Factors Affecting Infection of Water Oak, *Quercus nigra,* by *Tubakia dryina*

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ABSTRACT

Zhang, Y. C., and Walker, J. T. 1995. Factors affecting infection of water oak, *Quercus nigra,* by *Tubakia dryina.* Plant Dis. 79:568-571.

Tubakia dryina is commonly associated with leaf spots of water oak growing in the Piedmont of Georgia. The fungus was isolated from leaves, buds, or twigs collected from symptomatic trees from October 1991 through August 1992. The greatest isolation frequency (82 to 92%) occurred on leaves sampled in June, July, and August; a lower frequency (29 to 62%) was obtained for spring and fall months. Conidial germination on 2% water agar occurred from 10 to 35°C with maximum germination between 20 and 30°C after 24 h. The disease severity ratings of inoculated excised leaves and leaves on trees in the field increased with time and developed at a somewhat faster rate later in the season. Leaves on 1-year-old water oak seedlings, growing in soil treated with diuron + imazapyr at less than recommended rates, developed more severe disease when inoculated with *T. dryina* than seedlings treated with each herbicide alone or no herbicide.

Additional keywords: *Actinopelte,* Tubakia leaf spot

Water oaks (*Quercus nigra* L.) are a major component of the southern urban forest. Many of these stately trees were planted along streets across the South in the late 1800s and early 1900s along with willow oak (*Q. phellos* L.). These trees have been exposed to increasing environmental stresses, including extended droughts during the 1980s, soil compaction, construction damage to roots, insects, mites, and pathogens, in addition to other impacts, such as air pollutants, herbicides, paving, and bark damage from vehicles.

A fungal leaf disease that we observed frequently on water oaks in Georgia during the 1980s is caused by *Tubakia dryina* (Sacc.) Sutton, formerly *Actinopelte dryina* (Sacc.) Höhn (10). It was reported first on water oak in Georgia in 1943 (4). The fungus is widespread (1,2,3,13), but little information is available on factors affecting this leaf spot. Symptoms on water oak leaves include 1 to 2 mm brown circular spots with chlorotic halos that later enlarge to form irregular brown necrotic blotches along leaf veins and leaf mid-ribs. Diseased leaves may or may not become entirely chlorotic. Pycnothyria of *T. dryina* are abundant on infected leaves, occurring in concentric rings or scattered individually on leaf surfaces. Twig and branch dieback was commonly observed on street trees although this symptom may result from other causes. Conidiomata of *T. dryina* were observed on dead 1- to 2-year-old twigs of *Quercus robur* L. in western Germany, but it presumably was not the cause of dieback (2). The possible role of Tubakia leaf spot in the oak decline syndrome noted on different oak species in Europe and U.S. (5,11) is unknown.

Munkvold and Neely (7) found that this disease was associated with iron chlorosis of pin oak (*Quercus palustris* Münchh.) in Illinois and provided photographic documentation of the growth and development of *T. dryina* on living leaf tissue of northern red oak (*Quercus rubra* L.), shingle oak (*Quercus imbricaria* Michx.), and pin oak. It was confirmed that the fungus we observed on water oak in Georgia was *T. dryina* (D. Neely, *personal communication,* 1990).

Specific pre- and post-emergent herbicides are valuable management tools for controlling vegetation along parkways, pipelines, railroads, and landscaped areas. Tree sensitivity to herbicides varies with species, soil type, application rate, and amount of rainfall (8). Since we isolated *T. dryina* from declining water oaks along a railroad right-of-way where herbicide use was likely, we postulated that the disease might be more severe on trees growing in herbicide-treated soil than in nontreated soils.

The primary purposes of our investigations were to determine if *T. dryina* could be isolated from various water oak tissues, the time of year that water oak leaves were most susceptible to fungal invasion, the optimum temperature for germination of conidia, and the possible relationship between disease severity and prior exposure of trees to herbicides.

MATERIALS AND METHODS

Conidial germination. An isolate (OL-1) of *T. dryina* was obtained from diseased water oak leaves in Spalding County, Georgia. Subcultures of the fungus were grown for 2 weeks on potato-dextrose agar (PDA, 15 g per liter). Cultures were flooded with sterile distilled water and rubbed gently to dislodge conidia from sporodochia, then the conidial and mycelial suspension was filtered through sterile cheesecloth to obtain conidial suspensions. Conidial suspensions, containing 2.5×10^5 conidia per ml, were atomized onto petri dishes containing 2% water agar, and dishes were incubated at 10, 15, 20, 25, 30, and 35°C. Five dishes (replicates) were removed from each incubator after 6 (6 h light; 34.5 μ mol s^{-1} m^{-1}), 12 (8 h light), or 24 h (12 hr light) and placed at 0°C to retard further conidial germination. Germination percentage was determined by observing a minimum of 150 conidia at 200× magnification on each dish. A conidium was considered germinated if the length of a single germ tube exceeded half the conidial width. In most cases, there was only one germ tube per conidium. Mean and standard errors were calculated for the germination at each incubation period/temperature.

