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Susceptibility of *Camellia* to *Phytophthora ramorum*

Nina Shishkoff, Research Scientist, ARS-USDA, Foreign
Disease/Weed Science Research Unit, Frederick, MD 21702

Corresponding author: Nina Shishkoff. Nina.Shishkoff@ars.usda.gov

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Abstract

Camellia is a known host of *Phytophthora ramorum*, the "sudden oak death" pathogen. During 2003-2004, the organism was shipped from California throughout the U.S. on infected nursery stock, leading to a nationwide effort to recover the infected plants. This paper describes the symptoms on *Camellia* and the relative susceptibility of nine species and four hybrids. Camellias varied widely in susceptibility, with *Camellia* x 'Roni gaki' showing the worst overall symptoms, while some other cultivars showed little or none. Obvious symptoms include leaf lesions and stem blight; defoliation, while more difficult to observe, was also characteristic in camellias. The pathogen persisted in diseased plants, notably in stem tissue, for at least a month as demonstrated by isolation. One month after inoculation, roots remained asymptomatic, but the pathogen could be recovered from washed or surface-sterilized root pieces. These results will aid the ongoing national survey for *P. ramorum* at nurseries and forests.

Introduction

Phytophthora ramorum Werres, De Cock & Man in't Veld, causes leaf spots, twig blight, and cankers on a variety of plant hosts. It was first observed on nursery stock in Europe, then in California forests around the mid-1990s. In 2003, camellias infected with *P. ramorum* were found in California nurseries. Initially, only one cultivar, *C. sasanqua* 'Bonanza,' was found to be infected (4), but soon it became apparent that many cultivars were susceptible. In 2003-2004, shipments of camellia plants from California nurseries, some of which were infected with *P. ramorum*, were transported to other states and Canada, triggering a massive effort to recover these plants before the pathogen could spread. Since January 2005, the Emergency Federal Order Restricting Movement of Nursery Stock from California, Oregon, and Washington Nurseries requires inspection of nurseries that ship hosts of *P. ramorum* outside the regulated areas for the disease. In addition, many state agencies now conduct yearly nursery surveys to determine if the pathogen is present. In these surveys, symptomatic host plants are sampled; therefore it is important to recognize the various symptoms of the disease and to establish the relative susceptibility of *Camellia* species.

Inoculation of Plants and Rating of Disease

Nine species and four hybrids of *Camellia* were tested for susceptibility to *Phytophthora ramorum* (Table 1). In general, the plants were rooted cuttings with 8 to 25 leaves; some were older plants. The pathogen isolate (5-C) used in these experiments was originally recovered from *C. sasanqua* 'Bonanza' in California in 2003. Sporangia in solution were prepared as described previously (8), by placing mycelial plugs in a sterile soil extract solution for 48 h. Plants were inoculated using a large paintbrush to apply sporangial solution (approximately 5000 sporangia per ml) to leaves, buds, and stems. Plants were inoculated in batches of 15 to 20, with individuals of each tested species or cultivar separated into at least 2 batches. Each batch included a few rooted cuttings of *Rhododendron* 'Cunningham's White' used as positive controls; at 5000 sporangia per ml, this cultivar develops distinct, easy-to-rate symptoms that, for the genus, are considered severe. Inoculated plants were carefully

spaced in a dew chamber at 20°C for 3 to 4 days until symptom development, and then removed to insure that fallen leaves could be paired with the plant that had dropped them. Upon removal from the dew chamber, plants were rated for defoliation and the percent symptomatic area of each leaf. If a particular leaf symptom was not clearly caused by the pathogen, or if defoliation of nonsymptomatic leaves occurred, leaves were plated on PARP media (2).

Statistical analysis was done to compare disease severity among cultivars. Cultivars showing very little or no disease had to be excluded from data sets because the large number of zero data points made it impossible to get a normal distribution of data. Remaining data was log-transformed and analyzed by General Linear Models with Tukey's Studentized Range test using SAS (SAS Institute Inc., Cary, NC).

