Mycosphaerella Leaf Spot of Black Walnut

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ABSTRACT

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A leaf spot disease of black walnut (*Juglans nigra*) that causes premature defoliation in plantations has been found in Illinois, Indiana, Iowa, and North Carolina. The causal fungus is *Mycosphaerella juglandis*, heretofore known by the name of its anamorph, *Cylindrosporium juglandis*. Symptoms are described; etiology and the causal organism's characteristics are reviewed. Many *Juglans* species and hybrids are susceptible to infection by *M. juglandis*. *Carya ovata* and *C. illinoensis*, also Juglandaceae, are resistant. The disease can be controlled on black walnut by four spray applications of benomyl.

When plantations of black walnut (*Juglans nigra* L.) with poor growth characteristics were surveyed in southern Illinois in 1977, a small, angular leaf spot was often found. On the undersurfaces of the spots, conidiomata were present similar to those described by Wolf (4) as *Cylindrosporium juglandis* Wolf on English walnut (*J. regia* L.). Attempts by Wolf to infect *J. nigra* with *C. juglandis* were unsuccessful. In 1984, the teleomorph of the black walnut-associated fungus was found and described as *Mycosphaerella juglandis* Kessler (1). Symptoms, host range of the causal organism, and control of the leaf spot on black walnut are described in this paper.

MATERIALS AND METHODS

Isolation. Diseased leaflets were collected, stored in sealed white plastic bags, and transported to the laboratory. Lesions were inspected for conidiomata. Those bearing abundant conidiomata were placed in moist chambers and incubated overnight at room temperature. The next day, conidiomata with oozing spore masses or tendrils were selected and spore clusters were removed with a sterile dissecting needle to tubes of sterile distilled water. After agitation to disperse spores, the suspensions were plated onto sucrose-yeast extract agar (10:2 g) that contained 100 μg of streptomycin sulfate and 100 μg of penicillin G per gram. After several days, single isolated germinating conidia were transferred to agar slants and stored at 5 C.

Pathogenicity. Oatmeal agar cultures of five representative isolates were grown for 14–28 days at 24 C until abundant conidia had formed. Plates were flooded with sterile distilled water and conidia were dislodged with a sterile wire loop. For several inoculation test series, the resulting suspensions were adjusted to 1×10^4–6.9×10^5 spores per cubic centimeter with sterile distilled water. Plants were sprayed to runoff with plastic household sprayers. Inoculated potted trees were incubated for 2 or 3 days in moist chambers humidified by intermittent misting in a greenhouse maintained at 22–30 C. Leaves inoculated with spore suspensions in the field were enclosed in white plastic bags for 2 days to maintain wet surfaces during incubation.

Spore trapping. In two black walnut plantations, Vaseline-coated slides (3) were placed at the bottoms of the canopies of four trees. Slides were removed from the plantations weekly and replaced with fresh ones. Spore concentrations on the slides were determined by counting the spores observed in three transects (1.2×22 mm) over each slide at $\times 100$.

Host range. Several *Juglans* species and hybrids were evaluated for resistance to *M. juglandis*. Field plots had been established between 1968 and 1970 with selections of *J. nigra, J. regia, J. cinerea* L., *J. ailantifolia* Carriere, *J. ailantifolia* var. *cordiformis* (Maxim.) Rehder, *J. nigra* × *cinerea, J. nigra* × *ailantifolia* var. *cordiformis, J. regia* × *nigra, J. ailantifolia* var. *cordiformis* × *cinerea*, and *J. ailantifolia* × *cinerea*. Plots were arranged in a randomized complete block design with four trees per plot and with five replicates per selection. Spacing was 3.6×2.6 m. No fungicides were applied to the trees. Herbicides were applied to control herbaceous competition for the first 2 yr after planting. Subsequently, the plantation was mowed once a year. Trees were evaluated for natural infection in 1983 and 1984.

Control. Benomyl (50WP) at a concentration of 6 g a.i./L was applied with a Solo 423 mist blower four times at 2-wk intervals to 11-yr-old black walnut trees beginning on 20 June. Four liters of spray mixture covered 10 trees (average height 5 m). Fungicidal effectiveness was determined by comparing the percentages of leaflets yellowed, fallen, and bearing lesions on treated trees versus the unsprayed controls on 20 August—2 wk after the last spray application.

