

Differences in Susceptibility of Ornamental Holly Cultivars to *Sphaeropsis tumefaciens*

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Abstract:

Witches' broom, or *Sphaeropsis* gall, is an important disease of some holly species and cultivars in central and south Florida landscapes and nurseries. The fungal pathogen, *S. tumefaciens*, was isolated from symptomatic holly trees in Gainesville and Apopka landscapes. Agar plugs containing hyphae of the pathogen were used to inoculate pruning wounds made on 15 commercially available cultivars. A growing tip, secondary branch, and the main stem of each plant were inoculated in July 2005. By mid-August symptoms were apparent. Mean separations of lesion length showed statistically significant differences between cultivars. No cultivar was immune, and species of *Ilex* found most susceptible were *I. glabra* 'Nigra', *I. X* 'Mary Nell', *I. crenata* 'Compacta', and *I. crenata* 'Helleri'.

Objectives, Materials, and Methods:

The objective of this project was to quantify differences in the susceptibility of holly cultivars important to the Florida nursery and landscape industries. In order to accomplish this objective, virulent isolates of the pathogen were collected, cultured, and stored. Holly cultivars important to the industry were identified and obtained. An inoculation method was developed that would mimic natural inoculation in a landscape or nursery situation. A method for rating disease and quantifying differences in susceptibility was developed.

Diseased samples exhibiting galling, blighting, and witches' broom symptoms were collected from holly plants in Gainesville and Apopka landscapes. Affected galls and shoots were surface sterilized in a 10% household bleach solution and plated onto potato dextrose agar amended with antibiotics. Colonies with morphology similar to *S. tumefaciens* were transferred and stored for future use. Nine isolates were collected. Isolate UF05161 was selected for this trial and was increased on V8 juice agar.

Input on cultivar selection was sought from UF county faculty, holly producers, and colleagues in the UF IFAS Environmental Horticulture Department. Holly plants were purchased from Florida nurseries. Ten plants of 15 cultivars (150 total) were obtained and transplanted into 3 gal pots at the Environmental Horticulture Landscape Teaching Lab in Gainesville. Plants were fertilized with 65 g of Nutricote (17-8-7) on 14 JUL 2005. Plants were randomized into five blocks. Each block contained an inoculated and an un-inoculated control plant of each cultivar.

Plants were inoculated on 21 JUL 2005. Pruning cuts were made to a growing tip, a secondary branch, and a shoot off of the main stem. Wounds were wrapped with Parafilm M flexible film. An agar plug with fungal hyphae was placed directly on the wounds of inoculated plants prior to wrapping with Parafilm M. No agar plug was placed under film on the control plants.

On 16 AUG 2005 the Parafilm M was removed from pruning wounds. Wound sites were marked with colored tape, and lesion size around each wound was recorded in millimeters.

Data were analyzed with SAS computer software. Sources of variation explored with PROC GLM included: block, cultivar, inoculation, and the applicable two-way interaction terms. Fishers LSD t-test groupings were applied to means to show statistically significant differences at alpha level 0.05.

Results:

Lesions were observed at pruning sites of inoculated plants. Lesions of sunken, black to dark brown, necrosis had spread from the inoculation site down the stem (Fig. 1). Small black raised fruiting bodies (pycnidia) of the fungus were observed on some of the lesions. Cankers on secondary branches spread both up and down from inoculation sites. Cankers on these stems also were discolored and slightly sunken (Fig. 2). Cankers on main stems ranged from barely noticeable, to dark sunken lesions that had expanded up and down the stem under the bark of affected plants (Fig. 3 and 4).

Mean lesion size recorded for each cultivar at each pruning site is listed in Table 1. Cultivars with the greatest lesion size are considered most susceptible and cultivars with the smallest lesion size are considered least susceptible. An analysis of variance showed that cultivar, inoculation, and cultivar X inoculation were significant ($P > F$ less than 0.05).

Conclusions and recommendations:

Results of this study suggest that both statistically and biologically significant differences exist between and among cultivars of holly. Differences in cultivar rankings were observed among mean lesion length at different inoculation sites (growing tip, secondary branch, and main trunk). Inoculation was most consistent, and mean separations were greatest among data collected for growing tips.

Results presented in this report are preliminary. Cultivar rankings could change as disease continues to develop, so additional research is needed. Witches' brooming associated with woody gall symptoms did not occur in the 4-week-period after inoculation. It is likely, however, that these symptoms will occur later in the fall or in the spring of the year following inoculation as the woody hosts continue to grow.

I recommend that the current study be continued through this growing season and into a second season. Funding additional research with this project will provide valuable data regarding continued development of disease on these cultivars, pathogen dissemination, sanitation efforts, and fungicide management of this important disease.

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Selected References

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Table 1. Mean lesion size in millimeters for holly cultivars inoculated with *Sphaeropsis tumefaciens* at three pruning sites. Data were taken 3 weeks after inoculation.

<i>Ilex</i> sp. and Cultivar	Tip ^a		Branch		Main Stem	
glabra 'Nigra'	36	a	62	bc	11	bc
x 'Mary Nell'	34	a	28	bc	53	a
crenata 'Compacta'	31	ab	135	a	19	b
x attenuata	30	ab	12	c	9	bc
crenata 'Helleri'	27	abc	93	ab	12	bc
crenata 'Sky Pencil'	21	abcd	23	c	11	bc
x 'Nellie R. Stevens'	14	abcde	12	c	4	c
vomitorea pendula	14	bcde	12	c	1	bc
opaca 'Savannah'	8	cde	8	c	6	bc
cornuta 'Burfordii' dwarf	6	de	5	c	3	bc
vomitorea 'Pride of Houston'	5	de	8	c	6	bc
cornuta 'Carissa'	4	de	0	c	3	bc
vomitorea 'Schellings Dwarf'	3	de	5	c	1	bc
cornuta 'Rotunda'	0	e	6	c	0	bc
cornuta 'Delcambre'	0	e	3	c	2	c

^a Means in the same column followed by the same letter are not significantly different according to Fishers LSD t-test groupings, alpha = 0.05

Fig. 1

Stem blight symptoms observed on a shoot tip of *Ilex* x 'Nellie R. Stevens' inoculated with *Sphaeropsis tumefaciens* three weeks previous. Small erumpent black flecks on the blighted shoot are fruiting bodies (pycnidia) of the fungus.



Fig. 2

A dark sunken stem canker formed on this secondary branch of *Ilex compacta* 'Compacta' that was inoculated with *Sphaeropsis tumefaciens*. Disease symptoms were observed both up and down from the pruning wound inoculation site.



Fig. 3

The bark of this *Ilex* x 'Mary Nell' split indicating canker formation on the main stem and a successful inoculation with *Sphaeropsis tumefaciens*. Symptoms shown are three weeks after inoculation.



Fig. 4

A large, sunken, black, lesion on the main stem of *Ilex compacta* 'Compacta' three weeks after inoculation with *Sphaeropsis tumefaciens*.

