Phoma Blight of Fir and Douglas-Fir Seedlings in a California Nursery

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ABSTRACT

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Phoma eupyrena was consistently associated with needle cast and blight of red fir (*Abies magnifica*) and Douglas-fir (*Pseudotsuga menziesii*) seedlings at a nursery near the coast of northern California. Symptoms typically developed during the dormant period between the first and second growing season. Heavy rains and soil splash resulted in the buildup of soil cones around the stem and lower crown, followed by infection of lower needles or buds by the soilborne fungus. Inoculations of greenhouse-grown seedlings with *P. eupyrena* in soil resulted in symptoms identical to those observed in the field. Protective fungicides tested on red fir gave little or no control. The addition of a redwood mulch and the use of a shade cover reduced soil splash, soil cone formation, and disease incidence.

The USDA Forest Service Humboldt Nursery, located near Arcata in coastal northern California, lost more than 2 million 1-0 Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings to a needle blight disease in 1971 and again in 1975. Infection occurred following buildup of soil cones (collars) around stems and lower foliage during winter rains. The fungus most commonly isolated from affected needles and stems was a species of *Phoma* (12), and the disease became known as Phoma blight. Field tests in 1971 (R. S. Smith, M. D. Srago, and A. H. McCain, *unpublished*) indicated that chlorothalonil and captafol effectively controlled the disease. Chlorothalonil, applied at 2- to 4-wk intervals from October through April, has since been used in a routine preventive spray program. However, in spite of this spray program, Humboldt Nursery still lost 12% (10.3 million seedlings) of its Douglas-fir crops to this disease during 1979–1983.

In 1979, a disease problem appeared in the 1-0 and 2-0 beds of red fir (*Abies magnifica* A. Murr.) and to a lesser extent in white fir (*A. concolor* (Gord. & Glend.) Lindl. ex Hildebr.). Symptoms included dieback or tip blight starting at or near the buds and progressing down the stem. A species of *Phoma* morphologically indentical to the species associated with tip blight of Douglas-fir was isolated from affected tissues. From 1979 through 1983, the nursery lost 50% (1 million) of its red fir seedlings and 22% (670,000) of its white fir seedlings because of the suspected *Phoma* disease. None of 10 fungicides tested in 1982–1983 effectively controlled Phoma blight on red fir (8,9). The objectives of this study were to determine the etiology of the disease and to develop effective chemical and cultural control methods.

MATERIALS AND METHODS

Field symptoms and isolations. Observations on disease development in Douglas-fir and red fir were made over a period of years (8,9,12; R. S. Smith, M. D. Srago, and A. H. McCain, *unpublished*) and during this study. Seedlings in various stages of symptom development were taken to the laboratory for isolation of associated organisms. Necrotic stem and needle tissues were surface-sterilized in 0.6% sodium hypochlorite for 1–3 min and incubated on various agar media. Emerging fungi were transferred to water agar (WA) or cornmeal agar (CMA) slants for identification.

Pathogenicity studies. Seedlings of 1-0 red fir and Douglas-fir were lifted in June 1983 at Humboldt Nursery, transported to Berkeley, potted individually in autoclaved U.C. mix (11) in 12.7-cm-diameter pots, and maintained in an unheated greenhouse until inoculated in early January 1984.

Dormant seedlings were inoculated with isolates of *P. eupyrena* Sacc. from infected 2-0 red fir and Douglas-fir seedlings at Humboldt Nursery and maintained on CMA at 20 C, or soil from beds with red fir seedlings affected by Phoma blight at Humboldt Nursery and air-dried for 3 wk, then stored at 21 C.

Seedlings were inoculated by two methods. For mycelial inoculations, a 3-wk-old culture on CMA was cut into 3-mm cubes with a sterile scalpel. A cube was placed on an unwounded individual needle, an unwounded terminal bud, or into a scalpel cut to the cambial area on the stem. Thirty (10 needle-inoculated, 10 bud-inoculated, 10 stem-inoculated) red fir seedlings and 30 Douglas-fir seedlings were inoculated. Red fir were inoculated with an isolate from red fir and Douglas-fir with an isolate from Douglas-fir. Fifteen (five needle-inoculated, five bud-inoculated, and five stem-inoculated) red fir seedlings and 15 Douglas-fir seedlings were inoculated with cubes of agar only. After inoculation, each seedling was misted with water, covered with a plastic bag for 24 hr, and observed several times a week for 3 mo. Then pieces of tissue from inoculation points were surface-sterilized for 3 min with 0.6% sodium hypochlorite, rinsed in sterile water, and placed on potato-dextrose agar (PDA).

