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# Evaluation for Resistance to Powdery Mildew in *Cornus* Species and Hybrids Using a Leaf Disk Assay[1]

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## Abstract

Using a leaf disk assay, eight cultivars or/and breeding lines in *Cornus florida* L., *C. kousa* (Buerger ex Miq.) Hance, five cultivars in *C. kousa* × *C. florida*, one cultivar in *C. kousa* × *C. nuttallii* Aud. and one cultivar in (*C. kousa* × *C. nuttallii*) × *C. kousa* were evaluated for resistance to powdery mildew [*Erysiphe pulchra* (Cooke and Peck) U. Braun & S. Takamatsu (syn. *Microsphaera pulchra* Cooke and Peck)]. Flowering dogwoods (*C. florida*) cultivars were more susceptible than the other species and hybrids with the exception of the *C. florida* × *C. kousa* hybrid 'Ruth Ellen'. Resistance in kousa dogwoods and hybrids was manifested as restriction of hyphal growth and inhibition of sporulation. Although mildew colonies and sporulation were detected on leaves of all flowering dogwood cultivars, 'Karen's Appalachian Blush' and 'Worlds Fair' were more resistant than other cultivars. These dogwoods had significantly lower values of percent geminated conidia with branched hyphae, infection efficiency, sporulation and delayed latent period. The leaf disk assay provides a laboratory procedure to screen new cultivars and lines of dogwoods for resistance to powdery mildew.

**Index words:** dogwood, *Erysiphe pulchra*, *Microsphaera pulchra*, screening method.

**Species used in this study:** *Cornus florida* L.; *C. kousa* (Buerger ex Miq.) Hance; *C. nuttallii* Aud.; 'Rubra'; 'Red Pygmy'; 'Little Princess'; MW 95-25; 'Worlds Fair'; 'Karen's Appalachian Blush'; 'Ruth Ellen'; 'Constellation'; 'Aurora'; 'Celestial'; 'Stellar Pink'; 'Blue Shadow'; 'Milky Way'; 'Starlight', 'Venus'.

### Significance to the Nursery Industry

Powdery mildew is an important disease of flowering dogwood (*Cornus florida* L.) in nurseries and landscapes. Host resistance is a major strategy of integrated pest management since it lowers production cost and is environmentally safe. In past studies, resistance to powdery mildew was identified in some cultivars in flowering (2, 5, 6, 9, 12, 13) and kousa dogwoods (2, 6, 9), and some hybrids of *C. kousa* × *C. florida* (2, 6, 9) and *C. nuttallii* × *C. florida* (2). However, levels of resistance to powdery mildew were often variable across locations and years (2, 6, 9, 12). Rapid and reliable screening methods are critical for selecting resistant plants and developing powdery mildew management strategies. In this study, leaf disk assays were used to screen dogwood species and hybrids for resistance to powdery mildew. The method was time- and cost-effective and was consistent for evaluation of resistance to powdery mildew in dogwoods.

### Introduction

Using resistant cultivars is a sound strategy in the integrated management of powdery mildew [*Erysiphe pulchra* (Cooke and Peck) U. Braun & S. Takamatsu (syn. *Microsphaera pulchra* Cooke and Peck)] in flowering dogwood (*Cornus florida* L.) because it is effective, economical and environmentally friendly. Most flowering dogwood cultivars are susceptible to powdery mildew, but differences in susceptibility have been reported in flowering dogwood and most *C. kousa* cultivars and hybrids between *C. florida* and *C. kousa* (2, 6, 9, 12). Most evaluations for resistance to powdery mildew were conducted using natural inocula and expression of resistance for some cultivars varied considerably between years and locations (2, 6, 9, 12). Dogwood seedlings have been screened for resistance to powdery mildew in nurseries, but large number of plants and multiple years of evaluation were needed because of low frequency (~0.001) of resistant individuals in the general population and a high number of escapes from disease (12).

A cost- and time-effective method is desirable for screening and identifying dogwoods for resistance to powdery mildew. Leaf disk assays have been used to screen for powdery mildew resistance in melon (1, 3) and sweet cherry (8), and to investigate resistant components of flowering dogwoods to powdery mildew (5). The objective of this study was to evaluate *Cornus* species and hybrids for resistance to powdery mildew using an *in vitro* leaf disk assay.