RESULTS AND DISCUSSION

Symptoms. First lesions appeared 1–2 wk after black walnut leaves had reached their final size in late spring. Isolated necrotic lesions were angular and as large as 4 mm in diameter (Fig. 1A). A halo of chlorotic tissue often surrounded lesions. By midsummer, as lesion numbers increased from secondary infections, affected trees began to look chlorotic from a distance. Coalescing lesions produced a vein pattern (Fig. 1B) or a leaf scorch symptom (Fig. 1C). Lesion-bearing leaflets became increasingly chlorotic, and by mid-August, many of them abscised, particularly when the weather was dry.

Distribution. Mycosphaerella leaf spot

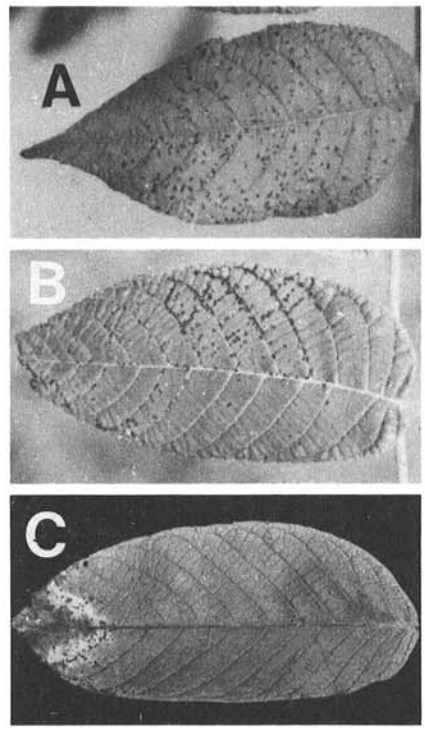

Fig. 1. Symptoms caused by *Mycosphaerella juglandis* on leaves of *Juglans nigra*: **(A)** typical scattered lesions, **(B)** vein pattern, and **(C)** leaf scorch.

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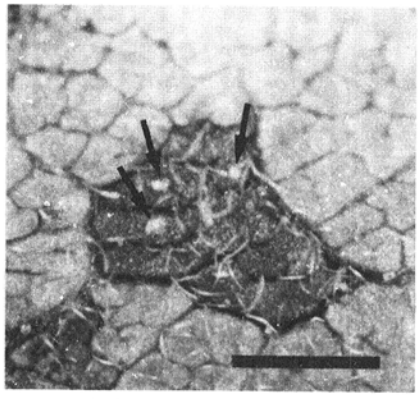

Fig. 2. White conidial masses (arrows) of *Cylindrosporium juglandis*, anamorph of *Mycosphaerella juglandis*, on lesion on *Juglans cinerea*. Scale bar = 0.25 mm.

Table 1. Trapping of conidia of *Cylindrosporium juglandis* on Vaseline-coated slides in relation to symptom development during 1978 and 1979

Dates	1978		1979	
	Conidia/mm²	Symptoms	Conidia/mm²	Symptoms
3–10 July	0.15[a]	Lesions	0	Lesions
10–17 July	0.30	···	0.99	···
17–24 July	0.47	···	4.19	···
24–31 July	6.58	···	11.20	···
31 July–8 August	16.57	Noticeable defoliation of infected leaves[b]	3.84	···
7–14 August	17.09	···	27.99	···
14–21 August	1.18	···	5.67	Noticeable defoliation
21–28 August	10.13	···	5.62	···
28 August–4 September	6.87	···	3.72	80% Defoliation
4–11 September	0.92	80% Defoliation	4.99	···
11–18 September	0.69		0.71	···
18–25 September	0.02	99% Defoliation	0.59	99% Defoliation

[a] From counts of conidia in three transects (1.2 × 22 mm) on each slide at ×100.
[b] Determined by counting abscission scars on leaf rachises.

was found on black walnut in plantations in Illinois, Indiana, Iowa, and North Carolina. Neely and Black (2), surveying roadside black walnut trees in five states, found small lesions of unknown cause in Ohio, Indiana, Kentucky, and Missouri. The size of their lesions (< 1 mm) and the concentration along veins suggest that they may also have been caused by *M. juglandis*.

Causal agent. Conidia-bearing conidiomata appeared on upper and lower lesion surfaces about 2 wk after lesions appeared but were much more common on the lower surface. When spores were released, conidiomata were easily detected by the presence of white spore masses on dry leaf surfaces (Fig. 2). When conidial masses were absent, conidiomata were difficult to detect unless the leaves were first wetted to cause swelling.

