Pathogenicity of *Tubakia dryina*

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ABSTRACT

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Tubakia dryina has been associated with leaf spots of *Quercus* spp. and trees of other genera, but its pathogenicity has not been established. Isolates of *T. dryina* were collected from several *Quercus* hosts. Excised leaves of *Q. alba, Q. rubra, Q. robur, Q. velutina, Q. imbricaria, Q. falcata* var. *pagodaefolia, Q. palustris*, and chlorotic *Q. palustris* developed necroses when inoculated with isolates from *Q. palustris* or *Q. imbricaria*. Excised leaves of *Acer saccharum, Nyssa sylvatica, Cercis canadensis, Carya ovata, Fraxinus americana*, and *Liquidambar stryaciflua* did not develop necroses after inoculation. An isolate of *T. dryina* from *Castanea pubinervis* also caused necroses on excised oak leaves but not on other leaves. Branches of *Q. rubra, Q. falcata* var. *pagodaefolia, Q. palustris*, and chlorotic *Q. palustris* developed symptoms after inoculation in the field with an isolate from *Q. palustris*. Symptoms developed more rapidly with later inoculations and were more severe on chlorotic *Q. palustris* than on any other species.

Additional keywords: *Actinopelte dryina*

Fungi in the genus *Tubakia* Sutton (12), formerly known as *Actinopelte* Sacc. (11), have been associated with foliar diseases of various oaks (*Quercus* spp.) and other deciduous trees (1,3,7,8). The distribution of the genus is probably worldwide (4), but in the United States, only *T. dryina* (Sacc.) Sutton has been reported, primarily in states east of the Mississippi River. In Illinois, the hosts of *T. dryina* include almost every oak species in the state (*Q. alba, Q. bicolor, Q. coccinea, Q. imbricaria, Q. falcata, Q. marilandica, Q. macrocarpa, Q. palustris, Q. rubra, Q. shumardii, Q. stellata*, and *Q. velutina*) and other tree species (*Acer saccharum, Cercis canadensis, Fraxinus americana, F. nigra, F. tomentosa, Nyssa sylvatica, Liquidambar styraciflua, Sassafras albidum, Rhus radicans, Ulmus alata*, and *Carya ovata*). Hosts in other regions include: *Q. glauca, Q. phillyraeoides, Castanea pubinervis*, and *C. sativa* in Japan; *Q. pedunculata* and *Q. pseudo-rubrae* in Italy; *Q. robur* in the Netherlands; and, in the United States, *Castanea vesca* and *Malus sylvestris* in New Jersey and *Eucalyptus* sp. in Louisiana (1,7,8).

In the Urbana-Champaign, Illinois, area, *T. dryina* frequently occurs on pin oak (*Q. palustris* Muench.) and northern red oak (*Q. rubra* L.) but is rarely observed on other species. It is most commonly observed on pin oaks that are chlorotic. On several occasions, spots associated with *T. dryina* have been observed on northern red oak leaves that are also infected with another locally common fungus, *Cylindrosporium quercus* Sorok. (9).

Symptoms associated with *T. dryina* on oak leaves are roughly circular, dark to reddish brown spots, 2–15 mm in diameter. The spots often coalesce to form irregular blotches, which sometimes follow the large leaf veins. The spots or blotches are usually adorned with numerous black pycnothyria, 70–100 μm in diameter, scattered or in concentric rings. Pycnothyria can occur on either the adaxial or abaxial leaf surface. Severely affected trees are prematurely defoliated (Fig. 1A–E, L–N).

The pathogenicity of *T. dryina* has not been proved. Most reports on the fungus have not included pathogenicity tests. Yokoyama and Tubaki (14) did not observe symptoms on seedlings of *Castanea pubinervis* (Hassk.) C. K. Schn., *Quercus glauca* Thunb., or *Q. phillyraeoides* A. Gray following inoculation with *T. dryina*. Holdenreider and Kowalski (5) also failed to induce symptoms following inoculation of *Q. robur* L. Kim and Wagner (6) reported symptom development on inoculated leaves of a "*Quercus* sp."