Isolations and inoculations: Confirmation of virulence. The virulence of *T. dryina* isolate OL-1 was confirmed by comparison with *T. dryina* ATCC isolates 22471 and 62255. Both surfaces of excised water oak leaves from healthy greenhouse seedlings were inoculated with each isolate. Leaves were rinsed in sterile distilled water before atomizing with conidial (1.5×10^5 conidia per ml) or mycelial suspensions; isolate 62255 produced few conidia. Single leaves were placed in each dish containing moistened filter paper. Each treatment was replicated seven times. The dishes were incubated at 15°C and the leaves examined every 3 to 4 days over 4 weeks for symptom development.

Monthly isolations. Oak leaves and buds were selected arbitrarily each month from October 1991 through August 1992 from three symptomatic water oak trees. Twig samples were taken from those twigs showing some dieback. Samples were surface disinfested with 1% NaOCl for 3 min, rinsed in sterile distilled water three times, plated on PDA, and incubated at 25°C with 12 h light/day. The percentage of leaves, buds, or twigs yielding *T. dryina* was calculated for each sampling date. The number of samples collected varied with sampling dates, but ranged from one to 10 for each tissue per tree.

Inoculation of excised leaves. Twenty leaves were collected on each of eight dates (13 April [bud-break], 23 April, 30 April, 14 May, 4 June, 7 July, 10 August,

Accepted for publication 15 March 1995.

and 15 September) from each of three nonsymptomatic field-grown water oak trees. Excised leaves were disinfested with 1% NaOCl for 3 min then rinsed three times with sterile distilled water. The abaxial surface of 10 leaves from each tree was atomized with a conidial suspension (1.2 to 1.8 × 10^5 ml^{-1}) from 2-week-old cultures of *T. dryina* (OL-1). Ten leaves from each tree were atomized with sterile distilled water as controls. Each leaf was then placed on a 1% water agar culture dish, the edges of the dish were wrapped with Parafilm and the dish was placed in a 25°C incubator with 12 h light/day. Leaves were examined weekly or biweekly for fungal sporulation and pycnothyria formation and rated for disease severity (DR): 0 = no spots observed on a leaf; 1 = ≤10% leaf area dead; 2 = 11 to 25% leaf area dead; 3 = 26 to 50% leaf area dead; 4 = 51 to 75% leaf area dead; 5 = >75% leaf area dead. Data collected from leaves collected on 13 April were excluded from analyses due to rapid death of leaves in the laboratory.

Leaf inoculations in field. To determine the development of Tubakia leaf spot on intact leaves under field conditions, leaves on five branches of a 15-year-old water oak were inoculated with conidial suspensions (1.2 to 1.8 × 10^5 ml^{-1}) of OL-1 isolate on each of seven dates: 23 April, 30 April, 14 May, 4 June, 7 July, 10 August, and 15 September 1992. Inoculated and noninoculated branches were enclosed in plastic bags for 72 h. Leaves were examined weekly or biweekly for disease development during the summer and individual leaves were rated in the same manner as in the detached leaf laboratory study. The DR data from both the laboratory and field inoculations were analyzed by covariate analysis (9). Linear regression analysis was performed on the DR data of leaves from both laboratory and field inoculations and the significance of any differences between the slopes of inoculated and noninoculated lines for each inoculation date was tested by covariate analysis.

Herbicide effects. To establish if Tubakia leaf spot disease was more severe on herbicide-treated oak trees than on nonherbicide-treated trees, diuron (Karmex 80% DF) and imazapyr (Arsenal 53%) were sprayed singly and in combination on 8 June to the soil surface of established 1-year-old water oak seedlings growing in 25 cm diameter containers of pasteurized greenhouse soil. Diuron was applied at the equivalent of 896 g.a.i. per ha^{-1} (1 lb formulation/A); imazapyr at 320 ml a.i. per ha^{-1} (0.6 pt formulation/A). These application rates were approximately one-fifth the recommended rates for field applications. Seedlings were placed in a polyethylene-covered, shaded greenhouse where temperatures ranged from 20 to 35°C. Trees were inoculated on 15 June by atomizing leaves with a conidial suspension of *T. dryina* (2.0 × 10^5 conidia per ml) and enclosed in polyethylene bags for 48 h. There were four replicates of inoculated trees and two replicates of noninoculated trees randomly arranged for each herbicide treatment. Disease ratings of individual leaves and stem growth measurements were made on 27 July, 6 weeks after herbicide applications. The total DR for all diseased leaves of each replicate was used in analysis. An insufficient number of seedling trees precluded repeating this experiment.

