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

Y.H. Li, M.T. Windham, R.N. Trigiano, D.C. Fare, J.M. Spiers, and W.E. Copes
Department of Entomology and Plant Pathology
University of Tennessee, Knoxville, TN 37996-4560

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**Abstract**

Using a leaf disk assay, eight cultivars 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 susceptiable 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 germinated 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’.

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**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) 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
cm (0.4 in) in diameter, were cut from leaves using cork bor-
ers. Leaf disks were placed randomly on two-layers of moist-
ened 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 labo-
atory experiments. Inoculation was conducted in a labora-
tory 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 cm², respectively, for three blocks. In-
culated 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 pa-
ers 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 us-
ing a Olympus BH-2 compound microscope (400x magnifi-
cation). One hundred germinated conidia on each leaf disk
were examined for the formation of branched hyphae and
percent germinated conidia with branched hyphae were re-
corded.

The number of mildew colonies on 1.6 cm (0.65 in) diam-
eter 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 effi-
ciency was defined as the percentage of inoculated conidia
that formed colonies on leaf disks and was calculated using the
equation

\[ IE(\%) = \frac{C}{S \times 3.14 \times r^2}, \]

where \( IE \) was infection efficiency, \( C \) was the number of colo-
nies on a leaf disk, \( S \) was the number of conidia inoculated
per cm² 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 micro-
scope 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 ob-
served 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
suspending was 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
suspiration were made for each leaf disk using a hemocy-
tometer and a compound microscope. Spore numbers per cm²
leaf area were calculated using the equation

\[ SP = \frac{C}{3.14 \times r^2}, \]

in which \( SP \) was spore numbers per cm² leaf disk area, \( C \) was
the mean number of conidia per leaf disk, and \( r \) was the ra-
dius of a leaf disk. Data of conidia per cm² 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, in-
fection 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

**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 ger-
minated conidia with branched hyphae and infection effi-
ciency 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 rela-
tionship with kousa dogwood cultivars and hybrids, but
the growth of hyphae was inhibited, and colonies were re-
stricted in all accessions with the exception of the *C. kousa ×
C. florida* hybrid ‘Ruth Ellen’. Resistance to powdery mil-
dew was reported among kousa cultivars and hybrids, but
resistance for some cultivars was variable for different loca-
tions 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 suscepti-
tible to powdery mildew than ‘Constellation’ (Table 1).

Significant differences in percent germinated conidia with
branched hyphae, infection efficiency, latent period and
sporulation per cm² leaf area were detected among the flow-
ering 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 Appa-
lachian Blush’ and ‘Worlds Fair’ had higher resistance. The
cultivar ‘Karen’s Appalachian Blush’ supported a signifi-
cantly less sporulation than the cultivar ‘Worlds Fair’ although
there were no significant differences in percent germinated
conidia with branched hyphae, infection efficiency and la-
tent period between these two cultivars. The higher resis-
tance exhibited by flowering dogwood cultivar ‘Karen’s Appa-
lachian 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** Mean of germinated conidia with branched hyphae,
latent period and sporulation per cm² leaf area for six
flowering dogwood cultivars and *C. kousa*, *C. nuttallii* and
hybrid dogwoods at *USDA Hardiness Zone 6* and 8.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>USDA Zone 6 (Mean)</th>
<th>USDA Zone 8 (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. kousa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. nuttallii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. flor.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Germinated Conidia with Branched Hyphae</td>
<td>50</td>
<td>20</td>
</tr>
<tr>
<td>Latent Period</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Sporulation per cm² Leaf Area</td>
<td>500</td>
<td>200</td>
</tr>
</tbody>
</table>

**Notes:**
1. D.A.I. = Days after inoculation
2. USDA Hardiness Zone 6 = 4-5
3. USDA Hardiness Zone 8 = 5-6
**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.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivar</th>
<th>GCBH (%)</th>
<th>IE (%)</th>
<th>LP (day)</th>
<th>SP±</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. florida</em></td>
<td>‘Rubra’</td>
<td>46.4a</td>
<td>8.33a</td>
<td>8.0a</td>
<td>26.6b</td>
</tr>
<tr>
<td></td>
<td>‘Red Pygmy’</td>
<td>22.7b</td>
<td>5.43b</td>
<td>8.7a</td>
<td>35.0a</td>
</tr>
<tr>
<td></td>
<td>‘Little Princess’</td>
<td>10.7c</td>
<td>7.61a</td>
<td>8.8a</td>
<td>27.2b</td>
</tr>
<tr>
<td></td>
<td>MW 95-25</td>
<td>9.6cd</td>
<td>2.56c</td>
<td>11.1ab</td>
<td>14.2c</td>
</tr>
<tr>
<td></td>
<td>‘Worlds Fair’</td>
<td>2.0de</td>
<td>1.54cd</td>
<td>15.6cd</td>
<td>16.0c</td>
</tr>
<tr>
<td></td>
<td>‘Karen’s Appalachian Blush’</td>
<td>1.6e</td>
<td>0.58d</td>
<td>19.3d</td>
<td>3.4d</td>
</tr>
<tr>
<td><em>C. kousa × C. florida</em></td>
<td>‘Ruth Ellen’</td>
<td>2.0e</td>
<td>1.43cd</td>
<td>14.4bc</td>
<td>16.5c</td>
</tr>
<tr>
<td></td>
<td>‘Constellation’</td>
<td>0.4e</td>
<td>0.07d</td>
<td>NAa</td>
<td>0e</td>
</tr>
<tr>
<td></td>
<td>‘Aurora’</td>
<td>0.2e</td>
<td>0.02d</td>
<td>NA</td>
<td>0e</td>
</tr>
<tr>
<td></td>
<td>‘Celestial’</td>
<td>0.9e</td>
<td>0.00d</td>
<td>NA</td>
<td>0e</td>
</tr>
<tr>
<td></td>
<td>‘Stellar Pink’</td>
<td>0.3e</td>
<td>0.00d</td>
<td>NA</td>
<td>0e</td>
</tr>
<tr>
<td><em>C. kousa</em></td>
<td>‘Blue Shadow’</td>
<td>0.1e</td>
<td>0.00d</td>
<td>NA</td>
<td>0e</td>
</tr>
<tr>
<td></td>
<td>‘Milky Way’</td>
<td>0.0e</td>
<td>0.00d</td>
<td>NA</td>
<td>0e</td>
</tr>
<tr>
<td><em>C. kousa × C. nuttallii</em></td>
<td>‘Starlight’</td>
<td>1.2e</td>
<td>0.04d</td>
<td>NA</td>
<td>0e</td>
</tr>
<tr>
<td>(C. kousa × C. nuttallii)</td>
<td>*C. kousa ‘Venus’</td>
<td>0.0e</td>
<td>0.00d</td>
<td>NA</td>
<td>0e</td>
</tr>
</tbody>
</table>

| P > F                   | <0.0001                | <0.0001  | 0.0008   | <0.0001 |
| LSD                     | 7.6                    | 1.67     | 3.9      | 6.74    |

*Sporulation were measured as the number of conidia per cm² leaf area at 28 days after inoculation. Data were analyzed using the square root transformation of conidia per cm² leaf area plus one.

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).

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**