The objectives of this study were to establish the pathogenicity of *T. dryina* on several of its reported hosts and to compare the ability of isolates from different tree species to colonize the leaves of other species. Many fungi now known as *T. dryina* were originally collected from diverse hosts and assigned to a variety of genera. Limber and Cash (8) reduced these species to synonymy with *T. dryina*. Glawe and Crane (2) found Illinois collections from different hosts to be morphologically indistinguishable. It remains to be determined whether collections from different hosts are equally indistinguishable in terms of pathogenicity.

MATERIALS AND METHODS

Symptomatic leaves of reported hosts were excised, and isolations were made in the laboratory. Conidiomata were picked from the leaf with a sterile needle and placed on potato-dextrose agar (PDA) containing streptomycin sulfate (0.2 g/L) in 9-cm-diameter culture dishes. The conidiomata were then covered with sterile distilled water to disperse the conidia. After 24 hr, single spores of the fungus were removed from the dishes and placed in culture tubes containing 8 ml of the same medium. The tubes remained on the laboratory bench at ambient temperature (22–26 C) until the colony covered the surface of the agar. The tubes were then stored at 8 C. Four to six replicates were prepared for each isolate. A total of 10 isolates were collected from *Q. palustris* (pin oak), *Q. rubra* (northern red oak), and *Q. imbricaria* (shingle oak). Isolate 22471 (from *C. pubinervis*) was obtained from the American Type Culture Collection.

The genus *Tubakia* can be readily identified by its pycnothyrium. To be certain of their identification, the isolates used in this study were compared with published descriptions of all five *Tubakia* species, with exsiccate specimens of *T. dryina* (including the type specimen) from the collection of the Illinois Natural History Survey, and with ATCC culture 22471.

Subcultures were increased on PDA to obtain conidia for inoculations. *T. dryina* forms abundant sporodochia in culture, from which the conidia were washed with sterile distilled water. No pycnothyria were formed in culture.

For greenhouse inoculations, a conidial suspension was prepared from isolate 5-1 (from pin oak). The suspension (2×10^5 ml^{-1}) was atomized onto the leaves of 12 seedlings of northern red oak; six of these had the leaves wounded by abrasion with Carborundum. Six additional seedlings, three with abraded leaves, were sprayed with sterile distilled water as controls. All seedlings were covered with plastic bags for 48 hr and observed twice weekly for symptoms. Trial 2 was performed 3 wk later with modifications—none of the leaves was