Infected camellia leaves developed dark, water soaked lesions with defined margins (Fig. 1). When lesions dried out, they turned brown and brittle, distorting thinner leaves. Generally lesions did not expand to encompass a whole leaf unless young leaves were involved, and then infections could travel down the petiole and into the stem. In some cultivars, the lesion was difficult to see on the upper surface of leaves, particularly in dark-pigmented leaves. Lesions on older leaves of the cultivar 'Korean Fire' appeared as faint ring spots on the upper surface and as dark spots on the lower surface (Figs. 2a and b). Lesions on young reddish leaves of 'Night Rider' were difficult to see on the upper surface, but obvious on the lower surface (Figs. 3a and b). Diseased camellia leaves frequently fell off 4 to 5 days after inoculation; some defoliation occurred even with leaves showing no visible lesions, although the pathogen could often be recovered from the surface-sterilized leaf. Significant defoliation of the cultivar 'Elina Cascade' occurred, although few symptomatic leaves were observed. In addition to leaf symptoms, branch die-back was sometimes observed, notably in *C. japonica* 'Crimson candles' (Fig. 4).

Results of camellia inoculations are summarized in Table 1. No generalizations could be made about susceptibility at the species level; some cultivars of *C. japonica* and *C. sasanqua* were very susceptible, while others were not. The hybrid 'Roni Gaki' showed the most severe symptoms among the taxa tested. *C. sasanqua* 'Midnight Lover' was quite susceptible, with defoliation up to 18.2% and observable symptoms on 18.8% of total leaves. *C. sasanqua* 'Angel's Kiss,' however, showed no symptoms. Similarly, while *C. japonica* 'Crimson Candles' was relatively susceptible (with 14% defoliation and 11.9% of leaves showing symptoms), *C. japonica* 'Black Magic' was asymptomatic. Disease on *C. oleifera* varied widely in severity from plant to plant; since this species can be propagated from seed, this may have been due to genetic variability. *C. sinensis*, the "tea" of commerce, was moderately susceptible.

Table 1. Responses of different species and cultivars of *Camellia* to infection by *Phytophthora ramorum*.

Camellia species and cultivars ^z	No. plants tested	% Defoliation ^{uy}	% symptomatic leaves ^{vy}	% dis /lvs tot ^{wy}	Lesion size ^x
<i>C. japonica</i> 'Black Magic'	4	0.0	0.0	0.0	0.0
'Korean Fire'	10	3.8 efg	21.4 bcde	0.9 bcd	4.4
'Koto no kaori'	8	33.3 abcd	42.7 abc	0.7 bcd	18.8
'Royal Velvet'	11	2.3	2.8	0.1	1.2
'Shibori kingyo'	8	29.4 abcde	74.9 ab	1.2 bcd	12.0
'Tamo-no-ura'	8	2.5	4.33	0.2	1.8
'Tsagawa shibori'	8	30.6 abcde	74.4 ab	1.0 bcd	16.9
<i>C. oleifera</i>	17	7.3	12.7	3.0	8.5
seedlings	16	0.3	8.6	3.0	12.8
'Lushan Snow'	6	1.6 efg	2.7 efgh	0.1 de	4.6
<i>C. sasanqua</i> 'Angel's Kiss'	6	0.0	0.0	0.0	0.0
'Asa Kura'	8	36.7 abcde	55.7 ab	1.4 bcd	20.2
'Bonanza'	6	9.5	16.4 defg	1.8 cde	5.4
'Hatsu Hikari'	7	13.8 abcdefg	9.8 defg	2.5 bcd	19.3
'Midnight Lover'	15	18.2 abcdef	18.8 cdef	2.6 bcd	9.4
<i>Camellia</i> hybrids					
x 'Crimson Candles'	8	14.4 abcdefg	11.9 cdef	2.7 bcd	13.2
x 'Fire and Ice'	8	0.1	0.6	0.0	1.8
x 'Night Rider'	8	5.4	10.6	0.5	12.4
x 'Roni Gaki'	12	33.0 ab	65.1 ab	12.2 a	17.1
<i>C. brevistyla</i>	7	0.0	0.0	0.0	0.0
<i>C. crapnelliana</i>	6	0.0	3.0	0.1	0.9
<i>C. cuspidata</i>	6	1.7	0.8	0.0	0.8
<i>C. octapetala</i>	5	5.9 def	12.9 cdefg	0.9 cde	3.3
<i>C. sinensis</i> var. <i>sinensis</i>	7	9.4 cdef	13.5 cdef	1.3 bcd	8.3
<i>C. sinensis</i> var. <i>sinensis</i> (seedlings)	10	5.1	11.8	1.2	6.30
<i>C. sinensis</i> (hardy form)	8	6.5 bcdefg	63.2 ab	1.2 bcd	36.2
<i>C. tsaii</i> var. <i>synaptica</i> 'Elina Cascade'	9	15.3 abcdefg	1.8 gh	0.8 de	12.2
<i>Rhododendron</i> 'Cunningham's White' (positive control)	36	1.6	47.7 ab	1.6 bc	6.2