RESULTS AND DISCUSSION

Conidial germination. Conidia of *T. dryina* germinated over a wide temperature range (Fig. 1). Minimum germination occurred at 10 and 35°C; maximum germination was between 20 and 30°C. Germination occurred with 6 h incubation at all temperatures except 10°C. After 24 h at 20, 25, and 30°C, germination was approximately 80%, somewhat higher than the maximum 54% reported on excised leaves of *Q. rubra* (7). Based on these results, we conclude that germination of *T. dryina* conidia is not limited by temperatures in the southern U.S. Kim and Wagner (3) reported that *A. dryina* did not germinate below 5 or above 37°C.

Isolations and inoculations: Virulence of isolates. More lesions developed on the adaxial than on the abaxial surfaces of excised water oak leaves inoculated with *T. dryina* conidia regardless of the isolate. Lesions usually developed within 2 weeks after inoculation. Although isolate OL-1 and ATCC isolates 22471 and 62255 caused similar symptoms, isolate 62255 caused fewer lesions than the other two isolates 1 month after inoculation. Lesions were confined by the small veins during the first week following inoculation, but frequently larger necrotic blotches developed, often including the mid-vein, within 2 weeks. Pycnothyria were observed along the mid-veins. Reisolation of the fungus confirmed that *T. dryina* was pathogenic. No symptoms were observed on leaves of the controls.

ATCC isolate 22471, originally isolated from *Castanea pubinervis* Schneid but not pathogenic to this host (13), caused severe symptoms on pin oak and shingle oak in Illinois (6). We found this isolate to be virulent on excised water oak leaves, causing symptoms very similar to those of the OL-1 isolate. The virulence of OL-1 isolate on other tree species was not investigated; however, the prevalence of other hosts of *T. dryina* in the southern U.S. suggests that inoculum may originate from sources other than water oak.

Monthly isolations. *T. dryina* was isolated from bud, leaf, and twig samples of symptomatic trees from October 1991 to August 1992; the greatest isolation frequency (92%) was from leaves collected in July (Table 1) and more isolates were ob-

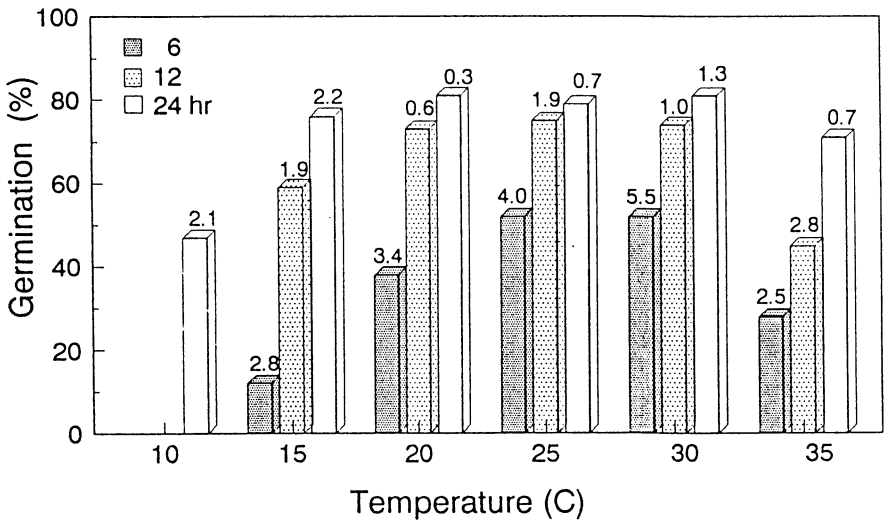

Fig. 1. Percentage of germination of *Tubakia dryina* conidia after 6, 12, and 24 h incubation on 2% water agar at specific temperatures. Figures above each column represent standard errors.

Table 1. Isolation frequency (%) of *Tubakia dryina* from leaf, bud, and twig samples taken from *Quercus nigra* trees from October, 1991 through August, 1992 in Griffin, Georgia

Month	Leaf[y]	Bud[y]	Twig[y]
1991			
October	29	. . .[z]	29
November	33	25	0
December	. . .	0	5
1992	. . .		
January	. . .	33	18
February	. . .	36	0
March	38	. . .	18
April	44	. . .	14
May	62	. . .	13
June	85	. . .	0
July	92	. . .	14
August	82	. . .	11
Mean	59	24	11

[y] Sample size ranged from 1 to 10 in sample date.
[z] Samples unavailable.