### Materials and Methods

Dogwood trees of fifteen cultivars in species of *C. florida*, *C. kousa*, *C. nuttallii* and their hybrids were obtained in 18.9 liter (#5) and 26.5 liter (#7) nursery containers or as bare root liners [76–91 cm (30–36 in.) or 152–183 cm (5–6 ft)] from Shadow Nursery (Winchester, TN) and the University of Tennessee (Table 1). Bare root trees were potted in 18.9 liter (#5) containers, with the exception of 1000 liter (32.8 $ft^3$) home-made container for 'Little Princess' and 170 liter container (Classic C-1800) for 'Worlds Fair', with a mixture of screened pine bark and sand. Trees were grown in a greenhouse under 50% shade cloth at the University of Tennessee Plateau Research and Education Center located near Crossville, TN. Fully expanded leaves were collected from the trees and rinsed in running water to remove dust and debris from leaf surfaces. Leaf disks, 1.6 cm (0.6 in) and 0.9

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cm (0.4 in) in diameter, were cut from leaves using cork borers. Leaf disks were placed randomly on two-layers of moistened filter papers in 9-cm (3.5 in) diameter Petri dishes for inoculation.

*C. florida* 'Cherokee Princess' trees infected with *E. pulchra* were maintained in a greenhouse at the University of Tennessee Knoxville campus and diseased leaves collected from the trees were used as the source of inoculum for laboratory experiments. Inoculation was conducted in a laboratory using the settling tower described previously (5). Each inoculation was considered as a block and each experiment consisted of three blocks with three leaf disks in each block per cultivar. The inoculated conidium density was 200, 126 and 235 conidia per $cm^2$, respectively, for three blocks. Inoculated leaf disks in Petri dishes were incubated at 22 ± 1C with a continuous photoperiod, which was provided by four 40-watt residential fluorescent bulbs that were 45 cm (17.7 in) above leaf disks. Distilled water was added to filter papers as needed to maintain high relative humidity in the dishes.

Inoculated 0.9 cm (0.4 in) diameter leaf disks were cleared, at 3 days after inoculation (DAI), with a solution of 0.15% trichloroacetic acid in chloroform-alcohol and stained with 0.6% coomassie brilliant blue R-250 in 10% trichloroacetic (10) as modified by Li (5) to observe germinated conidia with branched hyphae,. After rinsing with water, leaf disks were mounted in water on glass slides and covered with cover glasses. Cover glasses were sealed using Permount (Fisher Scientific, Fair Lawn, NJ) and the slides were observed using an Olympus BH-2 compound microscope (400× magnification). One hundred germinated conidia on each leaf disk were examined for the formation of branched hyphae and percent germinated conidia with branched hyphae were recorded.

The number of mildew colonies on 1.6 cm (0.65 in) diameter leaf disks was counted under a stereo microscope at 8 DAI. A colony was defined as a germinated conidium that formed more than five branched hyphae. Infection efficiency was defined as the percentage of inoculated conidia that formed colonies on leaf disks and was calculated using the equation

$$IE\ (\%) = C / (S \times 3.14 \times r^2),$$

where *IE* was infection efficiency, *C* was the number of colonies on a leaf disk, *S* was the number of conidia inoculated per $cm^2$ leaf area, and *r* was the radius of a leaf disk.

Formation of conidiophores and conidia on 1.6 cm (0.65 in) diameter leaf disks was examined using a stereo microscope at one day intervals from 1 to 28 DAI to determine latent period. Latent period was defined as the time at which the production of new conidiophores and conidia were observed from fungal colonies on inoculated leaf disks.

Sporulation was assessed at 28 DAI. Each inoculated 1.6 cm (0.65 in) diameter leaf disk was cut in half and placed in a 25 ml screw-top tube containing 8 ml of a 0.1% Tween 20 water solution and agitated using the maximum speed of a vortex mixer for 30 sec. After removing leaf disks, the conidial suspensions were centrifuged for 10 min at 1000 × g. The supernatant was discarded and pellets were resuspended in 0.3 ml of distilled water. Three estimates of conidia per ml suspension were made for each leaf disk using a hemocytometer and a compound microscope. Spore numbers per $cm^2$ leaf area were calculated using the equation

$$SP = C / (3.14 \times r^2),$$

in which *SP* was spore numbers per $cm^2$ leaf disk area, *C* was the mean number of conidia per leaf disk, and *r* was the radius of a leaf disk. Data of conidia per $cm^2$ leaf area were transformed using the square root of counts plus one in order to minimize the effects of zero values on ANOVA (7).