^u Defoliation = the number of leaves that had fallen off the plant divided by total leaves on the plant at inoculation. Leaves that fell were not necessarily symptomatic.

^v % symptomatic lvs = the number of leaves showing visible symptoms after inoculation divided by total leaves on the plant at time of inoculation. This included symptomatic leaves that had dropped.

^w % dis/lvs tot = the percent diseased tissue averaged over total leaves.

^x Lesion size is meant to give an idea of the average size of lesions on symptomatic leaves, derived by adding the % lesion size for each diseased leaf and then dividing by the number of diseased leaves total.

(footnotes continued)

Table 1 footnotes (continued).

^y Treatments followed by the same letter did not differ significantly at the 0.05 level. Treatments followed by no letters came from plants with little disease which were excluded from data sets. Remaining data was log-transformed and analyzed by General Linear Models with Tukey's Studentized Range test using SAS.

^z Breeders may be interested in additional information about the species and cultivars tested: 'Korean Fire' and 'Tamo no ura' were selection from wild plants. 'Bonanza' is also listed as *C. sasanqua* 'Bonanza' (Xhiemalis)] and 'Hatsu Hikari' as *C. sasanqua* 'Hatsu Hikari' (Xhiemalis)]; *Camellia* x 'Crimson Candles' is the F2 of *C. reticulata* x *C. fraterna*, *C.* x 'Fire 'n Ice' is a hybrid of *C. japonica* *tricolor* (Siebold) Red x *C. oleifera* 'Plain Jane', *C.* x 'Night Rider' is a hybrid of *C.* x *Williamsii* 'Ruby Bells' x *C. japonica* 'Kuro Tsubaki,' and I found no information on the parentage of *C.* x 'Roni Gaki.' Additional species tested were *C. brevistyla* (Hayata) Cohen-Stuart, *C. crapnelliana* Tutcher, *C. cuspidata* (Kochs) Wright ex. Gard. Chron., *C. octapetala* Hu, *C. sinensis* (L.) Kuntze var. *sinensis* (cutting-grown plants and seeds), *C. sinensis* ("hardy form," a selection from wild, not yet named), and *C. tsaii* Hu var. *synaptica* 'Elina Cascade.'

Fig. 1. Typical leaf symptoms of *P. ramorum* on *C. japonica* 'Tasagawa shibori.'

Fig. 2. Faint ringspot symptoms were barely visible on the upper surface of older leaves of *C. japonica* 'Korean Fire' (upper two leaves), while symptoms on younger leaves were more typical (lower leaf, Fig. 2a). Lesions on the same two older leaves were more obvious on the lower surface (Fig. 2b).

Fig. 3. Lesions were difficult to see on the upper surface of reddish young leaves of *Camellia* x 'Night Rider' (Fig. 3a), but were obvious on the same leaves seen from below (Fig. 3b).

Fig. 4. Tip die-back was pronounced on *C. japonica* 'Crimson Candles.' Red arrows indicate dead branch tips.