For soil inoculations, a cone of soil was formed around each seedling by applying wetted soil to the stem from the ground line to the foliage. Soils used were naturally infested soil, autoclaved naturally infested soil, and autoclaved naturally infested soil with *P. eupyrena* added (two plates of a 3-wk-old culture on CMA diced and added to about 575 cm^3 of autoclaved soil). Ten red fir seedlings and 10 Douglas-fir seedlings were used for each of the three soils. After formation of soil cones, each seedling was covered with a plastic bag and misted several times a week for 3 mo to maintain a high relative humidity similar to that which occurs at Humboldt Nursery. After symptom development, affected pieces of needles or stems were surface-sterilized for 3 min with 0.6% sodium hypochlorite, rinsed in sterile water, and placed on PDA.

Field study. A test of cultural measures alone or with fungicidal sprays for control of Phoma blight was conducted on 1-0 red fir from October 1983 to July 1984. Chlorothalonil (1.8 g a.i./L), mancozeb (1.9 g a.i/L), and tri-basic

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copper sulfate (1.9 g a.i./L) (fungicides effective for a period of time in 1982–1983 tests [8,9]) were tested singly and in combination with a redwood mulch (a mixture of redwood bark and sawdust) (Redwood Compost, Arcata Redwood Company, Arcata, CA) or shade cover (shade frames 1.2 × 3.7 m covered with lath to provide 40% shade) to reduce soil cone formation. A split-plot design was used, with mulch, shade cover, and an uncovered control as the main plots and three fungicide treatments and one untreated control as the subplots. The experiment was replicated five times down a 1.2-m-wide bed of 1-0 red fir. Each replicate covered a 22-m length of bed containing four subplots 1.2 × 1.8 m in each of three (no mulch or cover, mulch, cover) 7.2-m plots.

Plots and subplots were randomized in each replicate. Fungicides were applied at monthly intervals from the first week in October 1983 through the first week in June 1984. A spreader/sticker (1.3 ml/L) was added to mancozeb and tri-basic copper sulfate but not to chlorothalonil. Efficacy of treatments was evaluated by counting and removing dead seedlings at monthly intervals. In July 1984, all remaining seedlings were counted to obtain a total count per subplot, and the percentage of seedlings killed in each treatment was calculated.

RESULTS

Field symptoms and isolations. Disease development in Douglas-fir and red fir typically begins after heavy rains during the dormant period between the first and second growing season. Soil splash results in the buildup of soil cones around the stem and lower foliage (Fig. 1). Initial symptoms occur on the lower needles and spread upward. Needles turn chlorotic, then golden brown and are cast. Dieback or blight of terminal and lateral branches occurs on both Douglas-fir and fir but is more common on fir. The dieback starts at or near the buds, progresses down the stem, and may result in death of the seedling. Tissues formed in the second growing season usually remain unaffected. Seedlings that have reached sufficient height during the first growing season to be above soil buildup escape symptoms or lose only their lower needles and survive.