The percent germinated conidia with branched hyphae, infection efficiency, latent period and sporulation were analyzed using a randomized complete block design with subsamplings for each cultivar. Each run of inoculation through the settling tower was considered as a block and three leaf disks in a block for each cultivar were considered as subsamples. Means were compared using Fisher's significant difference at $P = 0.05$. Statistical analyses were completed using SAS software (Version 9.1, SAS Institute Inc., Cary, NC).

**Results and Discussion**

Overall, *C. kousa* dogwood cultivars and hybrids between *C. florida* and *C. nuttallii* were more resistant to powdery mildew than *C. florida* dogwood cultivars. Resistance was characterized as having lower values of germinated conidia with branched hyphae and infection efficiency and less or no sporulation (Table 1). Significant differences in percent germinated conidia with branched hyphae and infection efficiency were not detected among *C. kousa*, *C. nuttallii* and hybrid dogwoods (Table 1). In a previous study, conidia of *E. pulchra* germinated and formed secondary appressoria on dogwood leaves with different levels of resistance, but no branched hyphae formed without an established relationship with the host (4). The results in this study indicated that conidia of *E. pulchra* initially could establish a parasitic relationship with kousa dogwood cultivars and hybrids, but the growth of hyphae was inhibited, and colonies were restricted in all accessions with the exception of the *C. kousa* × *C. florida* hybrid 'Ruth Ellen'. Resistance to powdery mildew was reported among kousa cultivars and hybrids, but resistance for some cultivars was variable for different locations and years (2, 6, 9, 12). The hybrids, 'Ruth Ellen' and 'Constellation', were reported as susceptible in Asheville, NC (USDA Hardiness Zone 6) (9), but were considered highly resistant at Auburn University, AL (USDA Hardiness Zone 8) (2). In the present study, 'Ruth Ellen' was more susceptible to powdery mildew than 'Constellation' (Table 1).

Significant differences in percent geminated conidia with branched hyphae, infection efficiency, latent period and sporulation per $cm^2$ leaf area were detected among the flowering dogwood cultivars although fungal colonies of *E. pulchra* were observed on all these cultivars (Table 1). In the six flowering dogwood cultivars, 'Rubra', 'Red Pygmy' and 'Little Princess' were more susceptible; the breeding line MW 95-25 exhibited intermediate resistance; and 'Karen's Appalachian Blush' and 'Worlds Fair' had higher resistance. The cultivar 'Karen's Appalachian Blush' supported a significantly less sporulation than the cultivar 'Worlds Fair' although there were no significant differences in percent germinated conidia with branched hyphae, infection efficiency and latent period between these two cultivars. The higher resistance exhibited by flowering dogwood cultivar 'Karen's Appalachian Blush' agreed with the findings reported previously from *in vitro* and *in vivo* studies (5, 13). Hagan et al. (2) reported 'Worlds Fair' dogwood had the intermediate level of resistance to powdery mildew in a field trial in Alabama.

**Table 1. Comparisons of germinated conidia with branched hyphae (GCBH), infection efficiency (IE), latent period (LP) and sporulation (SP) of powdery mildew on *Cornus* species and hybrids.**

| Species<br>Cultivar | GCBH (%) | IE (%) | LP (day) | SP[x] |
|---|---|---|---|---|
| *C. florida* | | | | |
| 'Rubra' | 46.4a[y] | 8.83a | 8.0a | 26.6b |
| 'Red Pygmy' | 22.7b | 5.43b | 8.7a | 35.0a |
| 'Little Princess' | 10.7c | 7.61a | 8.8a | 27.2b |
| MW 95-25 | 9.6cd | 2.56c | 11.1ab | 14.2c |
| 'Worlds Fair' | 2.0de | 1.54cd | 15.6cd | 16.0c |
| 'Karen's Appalachian Blush' | 1.6e | 0.58d | 19.3d | 3.4d |
| *C. kousa* × *C. florida* | | | | |
| 'Ruth Ellen' | 2.0e | 1.43cd | 14.4bc | 16.5c |
| 'Constellation' | 0.4e | 0.07d | NA[z] | 0e |
| 'Aurora' | 0.2e | 0.02d | NA | 0e |
| 'Celestial' | 0.9e | 0.00d | NA | 0e |
| 'Stellar Pink' | 0.3e | 0.00d | NA | 0e |
| *C. kousa* | | | | |
| 'Blue Shadow' | 0.1e | 0.00d | NA | 0e |
| 'Milky Way' | 0.0e | 0.00d | NA | 0e |
| *C. kousa* × *C. nuttallii* 'Starlight' | 1.2e | 0.04d | NA | 0e |
| (*C. kousa* × *C. nuttallii*) × *C. kousa* 'Venus' | 0.0e | 0.00d | NA | 0e |
| *P* > *F* | <0.0001 | <0.0001 | 0.0008 | <0.0001 |
| LSD | 7.6 | 1.67 | 3.9 | 6.74 |

