Cylindrocladium crotalariae Kills Container-Grown Northern Red Oak (*Quercus rubra* L.)

Steven W. Oak and Joey D. Triplett

Plant pathologist and biological laboratory technician, USDA Forest Service, State and Private Forestry, Asheville, NC

The fungus Cylindrocladium crotalariae (Calonectria crotalariae) *was associated with root-diseased container-grown northern red oak (*Quercus rubra *L.) seedlings. Inoculation trials confirmed the pathogenicity of the fungus. This is the first reported occurrence of* C. crotalariae *root rot of northern red oak. Control measures are discussed.*

Northern red oak seedlings are grown at the USDA Forest Service Resistance Screening Center (Asheville, NC) for the purpose of producing basidiospore inoculum of the fusiform rust fungus (*Cronartium quercuum* f. sp. *fusiforme*) to infect slash (*Pinus elliottii* Engelm.) and loblolly (*P. taeda* L.) pines. The acorns are collected, heat-treated to destroy insect pests, dusted with captan (50 WP), and stored in plastic bags at 0.5 to 3.5 °C for no less than 3 months and up to 3 years. Acorns are germinated in a vermiculite medium in a mist bed. Single acorns are then planted in 4- by 4-inch pots filled with a steam-treated mixture of loamy field soil, sand, and peat moss (3:2:1, v/v mix). Pots are watered every 3 to 4 days by individual drip tubes connected to a common supply pipe. Normally, 90 percent of acorns germinate.

The seedlings are incubated in a greenhouse on raised benches at 21 °C and 24 hours of daylight-artificial light for 6 weeks, when leaves are inoculated for eventual basidiospore production. After the leaves are harvested, the seedlings are removed and the potting medium is saved to grow future crops. The soil is reused 4 to 5 times before being discarded. Pots are washed with 50 percent aqueous sodium hypochlorite (Clorox) and thoroughly rinsed with clean water before reuse. These methods have been successfully used since 1974.

In December 1981, a 5-week-old seedling lot had many ungerminated acorns; seedlings showing foliage dwarfing, marginal necrosis, or wilt; and dead seedlings (fig. 1). Only 25 percent of the seedlings had developed normally. The roots of diseased seedlings were sparse, with black and necrotic tap roots. Radicles of ungerminated acorns were completely rotted just below groundline, and parts of the acorn were discolored. White fungus mycelium was occasionally present. In a few cases, perithecia of a *Calonectria* sp. were observed on affected acorns.

Fungus isolations from symptomatic roots and acorns were made on Czapek's medium with 100 parts per million streptomycin sulfate added (CZK agar).

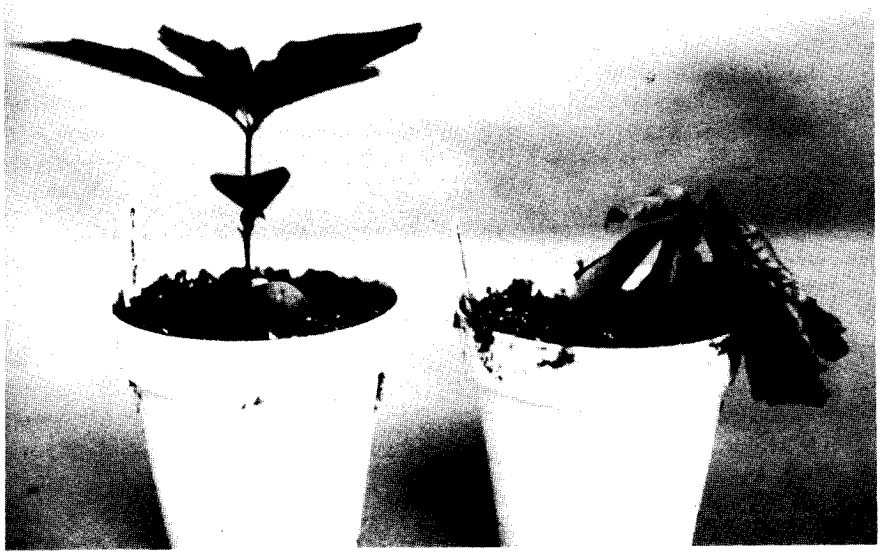

Figure 1—*Healthy and diseased 5-week-old northern red oak seedlings. Root system of diseased tree was sparse, discolored, and decayed.*

Additionally, alfalfa baiting (1), a rapid, qualititative method for detecting *Cylindrocladium* spp. from soil, was attempted on samples from the field-soil supply pile and from soil in pots with killed seedlings.