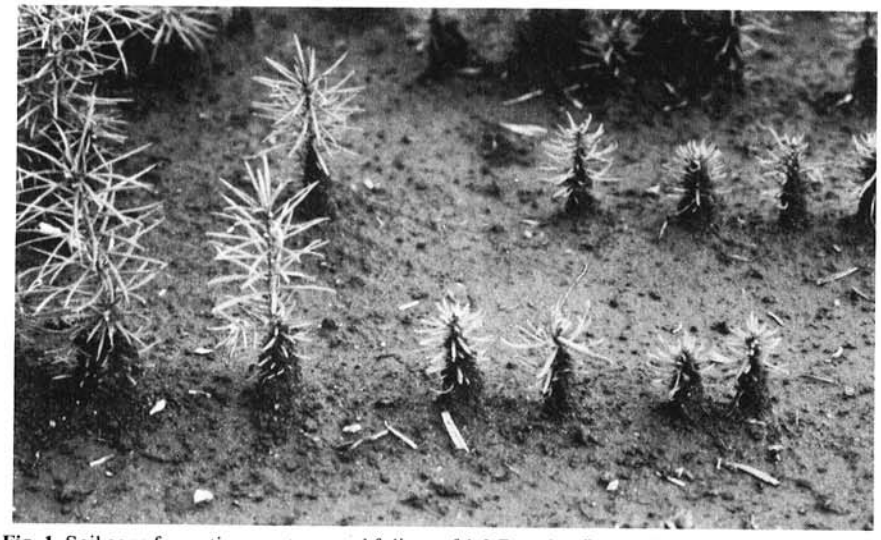

Fig. 1. Soil cone formation on stems and foliage of 1-0 Douglas-fir seedlings. The larger seedlings have outgrown the soil cones. Needle loss in lower foliage will occur, but the seedlings will survive through the second growing season. The smaller seedlings with soil cones extending to the upper crowns will not survive.

Table 1. Percentage of red fir seedlings killed by Phoma blight at the Humboldt Nursery as affected by fungicides and cultural treatments

	Fungicide[x]			
Cover	None	Chlorothalonil	Mancozeb	Tri-basic copper sulfate
None	20 a[y,z]	9	15	7
Lath	6 b	4	6	5
Mulch	6 b	3	5	4

[x] Fungicides applied with a pressurized sprayer at monthly intervals from 4 October 1983 through 5 June 1984 for a total of nine treatments. Rates used were 1.8 g a.i./L for chlorothalonil and 1.9 g a.i./L for mancozeb and tri-basic copper sulfate.

[y] Each value is the mean percentage of seedlings that died in five replicates over a 9-mo period.

[z] Cover means followed a common letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. F value for fungicide effects (2.49) or cover-fungicide interaction (0.82) were not significant ($P = 0.05$).

A species of *Phoma* was consistently isolated from affected tissues. Other fungi isolated infrequently included other species of *Phoma* and species of *Fusarium, Penicillium, Epicoccum, Trichoderma,* and *Phomopsis.* On the basis of pycnidia and conidia formed on dead needles in the field and oatmeal agar (OA) in the laboratory and of characteristics of chlamydospores formed on PDA, the fungus causing Phoma blight is *P. eupyrena.*

Pycnidia formed on dead needles in nursery beds and on OA are black, thin-walled, and typically separate. Conidia are one-celled, hyaline, ellipsoidal, and 3–5 × 1.5–2.5 μm. Colonies on OA and PDA are characterized by dense mycelial growth, which is dark green at first and then becomes black. Isolates typically form chlamydospores after a few days on PDA. The chlamydospores are hyaline at first, becoming pigmented, 4–7 μm in diameter, and are produced singly or in chains. The formation of dark chlamydospores in pairs or short chains is generally accepted as characteristic of *P. eupyrena* (2,10).

An isolate from red fir has been identified (R. James, *personal communication*) as *P. eupyrena.* Additional isolates from red fir and Douglas-fir are close to *P. eupyrena* but not identical with it because of the presence of some dictyochlamydospores in chains (A. Funk, *personal communication*). This characteristic is diagnostic for *P. glomerata* (Cda.) Wr. & Hochapf. (1).

Pathogenicity studies. Typical symptoms of blight observed in the field did not develop within 3 mo on seedlings inoculated with cubes of agar cultures. However, *P. eupyrena* was recovered from inoculated buds and wounded stems (9 of 10 bud-inoculated and 9 of 10 stem-inoculated red fir, 5 of 10 bud-inoculated and 6 of 10 stem-inoculated Douglas-fir). No other fungi grew from the surface-sterilized tissues when placed on agar. No fungi were recovered from inoculated needles or from tissues in the control treatments.

