

# Susceptibility of Evergreen Azalea Cultivars to Anthracnose Caused by *Colletotrichum acutatum*

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**SUMMARY.** *Colletotrichum acutatum* is the causal agent of anthracnose on azalea (*Rhododendron azalea*). This disease was observed in Fall 2002 on 'Palestrina' azalea in Italy. Severe outbreaks of this disease were recently observed on several cultivars of azalea grown in nurseries located in the Verbano-Cusio-Ossola Province in the Piedmont region of northern Italy. Information on susceptibility to *C. acutatum* among azalea cultivars currently grown in Italy was unavailable. Artificial inoculations were performed to evaluate the susceptibility of 70 evergreen azalea cultivars produced in the Lake Maggiore area. Sixty of the cultivars evaluated failed to develop disease symptoms and were considered resistant to *C. acutatum*. Symptomatic cultivars were placed into three susceptibility classes. The cultivars Addy Wery, Fior di Pesco Cavadini, Geisha Orangerot, Kermesina, Orion, Palestrina, and Snow developed severe anthracnose symptoms. The cultivars Conversation Piece, Eikan, and Martha Hitchcock showed moderate susceptibility.

**R**hododendrons (*Rhododendron* spp.) are popular and economically important ornamental plants in the Ericaceae family and are widely grown in the Piedmont region of northern Italy. The presence of acidic soils provides very favorable growing conditions. In the Lake Maggiore area, the market for acid-loving plants is valued at 13 million euros per year. Azalea is the most popular species grown, accounting for 50% of total production. More than one million plants are sold each year (Rabbogliatti, 2004), with a portion of the production grown for export.

Azaleas are susceptible to several foliar diseases (Benson and Williams-Woodward, 2001), including anthracnose, a fungal disease that causes leaf spots and defoliation. *Colletotrichum acutatum* was identified as the causal agent of anthracnose on azalea in Italy, where, in Fall 2002, the disease was observed on 'Palestrina' azalea

in several nurseries located in the Verbano-Cusio-Ossola province. The pathogen was isolated from infected leaves and stems. The mycelium produced cylindrical, tapered conidia measuring 4.8 to 7.2  $\mu\text{m}$  in length and 11.0 to 22.8  $\mu\text{m}$  in width (average,  $5.5 \times 15.2 \mu\text{m}$ ) (Garibaldi et al., 2004). Symptoms of this disease appear on both leaves and stems. Leaf lesions are 1 mm in diameter, irregularly round, dark-brown to black, and generally surrounded by a chlorotic halo. Lesions may coalesce, forming larger, irregular-shaped lesions. The disease causes defoliation of plants. Generally, the lower leaves are the most heavily infected. Brown, longitudinal lesions form along the entire stem.

Anthracnose on azalea was reported for the first time in Florida on swamp azalea (*Rhododendron viscosum*) and the causal agent was identified as *C. azaleae* (Ellis and Everhart, 1895). Since 1954, anthracnose on azalea was observed and described in Louisiana, where the

causal agent was identified as the conidial stage of *Glomerella cingulata*, the teleomorph of *C. gloeosporioides*. A susceptibility trial was carried out on 11 azalea cultivars belonging to the Kurume and Indica groups (Stathis and Plakidas, 1958). *Colletotrichum azaleae* is now considered synonymous with *C. gloeosporioides* (Farr et al., 1989; Von Arx, 1957). Severe outbreaks of anthracnose were recently reported on *Rhododendron* species growing in Sweden and Latvia. In this case, the causal agent of the disease was identified as *C. acutatum* (Vinnere et al., 2002). However, *C. dematium* was also isolated from some of the infected plants (Vinnere et al., 2002).

After the first appearance of anthracnose in 2002, severe outbreaks were observed in Italy on several cultivars of azalea grown in nurseries in the same area where the disease was first observed. There was no information available regarding the susceptibility to anthracnose among azalea cultivars produced and grown in Italy. The objective of this study was to determine levels of resistance to anthracnose among azalea cultivars grown in the Lake Maggiore area.

## Materials and methods

**PLANT MATERIAL.** The susceptibility of 70 evergreen azalea cultivars to anthracnose was evaluated (Table 1). Most cultivars belonged to the Kurume, Belgian, and Satsuky groups (Galle, 1987). Some of these cultivars are classified differently, following the Italian classification scheme (Scariot, 2006). Plants were supplied by Tecnoverde S.r.l. (Verbania, Fondotoce, Italy). One-year-old plants were grown in 10-cm-diameter plastic pots and arranged randomly in a complete-block design with three single-plant replications per each cultivar. Container substrate was 9 peatmoss : 1 perlite (by volume). The susceptibility trial was repeated twice.