Table 2. Regression of Tubakia leaf spot disease ratings on weeks in the laboratory experiment performed in 1992 in Georgia

	Inoculated				Control			
Date	Intercept	Slope	R^2	P-value	Intercept	Slope	R^2	P-value
23 April[z]	0.084	0.974	0.509	0.000	–0.344	0.587	0.570	0.000
30 April[z]	.0.270	0.387	0.517	0.000	–0.157	0.298	0.389	0.000
14 May[z]	0.137	0.316	0.542	0.000	–0.041	0.176	0.245	0.000
4 June	0.297	0.548	0.691	0.000	–0.194	0.494	0.554	0.000
7 July[z]	0.095	0.348	0.441	0.000	0.003	0.136	0.142	0.000
10 August	–0.119	0.582	0.508	0.000	–0.278	0.544	0.478	0.000
15 September[z]	–0.237	1.180	0.505	0.000	–0.123	0.643	0.300	0.000

[z] Slopes are significantly different between inoculated and control at the $P < 0.01$ on this date.

Table 3. Regression of Tubakia leaf spot disease ratings on weeks in the field experiment performed in 1992 in Georgia

	Inoculated				Control			
Date	Intercept	Slope	R^2	P-value	Intercept	Slope	R^2	P-value
23 April	0.434	0.069	0.258	0.000	–0.341	0.064	0.323	0.000
30 April[z]	0.310	0.093	0.314	0.000	–0.278	0.060	0.379	0.000
14 May[z]	0.233	0.079	0.323	0.000	–0.269	0.054	0.278	0.000
4 June[z]	0.098	0.097	0.394	0.000	–0.082	0.058	0.397	0.000
6 July[z]	0.268	0.111	0.341	0.000	–0.085	0.077	0.233	0.000
10 August[z]	0.105	0.201	0.372	0.000	–0.046	0.120	0.232	0.000
15 September	0.156	0.474	0.312	0.000	0.116	0.328	0.145	0.000

[z] Slopes are significantly different between inoculated and control at $P < 0.01$ on this date.

Table 4. *P* values for comparison of the slopes of regression lines between uninoculated and leaves inoculated with *Tubakia dryina* at different dates in the laboratory

Date	23 April	30 April	14 May	4 June	7 July	10 August
30 April	0.0001					
14 May	0.0001	0.0070				
4 June	0.0001	0.0001	0.0001			
7 July	0.0001	0.2106	0.2462	0.0001		
10 August	0.0001	0.0001	0.0001	0.4007	0.0001	
15 September	0.1152	0.0001	0.0001	0.0001	0.0001	0.0001

Table 5. *P* values for comparison of the slopes of regression lines between uninoculated and leaves inoculated with *Tubakia dryina* at different dates in the field

	Inoculation date					
Date	23 April	30 April	14 May	4 June	6 July	10 August
30 April	0.0001					
14 May	0.0604	0.0167				
4 June	0.0001	0.6087	0.0040			
6 July	0.0001	0.0925	0.0003	0.1288		
10 August	0.0001	0.0001	0.0001	0.0001	0.0001	
15 September	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

tained in June, July, and August than in other months. Neither buds taken in December nor twigs collected in November, February, or June yielded the fungus. No samples were taken in September and no isolations were attempted from fallen leaves. Although water oak is deciduous, some leaves frequently remain attached to the twigs throughout early winter and, if diseased, could be a source of inoculum for buds or twigs. No isolations were made from tissues of nonsymptomatic trees. *Tubakia dryina* may be present in young leaf tissue prior to symptom expression as it was in apparently healthy leaves and twigs of *Q. robur* in southern Bavaria (2).

Inoculation of leaves. There was a significant linear relationship between DR and weeks after inoculation in both laboratory and field experiments (Table 2 and 3). The low R^2 values were attributed to the variation of DR values within treatments (weeks). This was especially true for the field experiment, in which the interaction of the host and the pathogen probably was influenced by environmental conditions.

Excised leaves from nondeclining water oak were susceptible to isolate OL-1 of *T. dryina* when inoculated and incubated at 25°C with 12 h light/day. The DR increased with time (weeks) for both inoculated and control leaves. The differences between the slopes of regression lines of DR on weeks for inoculated and control leaves were significant for each date except for 4 June and 10 August (Table 2). A comparison of slopes indicates that the disease developed faster on the leaves inoculated on 23 April, 30 April, 14 May, 7 July, and 15 September than on the controls for each respective date. The lack of significant difference in slopes on other dates may have resulted from latent infections in the leaves.