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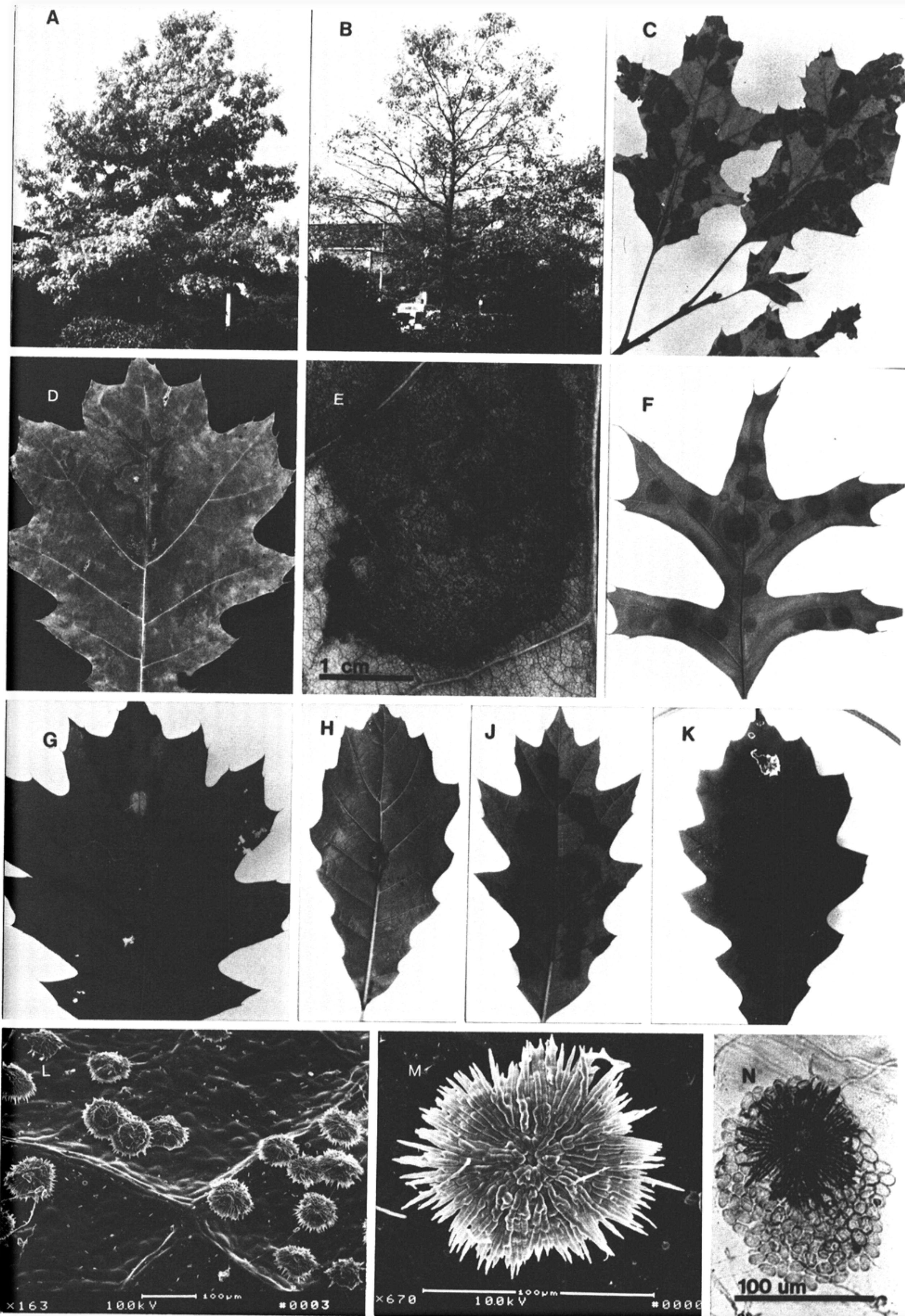

Fig. 1. Symptoms and signs of *Tubakia dryina* infection: **(A)** Noninfected chlorotic pin oak in mid-August. **(B)** Chlorotic pin oak showing severe symptoms in mid-August. **(C)** Leaves of pin oak showing small and large necrotic lesions. **(D)** Leaf of northern red oak showing necrosis along veins. **(E)** Leaf spot, with distribution of pycnothria. **(F)** Pin oak leaf 2 wk after inoculation. **(G)** Inoculated red oak leaf showing necrosis along veins. **(H)** Wounded but not inoculated control leaf after 2 wk. **(J and K)** Red oak leaves 2 wk after inoculation. **(L)** Scanning electron micrograph of pycnothyria on red oak leaf surface. **(M)** Scanning electron micrograph of typical pycnothyrium. **(N)** Typical pycnothyrium and conidia on collodion peel from leaf surface.

deliberately wounded and the seedlings were placed in a mist chamber for 4 days after the plastic bags were removed. Mist occurred for 5 sec-min^{-1} for 12 hr-day^{-1}.

During subsequent inoculations, a different technique was used. Before inoculation, a small spot of tissue along the midvein of each leaf was killed by being touched with a soldering gun. This technique was successful with inoculations with the oak anthracnose fungus, *Gnomonia quercina* Kleb. (10).

In trial 3, 18 northern red oak seedlings were placed in a growth chamber (24 C day, 20 C night with 14 hr of light, incandescent + fluorescent) and inoculated with a brush on the adaxial or abaxial surface, with or without scorching, with conidia of isolate 5-1 at 1.2×10^5 conidia-ml^{-1}.