Cylindrocladium crotalariae was isolated from root systems, acorns, and killed alfalfa sprouts from baited potting medium. This fungus is the asexual stage of *Calonectria crotalariae*. Alfalfa baits from the field-soil supply pile did not yield any *Cylindrocladium* isolates. Inspection of stored acorns showed them to be free of any decay or degradation.

Cylindrocladium spp. are some of the most damaging root disease fungi found in bareroot hardwood tree nurseries. Yellow-poplar *(Liriodendron tulipifera* L.) (4), black walnut (*Juglans nigra* L.) (3), sweetgum *(Liquid-ambar styraciflua* L.) (4), and cherrybark oak (*Quercus falcata* var. *pagodifolia* Ell.) (5) have been severely damaged. Microsclerotia are formed in soil and infected root tissue and are difficult to control completely, even with methyl bromide-chloropicrin fumigants (MC 33) (1). Root diseases caused by *Cylindrociadium* spp. have been infrequently found in forest stands (2, 7).

Because *C. crotalariae* was associated with the symptoms and was not known to damage northern red oak, we evaluated its pathogenicity.

Methods

The inoculum used for pathogenicity trials was prepared by inoculating single-spored isolates of *C. crotalariae* into 2-quart glass jars filled with autoclaved oat seeds. Jars were incubated until the oats were completely colonized and microsclerotia developed (about 6 weeks).

Two inoculation methods were used. The first involved planting germinated acorns into the potting medium routinely used at the Resistance Screening Center but infested with colonized oats at high and low rates (1:10 and 1:50, oats potting medium, respectively). In the second method, washed root systems of 1-month-old seedlings were dipped in water suspensions of microsclerotia. Suspensions were obtained by grinding and repeated decanting of colonized oats until only microsclerotia and water remained. Three dilutions were prepared representing high-, medium-, and low-inoculum doses. Ten root systems were dipped in the inoculum and washed, and the rinsate was collected. The inoculum dose per seedling root system was determined by dilution-plating the rinsate on CZK medium (30, 12, and 8 propagules per root system for high, medium, and low doses, respectively). Seedlings planted in potting medium with sterilized oats and seedlings given root dips into the washings of ground sterilized oats served as controls.

Inoculations were repeated with a 1:1 mix of screened peat moss/vermiculite to determine if potting medium influenced disease.

All control and inoculated seedlings were fertilized at establishment and every 4 weeks thereafter with a soluble fertilizer (15N-30P-15K) in water until the soil was thoroughly wetted. Pots were irrigated by hand as needed. Seedlings were incubated for 10 weeks in a greenhouse under natural light between 12 and 30 °C. The media incorporation trial was terminated after the incubation period, and all seedlings were removed from their pots. Roots were evaluated for disease symptoms and cultured on CZK agar after surface sterilization (2 minutes in 10 percent aqueous sodium hypochlorite). Seedlings inoculated by root dip were removed immediately after they died and were similarly cultured.

Results

Some northern red oak seedlings were killed in all inoculation trials regardless of inoculation method, potting medium, or inoculum rate (table 1). The principal symptom for the medium incorporation method was damping-off (fig. 2)--only 6 of the 33 total killed seedlings initiated shoot growth before

Table 1—*Percentage of container grown northern red oak seedlings with symptoms of root disease after two root inoculation methods with* Cylindrocladium crotalariae *(incubation period = 10 weeks).*

Inoculum rate	Percentage diseased seedlings[a]					
	Inoculum in medium			Root dip inoculation		
	Soil	Mix	Average	Soil	Mix	Average
Low	66	83	75	0	33	17
Medium	NA	NA	NA	33	42	38
High	83	42	63	33	33	33
Average	75	63	69	22	36	29

[a] Symptoms include damping-off, dwarfed foliage, marginal leaf necrosis, and wilting. NA = not applicable.