A comparison of slopes of the regression lines of DR on weeks for leaves inoculated on different dates in the laboratory shows that the disease development rate was rapid on the leaves inoculated on 23 April, decreased on 30 April and 14 May, increased on 4 June, decreased again on 7 July, and increased on 10 August and 15 September (Tables 2 and 4). This suggests that excised leaves were more susceptible to the isolate OL-1 in late April, about 2 weeks after bud break, and in June, August, and September, than in May and July under the same environmental conditions.

Tubakia leaf spot developed less rapidly or had a longer incubation period on inoculated leaves in the field than it did on detached leaves in the laboratory. The slopes of the regression lines of the inoculated leaves were significantly greater than those of noninoculated ones for each date except 23 April and 15 September (Table 3).

The leaf spot disease developed faster on the leaves inoculated in late season than in early season(Tables 3 and 5). The disease developed slower on the leaves inoculated on 23 April and 14 May than on those inoculated on any other dates. There were no differences in the disease development rate among the leaves inoculated on 30 April, 4 June, and 6 July. The disease development rate of the leaves inoculated on 14 May was slower than that of those inoculated in adjacent months, which collaborates laboratory results. The disease development rate was the fastest on the leaves inoculated on 10 August and 15 September.

Our results confirm similar findings of Munkvold and Neely (6) who found that this disease on northern red oaks developed at a slower rate when inoculations were made in June as opposed to July and August, and that detached leaves were more susceptible than attached ones. The lower DR on attached leaves in the field than on excised leaves in the laboratory may be attributed to the decreased resistance of the leaves to the disease after being detached, or to environmental differences during incubation, or both. The physiological changes that can occur in leaves following detachment may have considerable effects on the early stages of a host-parasite relationship. Although the inoculation of excised leaves under controlled laboratory conditions does provide useful information on pathogenicity, disease progress, and host susceptibility (6), we cannot explain why the excised leaves

Table 6. Influence of diuron and/or imazapyr soil treatment (8 June) on subsequent disease ratings and new stem growth of water oak 6 wk after inoculation with *Tubakia dryina* (OL-1) on 15 June 1992

Herbicide and rate	Inoculation	Disease severity (DR)	Stem growth (cm)
Diuron	Yes	8.5 a[z]	30.7 a
(1 lb/acre)	No	6.0 a	21.6 b
Imazapyr	Yes	13.0 a	15.0 bc
(0.6 pts/acre)	No	5.5 a	8.2 cd
Diuron(1 lb/acre)+	Yes	45.3 b	4.6 d
Imazapyr(0.6 pts/acre)	No	15.5 a	6.0 d
None	Yes	5.8 a	23.7 b

[z] Values with the same letter within columns are not significantly different from each other at $P < 0.05$.

inoculated on 23 April showed the highest disease development rate in the laboratory experiment whereas the attached leaves inoculated on this same date had the lowest rate in the field experiment. Additional information is needed to ascertain what differences exist between excised and attached leaves that can influence Tubakia leaf spot disease.

Herbicide effects. The combination of diuron and imazapyr herbicides with *T. dryina* inoculation significantly ($P = 0.05$) increased the DR of Tubakia leaf spot compared with the DR of trees receiving a single herbicide with or without inoculation, or when no herbicide was applied (Table 6). All other herbicide treatments, with or without *T. dryina* inoculation, did not affect disease development. Treating soil with imazapyr and diuron together inhibited stem elongation. Our data indicate that these herbicide combinations may predispose water oak to *T. dryina*; however, additional research in field situations is essential before the effects of these and other herbicides on this disease can be fully assessed.

The wide host range of *T. dryina* and its survival in various oak tissues throughout the year suggest that the fungus is omnipresent, and perhaps this disease is another case of a ubiquitous pathogen that is not very aggressive unless the host is subjected to certain environmental stresses. The exact role of drought stress on predisposition to this disease remains uncertain. The Piedmont of Georgia was deficient in rainfall during the 1980s when the disease was frequently observed. Severe droughts occurred in 1984 and 1986; eight of the last 11 years had 12% less precipitation than the 50-year mean for this region (12).

ACKNOWLEDGMENTS

We thank John Melin for his technical assistance, Jerry Davis and Mary Kay Rayens for statistical advice, and Laurel Oxner and Belinda Purser for typing the manuscript. Credit is also due to Dan Neely for his verification of *Tubakia dryina* on water oak leaves. This work was supported, in part, by Hatch and state funds provided to the University of Georgia, College of Agricultural and Environmental Science, Georgia Station, Griffin.

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