Excised leaves were inoculated in the laboratory to test a wide range of tree species. The tree species used in this experiment were either known hosts of *T. dryina* or closely related species. Oak species were northern red (*Q. rubra* L.), black (*Q. velutina* Lam.), white (*Q. alba* L.), shingle (*Q. imbricaria* Michx.), English (*Q. robur* L.), pin (*Q. palustris* Muench.), and cherrybark (*Q. falcata* var. *pagodaefolia* Ell.); chlorotic pin oak was added later. Nonoak species inoculated were sugar maple (*Acer saccharum* Marsh.), eastern redbud (*Cercis canadensis* L.), white ash (*Fraxinus americana* L.), black tupelo (*Nyssa sylvatica* Marsh.), shagbark hickory (*Carya ovata* (Mill.) K. Koch), sweetgum (*Liquidambar styraciflua* L.), and Chinese chestnut (*Castanea mollissima* Blume.). Leaves excised at the base of the petiole were placed at random with either the adaxial or abaxial surface up on moist filter paper in 15-cm-diameter culture dishes. Conidial suspensions were prepared by grinding an agar colony of *T. dryina* in a blender with sterile distilled water, filtering through several layers of cheesecloth, and diluting the suspension to the desired concentration using a hemacytometer. A disposable pipette was used to distribute the suspension evenly over the leaf surface (2 ml per leaf). The leaves were incubated in covered culture dishes at room temperature for 2 wk. The filter paper beneath each leaf was moistened as needed to maintain humidity. Disease severity (an estimate of percentage of leaf area killed) was rated 1 and 2 wk after inoculation. After 2 wk, the fungus was reisolated from one leaf of each species and grown on PDA to certify its identity.

The first trial was designed to detect differences in disease severity resulting from different conidial concentrations. Concentrations tested were 2×10^6, 2×10^5, 2×10^4, 2×10^3, and zero conidia-ml^{-1}. Ten leaves of northern red oak were inoculated with each of the concentrations. Symptom severity was not significantly different for the different conidial concentrations. Therefore, subsequent trials employed conidial concentrations on the order of 10^5 conidia-ml^{-1}. In the next four trials, all of the tree species were tested (10 leaves inoculated and five treated with sterile distilled water as controls), using isolate 5-1. Some inoculated leaves and controls did not receive the soldering gun treatment. This experiment was repeated using isolate 5-6 (from shingle oak). When the same experiment was repeated using isolate 22471, the tree species used were northern red oak, pin oak, shingle oak, sugar maple, sweetgum, shagbark hickory, European beech, and Chinese chestnut. In another experiment, leaves naturally infected with *C. quercus* were inoculated with *T. dryina* (isolate 5-1), as described above. Five leaves infected with *C. quercus* and five healthy leaves were inoculated, and water was spread on five healthy leaves as controls.

Established trees at the Illinois Natural History Survey arboretum in Urbana were inoculated with isolate 5-1 on 26 June, 17 July, and 11 August 1987. Treatments consisted of inoculating all leaves on a single branch with conidia (10^5 ml^{-1}) or sterile distilled water applied with a brush. After inoculation, branches were covered with plastic bags for 48 hr. Disease ratings were made weekly. Trial 1 (26 June) involved only *Q. rubra*; 10 trees were inoculated and five were brushed with sterile distilled water. Trial 2 (17 July) involved northern red oak, pin oak, and chlorotic pin oak; five trees of each species were inoculated and five

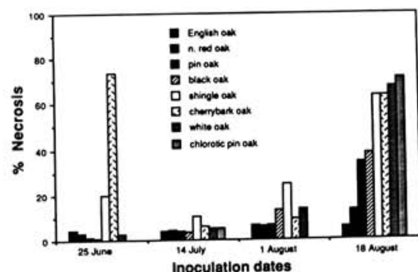

Fig. 2. Mean disease severity of excised oak leaves 1 wk after inoculation with *Tubakia dryina* (isolate 5-1). Each bar represents the mean of 10 leaves.

