth; center portion of colony is at first light grey, but becomes darker with age.
3. Mature colonies (4 weeks old) have concentric grey and white zones (figure 4). Submerged mycelium is dark green to grey; aerial mycelium is white and light grey to olivaceous (does not become as dark as 83-12).

4. No chlamydospores formed in culture after 4 weeks.
5. No pycnidia formed on either PDA or OMA after 4 weeks.
6. The following techniques were used to try to induce sporulation:
 - a. Exposure of OMA and PDA colonies to black light for 4 days (this will induce sporulation of most Botrytis isolates).
 - b. Growth on OMA for 4 weeks (all Phoma isolates I have previously grown will sporulate on OMA after this much time).
 - c. Pair cultures on PDA and OMA with isolate 83-12 and a stock of Phoma herbarum)pycnidia should form where the cultures come in contact).

None of these procedures were successful in causing sporulation.

Conclusions

1. Isolate 83-12 is probably Phoma eupyrena Sacc.; identify of isolate 83-12 is presently unknown.
2. Its habit of growth and the fact that it was isolated from the same host species as isolate 83-12, indicates that isolate 83-13 may be related to P. eupyrena. However, its lack of characteristic chlamydospores and failure to sporulate raises questions as to its affinities.
3. Identification of Coelomycetes with 1- or 2-celled conidia and separate pycnidia can be confusing. Growth characteristics, chlamyospore production, reaction with NaOH, and crystal formation are more helpful than spore size, shape, and septation in identification.

Characteristics of Phoma eupyrena

1. Phoma eupyrena is a common soil-inhabiting fungus which has been reported in Europe, India, Southeast Asia, and the United States.
2. The fungus is easily isolated from soil because of its production of chlamydospores.
3. It has been commonly reported from sandy forest nursery soils.
4. The fungus was first described as parasitizing potatoes, but has also been isolated where potatoes have never been grown.
5. The fungus is an initial reinvader of sterilized soils.

6. P. eupyrena has been isolated from various substrates including the rhizospheres of potatoes, wheat, and grass, aerial portions of grasses and weeds, and various necrotic tissues.
7. The fungus is usually considered a weak parasite. I have isolated it from above-ground cankers of Russian-olive seedlings and the tips of 1-0 Engelmann spruce seedlings with dieback symptoms. Pathogenicity was not confirmed in these two instances.

REFERENCES

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Figure 1. Young (4-day-old) colony of *Phoma eupyrena* (isolate 83-12) growing on PDA.



Figure 2. Four week-old cultures of *Phoma eupyrena* (isolate 83-12) growing on PDA and OMA.



Figure 3. Chlamydozoospores produced by *Phoma eupyrena* on PDA x 450.



Figure 4. Four week-old culture of isolate 83-13 growing on PDA.