Conidiomata were dark and measured 50–100 μm in diameter. Although more commonly present on large lesions, they could be found on lesions as small as 0.6 mm in diameter on *J. nigra*. The smallest lesions (0.4 mm in diameter) to bear conidiomata were found on *J. ailantifolia*. Conidia were hyaline and contained as many as nine cells. Dimensions were 28–46 × 2.1–3.5 μm.

Conidia produced in conidiomata on oatmeal agar were similar to those produced in nature. Conidia were also produced on scattered conidiophores on agar media. These conidia were more variable in morphology. Conidial germination occurred either apically from end cells or laterally from middle cells. In culture, the fungus grew slowly and averaged 1 mm or less of radial growth per day on oatmeal agar at 25 C. Maximum growth occurred near 27 C. A temperature of 32 C was unfavorable for growth and mycelia did not develop above 34 C.

In culture, early conidial production could be encouraged by inoculating plates with spore suspensions, either by flooding the plates with suspensions or by point or streak inoculation with a transfer loop. Sporulation was most abundant on potato, intermediate on sucrose-yeast extract, and least on cornmeal and oatmeal agar media. The heavier sporulation on potato and sucrose-yeast extract agars was due in part to production of conidia on moniliaceous conidiophores rather than in conidiomata. Moniliaceous conidia were more irregular in shape, slightly shorter, and straighter than those produced in conidiomata.

Although pycnidia, which produce small spermatialike cells, appear on agar media, particularly on cornmeal and oatmeal agars, no teleomorphic state has been found in culture. In nature, the teleomorph develops on fallen, infected leaflets. Pseudothecia are present by February in southern Illinois. Mature asci bearing ascospores appear about the first of May (1).

Etiology. Primary infections from ascospore inoculum as indicated by spore trapping occurred in May and early June. Conidiomata appeared on primary lesions after 10–14 days. As ascertained by trapping them on Vaseline-coated microscope slides, conidia were disseminated from tree to tree primarily by windblown rain and within crowns by rain splash. Conidia first appeared on trap slides toward the end of June, increased gradually during July, and reached maximum population in August (Table 1). As leaves fell in August and September, the number of conidia trapped gradually decreased.

Lesions appeared 1–3 wk after leaf inoculation with conidia in both greenhouse and field studies. Lower leaf surfaces were more susceptible to infection than were upper leaf surfaces. Lower leaf surfaces of seedlings sprayed with conidial suspension averaged five times as many lesions as did sprayed upper surfaces.

Inoculations of white ash (*Fraxinus americana* L.), sweetgum (*Liquidambar styraciflua* L.), red oak (*Quercus rubra* L.), white oak (*Q. alba* L.), sycamore (*Platanus occidentalis* L.), shagbark hickory (*Carya ovata* (Mill.) K. Koch), and pecan (*C. illinoensis* (Wang) K. Koch) were negative. Inoculations of *J. regia*, *J. major*, *J. microcarpa*, and *J. nigra* × *microcarpa* were positive, and lesions similar to those on *J. nigra* developed. Although Wolf (4) was unable to infect black walnut with conidial inoculum from English walnut, no difficulty was encountered in doing so in this study. These positive inoculation results and the similarity of anamorph states as described by Wolf (4) on *J. regia* to the one on *J. nigra* (1) indicate that Wolf's *Cylindrosporium juglandis* can be accepted as the anamorph of *M. juglandis*.

Table 2. Effectiveness of benomyl in controlling Mycosphaerella leaf spot in an 11-yr-old black walnut plantation

Treatment	Mean percentage of leaflets		
	With lesions	Yellowed	Fallen
Benomyl[a] (6 g a.i./L)	2.4	1.8	2.8
Unsprayed control	78.6	24.3	32.5

[a] Applied with a Solo 423 mist blower four times at 2-wk intervals beginning on 20 June; symptoms evaluated 2 wk after final spray.

The host range of *M. juglandis* within the genus *Juglans* appears to be wide. Lesions similar to those found on *J. nigra* and bearing fruiting bodies were found on *J. cinerea*, *J. ailantifolia*, *J. regia*, and *J. ailantifolia* var. *cordiformis* and on hybrids *J. ailantifolia* × *cinerea*, *J. ailantifolia* var. *cordiformis* × *cinerea*, *J. nigra* × *ailantifolia* var. *cordiformis*, and *J. regia* × *nigra*.

Control. Benomyl controlled the disease in the field when applied four times at 14-day intervals with a mist blower beginning on 20 June in 1979 (Table 2).

LITERATURE CITED

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