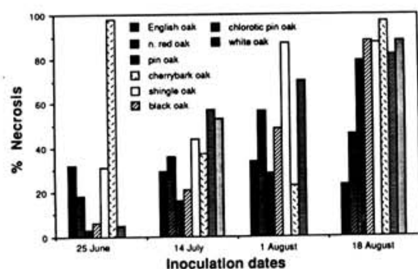

Fig. 3. Mean disease severity of excised oak leaves 2 wk after inoculation with *Tubakia dryina* (isolate 5-1). Each bar represents the mean of 10 leaves.

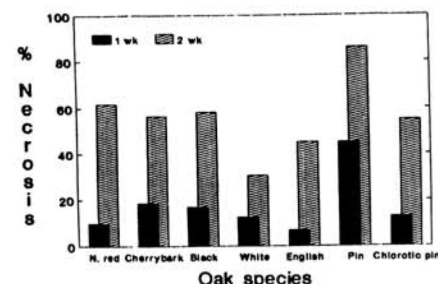

Fig. 4. Mean disease severity of excised oak leaves after inoculation with *Tubakia dryina* (isolate 5-6). Each bar represents the mean of 10 leaves.

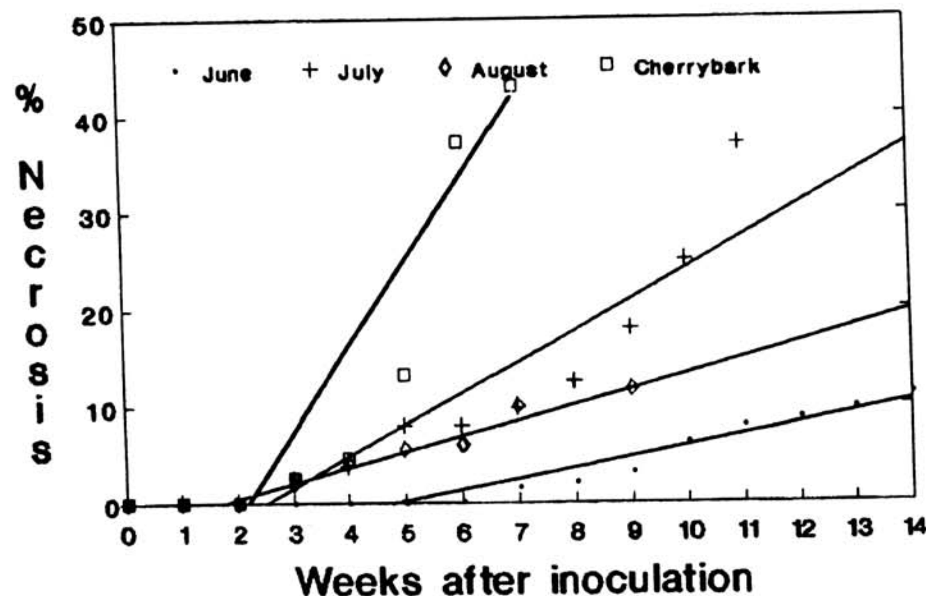

Fig. 5. Mean disease severity and regression lines for northern red oaks inoculated with *Tubakia dryina* on 26 June, 17 July, and 11 August 1987 and for cherrybark oak inoculated 11 August 1987. Each point represents the mean of five branches. Northern red oak: June $y = 1.13x - 5.367$, $r^2 = 0.580$; July $y = 3.204x - 7.931$, $r^2 = 0.641$; August $y = 1.607x - 2.731$, $r^2 = 0.744$. Cherrybark oak: $y = 8.714x - 19.057$, $r^2 = 0.487$.

were brushed with sterile distilled water. Trial 3 (11 August) was the same as trial 2, with the addition of cherrybark oak.