Pathogen Persistence Over Time

Some inoculated plants were incubated for approximately one month in the greenhouse, and then sampled to determine whether the organism was alive. Plants were dissected, and leaves, buds, roots, young shoot tips (the top 60 to 70 cm) and stem segments were plated on PARP media to determine if the pathogen was still present in plant tissue. Randomly selected stem segments were excised with a scalpel from the trunk and all major secondary branches. Fallen leaves were also collected and cultured.

After a month almost all symptomatic leaves had fallen off the plants. *P. ramorum* was recovered from fallen leaves on the surface of soil. On plants, the organism could be recovered most frequently from shoot tips that had suffered die-back (Table 2).

Table 2. Recovery of *P. ramorum* from various plants parts one month after inoculation.^x

Camellia species and cultivars	No. tested	Percent of plant parts infected				
		buds	shoot tips	Stem segments	fallen leaves	leaves on plant
<i>C. japonica</i> 'Korean Fire'	9	0.0	18.7	2.8	48.4	2.8
'Koto no kaori'	8	2.4	3.1	0.0	6.5	3.4
'Shibori kingyo'	8	7.7	0.0	10.4	5.6	7.5
'Tsagawa shibori'	8	7.0	16.7	18.8	0.0	6.3
<i>C. oleifera</i> 'Lushan Snow'	6	3.7	14.9	0.0	4.2	2.6
<i>C. sasanqua</i> 'Hatsu Hikari'	7	6.9	28.9	16.2	0.0	4.7
'Midnight Lover' ^y	9	0.0	18.0	32.7	16.5	2.8
<i>Camellia</i> hybrids x 'Fire and Ice'	8	6.5	11.6	0.0	7.1	5.1
x 'Night Rider'	8	2.8	14.2	2.1	11.3	5.0
x 'Roni Gaki'	12	3.3	14.4	23.8	0.0	3.1
<i>C. sinensis</i> var. <i>sinensis</i> seedlings	4	0.0	50.0	25.0	55.6	19.2
<i>C. sinensis</i> (hardy form)	8	0.8	33.9	44.0	1.4	7.0
<i>C. tsaii</i> var. <i>synaptica</i> 'Elina Cascade'	9	1.2	33.5	5.0	29.0	2.2

^x As determined by plating onto PARP.

^y 'Midnight lover' was checked for disease after 78 days.

Infection of Roots

Sections of *C. sasanqua* 'Bonanza' were rooted in 3-inch pots of Turface MVP, a fired montmorillonite clay soil conditioner (Profile Products LLC, Buffalo Grove, IL), and seeds of *C. oleifera* and *C. sinensis* were germinated in 3 inch pots of Turface; plant roots were then drenched with 15 ml of a sporangial solution (approximately 5000 sporangia per ml) of *P. ramorum*. Plants were incubated under greenhouse conditions for a month, then root samples were either washed and directly plated onto PARP media, or surface-sterilized in 0.025% sodium hypochlorite for 5 to 10 min before plating. Roots were asymptomatic but *P. ramorum* was recovered from both washed roots and those surface-sterilized ones at rates of 11 to 38% and 5 to 18%, respectively.

Conclusion

The differing level of susceptibility of camellia species and cultivars to *P. ramorum* means that breeders have ready sources of resistance available. However, when quarantine makes the detection of a pathogen important, less obvious symptoms are not a great advantage. Detection is currently carried out by nursery inspectors looking for symptomatic plants and collecting samples. Because camellias drop leaves in response to infection, an inspector might have to look not only for leaf spots, but for defoliation and shoot die-back. Because the organism can be isolated from apparently symptomless leaves that have fallen onto the soil surface, perhaps fallen leaves should be tested as part of the sampling procedure. Cultivars with high levels of disease might prove to be useful as "canaries in the coal mine"; interspersed among less susceptible cultivars, they would show symptoms more readily.

Phytophthora ramorum has been found to infect roots under laboratory or greenhouse conditions (3,6,7) and such roots are often symptomless. In addition, surveys of containerized plant nurseries and greenhouses have shown that pathogenic *Phytophthora* and *Pythium* spp. are commonly present in potting mix of symptomless plants (1,5). These findings suggest that a bulk soil assay or a test of irrigation runoff might be useful in detecting *P. ramorum*.

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