Figure 2—*Medium-incorporated inoculation method. Controls at left and damped-off inoculated at right.*

death. Trees that died after initiating growth showed top symptoms that included dwarfed foliage, marginal leaf necrosis, and wilting. Roots of the damped-off seedlings were well decayed and often consisted of only a stub protruding from the germinated acorn. Root systems of seedlings without top symptoms at the end of the evaluations were well developed and fibrous, regardless of treatment.

Seedlings in the root dip method began dying 13 days after inoculation. Roots of killed and symptomatic seedlings showed varying degrees of necrosis, a water-soaked appearance in the laterals, and black discoloration in the woody part of the tap root.

One control seedling in the artificial medium/oat incorporation (high rate) treatment never completed germination. Two control seedlings in the root dip treatment showed marginal leaf necrosis and leaf dwarfing, but roots of these trees were asymptomatic.

Cylindrocladium crotalariae was reisolated from 43 percent of all symptomatic seedlings in the inoculation trials, but a higher percentage of the successful isolations were obtained from the root dip method than from the medium incorporation method (57 and 33 percent, respectively). Only saprophytic fungi were associated with the ungerminated control.

Control measures and discussion

When the outbreak was detected, all oaks were removed from the greenhouse and discarded, as was all previously used soil. Tools, pots, greenhouse benches and floors, and the drip tube watering system were thoroughly washed with 50 percent aqueous sodium hypochlorite and rinsed with clean water. A new crop of oaks was planted with the operational potting medium (steam-treated field soil, sand, and peat moss in 3:2:1, v/v mix). This crop grew normally for 2 to 3 weeks but then developed symptoms of root disease. Fruiting of *Calonectria crotalariae* (sexual stage of *Cylindrocladium crotalariae*) was observed on seedling roots and acorn tissue. After repeating the hypochlorite cleaning, oaks were planted in artificial medium (1:1, peat moss/vermiculite). Several subsequent oak crops have been grown in artificial medium without recurrence of the root disease. We suspect that field soil used in the potting medium mix was infested with the root disease organism.

Control recommendations based on our experience and the known action of *Cylindrocladium* spp. in bareroot hardwood nurseries (1, 3) can be suggested.

- ?? Avoid using field soil as a growing medium.
- ?? If field soil must be used, fumigate with methyl bromide-chloropicrin fumigants (MC 33 preferred).
- ?? Attempt to use commercially formulated or other "defined" potting media. Consider treating this material to reduce or eliminate potential pests as added insurance.
- ?? Avoid reusing potting medium from containers where seedlings have died; pests that may have had a role in the mortality could still be present in the medium.
- ?? Do not rely on steam treatment for total control of soilborne disease organisms.
- ?? Frequent reconnaissance of plant beds can aid in detecting problems before they limit production. Timely and aggressive pest control and culling of damaged stock, when necessary, will minimize losses.

Literature Cited

1. Cordell, C.E. The biology of *Cylindrocladium* spp. in southern forest tree nurseries. Ph.D. Thesis, Duke University; 1976. 189 p.
2. Cordell, C.E.; Rowan, S.J. *Cylindrocladium scoparium* infection in a natural sweetgum stand. Plant Disease Reporter 59: 775-776; 1975.
3. Cordell, C.E.; Skilling, D.D. *Cylindrocladium* root rot. In: Peterson, G.W.; Smith, Jr., R.S. (eds.). Forest nursery diseases in the United States. Agric. Handb. 470. Washington, DC: U.S. Department of Agriculture, Forest Service; 1975.126 p.
4. Cordell, C.E.; Juttner, A.S.; Stambaugh, W.J. *Cylindrocladium floridanum* causes severe mortality of seedling yellow-poplar in a North Carolina nursery. Plant Disease Reporter 55: 700-702; 1971.
5. Kuhlman, E.G.; Cordell, C.E.; Filer, T.H. Cylindrocladium root rots of sweetgum seedlings in southern forest tree nurseries. Plant Disease 4: 1079-1080; 1980.
6. Ross, E.W. Association of *Cylindrocladium scoparium* with mortality in a 27-year-old yellow poplar plantation. Plant Disease Reporter 51: 38-39; 1967.
7. Smyly, W.B.; Filer, Jr., T.H. *Cylindrocladium scoparium* associated with root rot and mortality of cherrybark oak seedlings. Plant Disease Reporter 61: 577-579; 1977.