RESULTS

Inoculations in the field and laboratory confirmed the pathogenicity of *T. dryina*, whereas inoculations in the greenhouse and growth chamber did not. Excised oak leaves displayed severe symptoms of infection by *T. dryina* 1 or 2 wk after inoculation. Preinoculation treatment with the soldering gun was not necessary to produce symptoms on excised leaves. Both the isolate from pin oak and the isolate from shingle oak infected all of the oak species tested (Figs. 2, 3, and 4). Infection was characterized by a dark brown or water-soaked area on the leaf, accompanied by abundant formation of conidiomata (Fig. 1H,J,K). Disease severity increased overall with later inoculation dates. No consistent rank of susceptibility was evident among the oak species. Controls showed no necroses other than the small, tan-colored lesion caused by the soldering gun. The oak isolates did not infect sugar maple, sweetgum, black tupelo, Chinese chestnut, white ash, shagbark hickory, or eastern redbud, although conidiomata and conidia did form on the dead tissue killed by the soldering gun.

Inoculation of excised leaves with ATCC isolate 22471 produced severe symptoms on pin oak and shingle oak but none on northern red oak. *T. dryina* was reisolated from those leaves with symptoms. Necrosis occurred on leaves of Chinese chestnut, but the fungus could not be reisolated from the leaves. No symptoms appeared on leaves of any of the other tree species tested. Conidiomata and conidia were formed only on pin oak and shingle oak.

Leaves infected with *C. quercus* and inoculated with *T. dryina* showed severe symptoms after 1 wk. However, these symptoms did not differ significantly from those of inoculated leaves that were not infected with *C. quercus*.

In field trials on oak, all inoculated treatments developed typical symptoms (Fig. 1C,F,G). Regression lines were fitted to the disease progress data (Figs. 5–7). Slopes were compared between species and inoculation times. Northern red oaks inoculated in July or August developed symptoms faster ($P = 0.0001$) than those inoculated in June. Disease severity was significantly greater for chlorotic pin oak than for nonchlorotic pin oak and greater for pin oak than for northern red oak in the July and August inoculations ($P = 0.0001$). By late summer, some control trees had also developed symptoms (especially chlorotic pin oak), but symptoms on inoculated trees were significantly more severe.

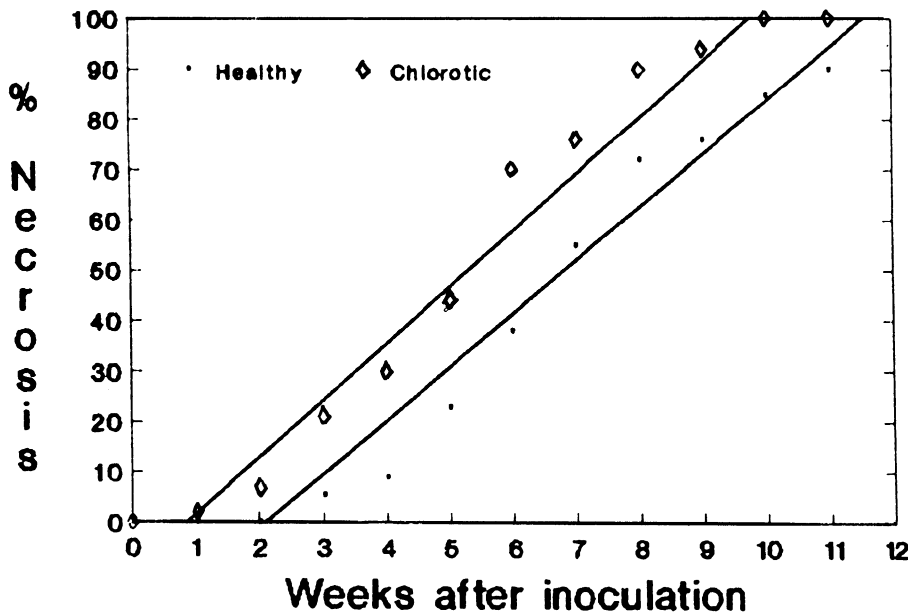

Fig. 6. Mean disease severity and regression lines for healthy and chlorotic pin oaks inoculated with *Tubakia dryina* on 17 July 1987. Each point represents the mean of five branches. Chlorotic $y = 11.227x - 9.727$, $r^2 = 0.876$; healthy $y = 10.544x - 22.044$, $r^2 = 0.808$.