Typical blight symptoms developed on both red fir (7 of 10) and Douglas-fir (4 of 10) seedlings treated with naturally infested soil and with the same soil to which *P. eupyrena* was added after autoclaving (7 of 10 red fir and 7 of 10 Douglas-fir). *P. eupyrena* was isolated from symptomatic seedlings. No symptoms developed on seedlings treated with autoclaved soil, and *P. eupyrena* was not isolated from them.

Field study. A significant F value was found for cover effects but not for fungicide effects or for cover-fungicide interaction (Table 1). Both a mulch treatment and a lath cover reduced soil cone formation compared with uncovered plots and reduced disease incidence from 20 to 6%. The mulch treatment resulted in the formation of mulch cones rather than

soil cones. The mulch cones had no apparent adverse effect on the seedlings and prevented soil from building up around stems and lower foliage.

DISCUSSION

P. eupyrena is pathogenic on red fir and Douglas-fir seedlings and appears to be responsible for the considerable loss of seedlings at Humboldt Nursery. Inoculations under controlled conditions have confirmed pathogenicity. *P. eupyrena*, like other species of *Phoma*, is generally considered a weak pathogen or secondary invader of plants. However, the consistent association of this fungus with diseased seedlings at Humboldt Nursery, the lack of other fungi consistently associated with affected seedlings, and the recovery of the fungus from dead and dying seedlings after its introduction into the soil under controlled conditions indicate a causal role of this fungus in the observed disease. *P. eupyrena* has been associated with chlorosis of lodgepole pine seedlings (4), needle and twig blight of Engelmann spruce (5), mortality of Mugo pine (6), and tip blight of ponderosa pine seedlings (7) at nurseries in Nebraska and Idaho.

Observations over a period of years have led to the following suggested disease cycle and epidemiological behavior of Phoma blight at Humboldt Nursery. *P. euyprena* is a common soil-inhabiting fungus (3) and an initial reinvader of fumigated soil (13). The initial source of inoculum at Humboldt is not known. Soil splash during winter months results in buildup of soil cones around seedlings. This soil covering may reduce seedling vigor and provide a favorable environment for the fungus and for infection. The fungus invades needles or dormant buds from the soil. Recovery of the fungus from senescent bud scales and dead tissues associated with wounded stems after 3 mo in our inoculation study suggest that chlamydospores of the fungus may remain quiescent on host tissues for long periods until conditions favorable for infection occur. Pycnidia formed on dead needles may provide additional inoculum. Soil splash, as well as flowing water, during prolonged periods of heavy rain typical at Humboldt result in spread of inoculum through beds. Prolonged periods of heavy rainfall (102 cm total in December, January, and February) resulted in the buildup of soil cones and preceded the spread of the disease downslope through experimental beds in March 1983 (8,9).

Protective fungicides tested on red fir gave little or no control, perhaps because the tests were conducted during two abnormally wet winters. Production beds and the experimental bed went untreated for 8 wk in January and February 1983 because of almost daily rain. This 8-wk period coincided with the time that the disease appeared.

Preventing or reducing soil cone formation should reduce disease incidence. Less disease was observed in areas where moss was present on the soil. The moss apparently suppressed soil splash and buildup of soil cones. Mulch and lath cover reduced soil cone formation and reduced disease incidence. White fir seedlings in production beds were less affected than red fir seedlings, possibly because white fir grow larger than red fir during the first growing season and therefore outgrow the effects of soil cone buildup. The larger seedlings also provide a dense canopy that intercepts raindrops and reduces soil splash.

Early sowing of seed can increase first-year seedling growth and apparently suppress the disease. Since 1978, sowing of seed in March at Humboldt Nursery has resulted in greater top growth of Douglas-fir seedlings during the first year than was typical in prior years when seed was sown in May. Less damage and mortality have occurred since this cultural change, apparently because seedling foliage is sufficiently above the soil cone that it seldom becomes infected. However, since cool wet spring weather often delays sowing, our current recommendation is to sow seed as early as practicable and to apply a redwood mulch to beds of 1-0 red fir, white fir and Douglas-fir before the winter rainy season. Additional tests are needed to determine the effectiveness of different types of mulch and the most efficient method and time of application.

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