**PATHOGEN AND INOCULATION METHOD.** Plants were artificially

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## Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
29.5735	fl oz	mL	0.0338
2.5400	inch(es)	cm	0.3937
1	micron	$\mu\text{m}$	1
$(^{\circ}\text{F} - 32) \div 1.8$	$^{\circ}\text{F}$	$^{\circ}\text{C}$	$(1.8 \times ^{\circ}\text{C}) + 32$



Table 1. Evergreen azalea cultivars resistant to anthracnose incited by *Colletotrichum acutatum*.

Cultivar	Hybrid group <sup>z</sup>	Parentage <sup>y</sup>
Alladin Scout	Unknown	Unknown
Amoena	Kurume (Amoena)	Unknown
Apricot	Kurume	Unknown
Arabesk	Kurume	Unknown
Athena	Unknown	Unknown
Blaauws Pink	Kurume	Unknown
Blauwe Donav	Kurume	Unknown
Cherish	Unknown	Unknown
Donatine	Belgian	Unknown
Feuerzauber	Belgian	Seedling Friedhelm Scherrer × Kirin or Rex
Friedhelm Scherrer	Unknown	Unknown
George Arends	Kurume	Unknown
Girards Fuchsia	Kurume	[(Sandra Ann × EV 1689) × (Herbert × Hot Shot)] × Sandra Ann
Girard's Rose	Kurume	[(Fedora × El Capitan) × (Boudoir)] × Bouroir
Gloria	Belgian	Unknown
Hampton Beauty	Unknown	Unknown
Hatsu Giri	Kurume	Unknown
Helena	Kurume	Eden Jane Alcott × Pink Pearl
Hino Crimson	Kurume	Amoenum × Hinode Giri
Hogi Kasane	Unknown	Unknown
Irish Lace	Unknown	Unknown
Iveryana	Unknown	Unknown
Janique	Kurume	Unknown
Johanna	Kurume	Florida seedling
Kathy Ann	Kurume	Unknown
Kermesina Rosata	Kurume	Kermesina sport
Kermesina Variegata	Kurume	Unknown
Kirin	Kurume	Unknown
Koli	Belgian	Seedling Petrick Alba × Rex
Lily Marleen	Kurume	Little Ruby × Dr. W. F. Wery
Marana	Unknown	Unknown
Massasoit	Kurume	Unknown
Mevrouw G. Kint	Belgian	Glaser number 10 sport
Mevrouw G. Kint White	Belgian	Sport of Mevrouw G. Kint (Glaser number 10 sport)
Miss Augusta	Unknown	Unknown
Moederkesdag	Kurume	Prof. Wolters × Hinode Giri
Multiflorum	Kurume	Unknown
Nanny	Kurume	Unknown
Nazarena	Kurume	Unknown
Orchid Empress	Unknown	Unknown
Ostalett	Belgian	Unknown
Panfilia	Belgian	Unknown
Patricia Barmold	Kurume	Kermesina × Blauwe Donav
Perla del Verbano	Kurume (Japonica)	Unknown
Promise	Unknown	Unknown
Purple Splendor	Kurume	<i>poukhanense</i> × Hexe
Remembrance	Kurume	<i>indicum</i> × Hazel Dowson
Rex	Kurume	Kirin × Hinomayo
Sachsenstern	Belgian	Unknown
Silvester	Kurume	Aladdin × Amoena
Stella Maris	Belgian	Rosali sport
Stewartstownian	Kurume	Unknown
Tamia	Belgian	Unknown
Tapestry	Unknown	Unknown
Thesla	Belgian	Unknown
Timeless	Unknown	Unknown
Treasure	Kurume	Unknown
Vuyk's Rosyred	Kurume	Unknown
Vuyk's Scarlet	Kurume	Unknown
White Lady	Belgian	Sport of Memoria Karl Glaser (Gustav Hacker sport)

<sup>z</sup>The hybrid group follows the classification reported in Galle (1987).<sup>y</sup>The parentage follows the classification reported in Galle (1987).