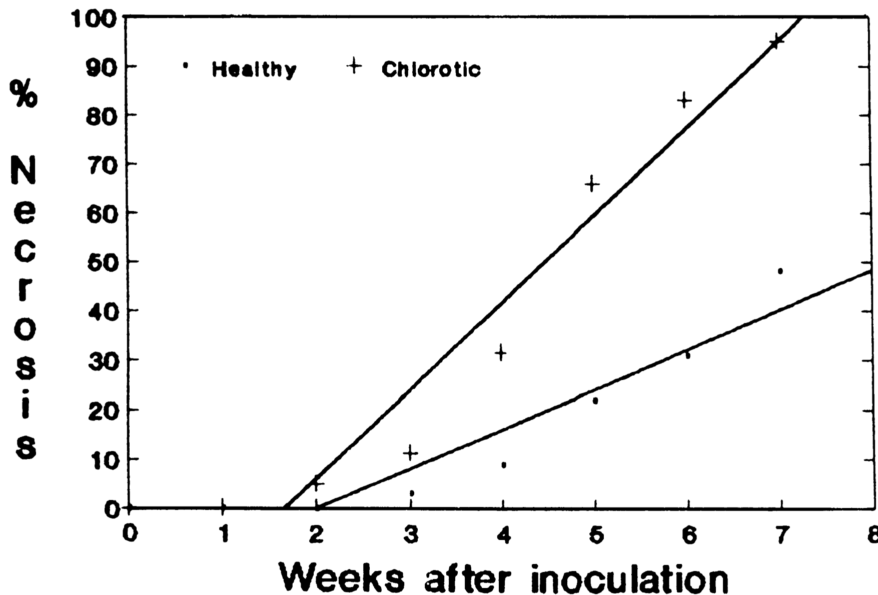

Fig. 7. Mean disease severity and regression lines for healthy and chlorotic pin oaks inoculated with *Tubakia dryina* on 11 August 1987. Each point represents the mean of five branches. Chlorotic $y = 17.786x - 29.6$, $r^2 = 0.792$; healthy $y = 8.029x - 15.943$, $r^2 = 0.815$.

DISCUSSION

This research showed that *T. dryina* was pathogenic to a number of oak species but was not pathogenic to nonoak hosts. Faster disease development in July and August might be due to increased leaf susceptibility (possibly the onset of senescence) and/or more favorable conditions for disease. Naturally occurring infections are not usually evident until mid- to late July. The stress associated with chlorosis of pin oak favored more severe symptoms.

In the laboratory experiments using excised leaves, *T. dryina* infected all oak species tested. That it did not infect the leaves of other host species suggests that the pathogenicity of *T. dryina* to nonoak species is very limited. Indeed, it is likely that the fungus is only a saprophyte or secondary colonizer of nonoak species. The excised leaves are believed to be more susceptible to infection than leaves in the field because of high humidity, high inoculum levels in laboratory inoculations, and stress associated with excision; if infection did not occur in the excised leaf inoculations, it seems unlikely that it could occur in nature. The saprophytic ability of *T. dryina* is well documented (8,13,14); Tubaki and Yokoyama (13) found that *T. dryina* colonized dead leaf tissue of all tree species tested. We have no explanation for the failure of inoculations in the growth chamber experiment; some environmental requirement(s) for infection

apparently were not met. In greenhouse inoculations, no leaf tissue was killed before inoculation, and inoculations were performed in the spring and early summer, when the leaves may not be susceptible.

The inoculation technique used in this study was quite successful, and it could be used in further studies testing the pathogenicity of *T. dryina* isolates on a number of reported hosts in the field. The technique could prove useful in studies with other foliar pathogens of deciduous trees.

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