[x]Sporulation were measured as the number of conidia per $cm^2$ leaf area at 28 days after inoculation. Data were analyzed using the square root transformation of conidia per $cm^2$ leaf area plus one.

[y]Means followed by the same letter in a column for each variable are not significantly different from each other at the $P = 0.05$ level using Fisher's least significant difference (LSD).

[z]Conidiophore and conidium formation was not detected.

Screening for disease resistance is a critical step to select breeding lines and develop resistant cultivars. Field studies to evaluate resistance to powdery mildew have relied on natural inocula. Multi-year experiments were needed because the results were unreliable during the years with low levels of disease (2, 11, 12, 13). The leaf disk assay approach used in this study provides a new technique to screen dogwood genotypes for resistance to powdery mildew in a laboratory.

## Literature Cited


1. Cohen, R. 1993. A leaf disk assay for detection of resistance of melons to *Sphaerotheca fuliginea* race 1. Plant Dis. 77:513–517.

2. Hagan, A.K., B. Hardin, C.H. Gilliam, G.J. Keever, J.D. Williams, and J. Eakes. 1998. Susceptibility of cultivars of several dogwood taxa to powdery mildew and spot anthracnose. J. Environ. Hort. 16:147–151.

3. Kusuya, M., K. Hosoya, K. Yashiro, K. Tomita, and H. Ezura. 2003. Powdery mildew (*Sphaerotheca fuliginea*) resistance in melon is selectable at the haploid level. J. Exp. Bot. 54:1069–1074.

4. Li, Y.H., M.T. Windham, R.N. Trigiano, D.C. Fare, J.M. Spiers, and W.E. Copes. 2005. Spore germination, infection structure formation, and colony development of *Erysiphe pulchra* on dogwood leaves and glass slides. Plant Dis. 89:1301–1304.

5. Li, Y.H., M.T. Windham, R.N. Trigiano, D.C. Fare, J.M. Spiers, and W.E. Copes. 2006. Development of *Erysiphe pulchra*, the causal agent of powdery mildew, on resistant and susceptible dogwood (*Cornus florida*) leaf disks. Can. J. Plant Pathol. 28:71–76.

6. Johnson, M.P., J.R. Hartman, R.E. McNiel, and W.M. Fountain. 2001. Evaluation of dogwood and birch species and cultivars for resistance to key insect pests and diseases. J. Environ. Hort. 19:73–78.

7. May, R.A. and R.N. Trigiano. 1991. Somatic embryogenesis and plant regeneration from leaves of *Dendranthema grandiflora*. J. Amer. Soc. Hort. Sci. 116:366–371.

8. Olmstead, J.W. and G.A. Lang. 2000. A leaf disk assay for screening sweet cherry genotypes for susceptibility to powdery mildew. HortScience 35:274–277.

9. Ranney, T.G., L.F. Grand, and J.L. Knighten. 1995. Susceptibility of cultivars and hybrids of kousa dogwood to dogwood anthracnose and powdery mildew. J. Arboriculture 21:11–16.

10. Schiffer, R., R. Görg, B. Jarosch, U. Beckhove, G. Bahrenberg, K. Kogel, and P. Schulze-Lefert. 1997. Tissue dependence and differential cordycepin sensitivity of race-specific resistance responses in the barley-powdery mildew interaction. Mol. Plant-Microbe Interact. 10:830–839.

11. Windham, M.T., R.N. Trigiano, and A.S. Windham. 2005. Susceptibility of *Cornus* species to two genera of powdery mildew. J. Environ. Hort. 23:190–192.

12. Windham, M.T. and W.T. Witte. 1998. Naturally occurring resistance to powdery mildew in seedlings of *Cornus florida*. J. Environ. Hort. 16:173–175.

13. Windham, M.T., W.T. Witte, and R.N. Trigiano. 2003. Three white-bracted cultivars of *Cornus florida* resistant to powdery mildew. HortScience 38:1253–1255.