inoculated with a spore and mycelium suspension ( $10^5$  cfu/mL) of *C. acutatum* prepared from 20-d-old cultures grown on potato dextrose agar maintained at  $20 \pm 1$  °C. In the first trial, azaleas were inoculated with an isolate of *C. acutatum* isolated from infected ‘Palestrina’ azalea. In the second trial, a mixture of three isolates of the same fungal species from infected specimens of azalea ‘Palestrina’, ‘Fior di Pesco Cavadini’, and ‘Addy Wery’ were used. Fifteen milliliters of the spore and mycelium suspension were applied using a manual sprayer to inoculate each plant, thoroughly coating all the leaves. Plants inoculated with deionized water served as controls. Inoculated and noninoculated plants were held inside the greenhouse in a high-humidity chamber for 3 d after inoculation at 16.5 to 26.5 °C (average, 18.9–19.5 °C) and 61.3% to 75.8% relative humidity. Plants were then placed on a greenhouse bench. Relative humidity and air temperature values were measured by a data logger (Fourier Systems, New Albany, IN) placed among the plants.

**DISEASE ASSESSMENT AND STATISTICAL ANALYSIS.** Disease development was evaluated daily, starting at the first appearance of symptoms. The number of leaf lesions was counted randomly on 15 leaves per plant. Most tested cultivars (85.7%) failed to develop disease symptoms and were considered resistant (no leaf

spotting present in trials 1 and 2) to *C. acutatum* (Table 1). The average number of spots per leaf was calculated for each susceptible cultivar (Table 2), and data collected were subjected to analysis of variance. Means were separated by the LSD test (SPSS for Windows version 11.0.1; SPSS, Chicago). Symptomatic cultivars were grouped into three susceptibility classes: moderately susceptible (average number of spots/leaf, 0.1–5), susceptible (average number of spots/leaf, 5.1–20), and highly susceptible (average number of spots/leaf, >20).

## Results and discussion

The inoculation method used resulted in consistent expression of plant symptoms, which developed within 3 d of inoculation on the most susceptible cultivars. Sixty of the tested cultivars (85.7%) were resistant to *C. acutatum* (Table 1). Only seven of the cultivars (10%) developed severe anthracnose symptoms: ‘Addy Wery’, ‘Fior di Pesco Cavadini’, ‘Geisha Orangerot’, ‘Kermesina’, ‘Orion’, ‘Palestrina’, and ‘Snow’ (Table 2). ‘Conversation Piece’, ‘Eikan’, and ‘Martha Hitchcock’ (4.3% of the tested cultivars) showed moderate susceptibility (Table 2). The only significant difference between the two experiments carried out was for ‘Snow’, which was susceptible in the first trial and highly susceptible in the second trial. There

is no apparent connection between degree of susceptibility and common parents (when known). Among the Kurume group there are susceptible cultivars, such as ‘Addy Wery’ and ‘Palestrina’, and resistant cultivars, such as ‘Arabesk’ and ‘Hino Crimson’. ‘Snow’ was one of the most susceptible to *C. acutatum* under our conditions, even though this cultivar was the most resistant in a 1958 trial performed in the United States (Stathis and Plakidas, 1958). Such discrepancy may suggest either variability in susceptibility of ‘Snow’ to different *Colletotrichum* species or the diffusion of new, more aggressive *C. acutatum* strains.

During the past 3 years, the development of anthracnose on azalea in Italy caused severe financial losses to the industry in the Lake Maggiore area, where production of this species is concentrated. However, our results have demonstrated that among the more popular azalea cultivars grown in the Piedmont region, the majority are resistant to anthracnose. This provides growers a good range of choice when selecting plant material to grow, without having to incur unnecessary risk resulting from losses from the cultivation of highly susceptible cultivars. The variability of *Colletotrichum* species (Abang et al., 2006; Denoyes et al., 2003; Sreenivasaprasad and Talhinhas, 2005) and the possibility for *Colletotrichum* species to infect the same

Table 2. Evergreen azalea cultivars susceptible to anthracnose incited by *Colletotrichum acutatum*.

Cultivar	Hybrid group <sup>z</sup>	Average leaf spots (no.) <sup>y</sup> and susceptibility class <sup>x</sup>				Parentage <sup>w</sup>
		Trial 1		Trial 2		
Addy Wery	Kurume	11.1 bc	S	8.3 abc	S	Malvatica × Flame
Conversation Piece	Satsuki	1.0 a	MS	4.8 ab	MS	(Emile Russave × Carol) × Eikan
Eikan	Satsuki	1.3 a	MS	2.4 a	MS	Asahi Zuru seedling
Fior di Pesco Cavadini	Kurume (Japonica)	17.3 c	S	13.4 bcd	S	Unknown
Geisha Orangerot	Kurume	11.9 bc	S	17.0 cd	S	Unknown
Kermesina	Kurume	10.3 abc	S	17.1 cd	S	Unknown
Martha Hitchcock	Satsuki	1.2 a	MS	1.4 a	MS	Mucronatum × Shinnyo no Tsuki
Orion	Kurume	10.2 abc	S	19.9 d	S	Unknown
Palestrina	Kurume	6.7 ab	S	21.4 d	S	Kaempferi × J.C. van Tol
Snow	Kurume	18.3 c	S	33.6 e	HS	Unknown

<sup>z</sup>The hybrid group follows the classification reported in Galle (1987).

<sup>y</sup>Data were subjected to analysis of variance and least significant difference test. Values followed by the same letter are not significantly different ( $P \leq 0.05$ ).

<sup>x</sup>MS, moderate susceptible (average number of spots, 0.1–5); S, susceptible (average number of spots, 5.1–20); HS, highly susceptible (average number of spots, >20).

<sup>w</sup>The parentage follows the classification reported in Galle (1987).



host (Howard et al., 1992), suggest that the susceptibility of azalea cultivars to anthracnose be monitored and also that *Colletotrichum* species isolated in nurseries be monitored to determine whether resistant strains are developing.

### Literature cited

- Abang, M.M., R. Asiedu, P. Hoffmann, G.A. Wolf, H.D. Mignouna, and S. Winter. 2006. Pathogenic and genetic variability among *Colletotrichum gloeosporioides* isolates from different yam hosts in the agroecological zones in Nigeria. *J. Phytopathol.* 154:51–61.
- Benson, D.M. and J.L. Williams-Woodward. 2001. Azalea diseases, p. 81–88. In: R.K. Jones and D.M. Benson (eds.). *Diseases of woody ornamentals and trees in nurseries*. APS Press, St Paul, MN.
- Denoyes, R.B., G. Guérin, C. Délye, B. Smith, D. Minz, M. Maymon, and S. Freeman. 2003. Genetic diversity and pathogenic variability among isolates of *Colletotrichum* species from strawberry. *Phytopathology* 93:219–228.
- Ellis, J.B. and B.M. Everhart. 1895. New species of fungi. *Torrey Bot. Club Bul.* 22:434–440.
- Farr, D.F., G.F. Bills, G.P. Chamuris, and A.Y. Rosman. 1989. *Fungi on plants and plant products in the United States*. APS Press, St Paul, MN.
- Galle, F.C. 1987. *Azaleas*. Timber Press, Portland, OR.
- Garibaldi, A., D. Bertetti, O. Vinnere, and M.L. Gullino. 2004. Presence of *Colletotrichum acutatum* causing leaf spot on *Azalea japonica* in Italy. *Plant Dis.* 88:572 (abstr.).
- Howard, C.M., J.L. Maas, C.K. Chandler, and E.E. Albregts. 1992. Anthracnose of strawberry caused by the *Colletotrichum* complex in Florida. *Plant Dis.* 76:976–981.
- Rabbogliatti, I. 2004. Il distretto floricolo del Lago Maggiore: Situazione attuale e prospettive future. *Proc. Symp. L'azalea, nuovi orizzonti nella ricerca, nella produzione e nell'impiego*. p. 14–19.
- Scariot, V. 2006. The DNA-typing of ornamental plants: Evergreen azaleas (*Rhododendron* spp.) and old garden roses (*Rosa* spp.). University of Turin, Italy, PhD Diss.
- Sreenivasaprasad, S. and P. Talhinhas. 2005. Genotypic and phenotypic diversity in *Colletotrichum acutatum*, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. *Mol. Plant Pathol.* 6:361–378.
- Stathis, P.D. and A.G. Plakidas. 1958. Anthracnose of azaleas. *Phytopathology* 48:256–260.
- Vinnere, O., J. Fatehi, S.A.I. Wright, and B. Gerhardsson. 2002. The causal agent of anthracnose of *Rhododendron* in Sweden and Latvia. *Mycol. Res.* 106:60–69.
- Von Arx, J.A. 1957. Die arten der gattung *Colletotrichum* Cda. *Phytopathol. Z.* 29:413–468.