

## Woody Landscape Plant Breeding in Minnesota (45° N): It's not all about Cold Hardiness

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### Abstract

The Woody Landscape Plant Breeding project at the University of Minnesota has been in existence since 1954. The project was initiated largely to develop an extended palette of cold-hardy woody landscape plants for northern landscapes. Since its inception, it has been responsible for the release of 49 woody plant cultivars including large stature shade trees, small stature flowering trees, shrubs, roses, and deciduous azaleas. Given the project's success at building a cold-hardy germplasm pool for a number of woody taxa, recent breeding efforts have been directed towards other traits such as disease resistance in several plant taxa. Projects include an examination of the nature of resistance to powdery mildew disease (*Microspphae* spp.) in deciduous azalea (*Rhododendron* subg. *Pentanthera* (G. Don) Pojark) species and cultivars in both field and growth chamber studies; a physiological race and an Amplified Fragment Length Polymorphism (AFLP) molecular characterization of the rose black spot pathogen (*Diplocarpon rosae* Wolf) and identification of resistance genes to the disease in cultivated rose (*Rosa* ×*hybrida*). Recently, a high-throughput screening protocol to facilitate the identification of resistance to golden canker disease incited by the fungal pathogen (*Cryptodiaporthe corni* (Wehm.) Petrak) in a tree native to the upper midwestern U.S., Pagoda dogwood (*Cornus alternifolia* L.) has been initiated.

### BACKGROUND

The Woody Landscape Plant Breeding project was initiated at the University of Minnesota in 1954 with the mandate to develop landscape plants capable of thriving in the harsh winter environment of the region. The regions' winter is largely defined by the USDA Plant Hardiness zone 3/4 criteria, with winter low temperatures ranging from -20°C (-4°F) to -40°C (-47°F). The projects' activities have primarily been carried out at the Horticultural Research Center, located within the Minnesota Landscape Arboretum proper in Excelsior, Minnesota (44° 51' 30" N) (Fig. 1). In the course of its history the project has been led by three individuals, Dr. Leon Snyder from 1954 to 1976, Dr. Harold Pellett from 1976 to 2001, and Dr. Stan Hokanson since 2001.

### CULTIVARS

Forty-nine woody landscape plant cultivar releases are associated with research carried out at the Horticultural Research Center in Excelsior, Minnesota (<http://www.maes.umn.edu/releases/index.asp>). The releases from the project constitute a wide diversity of woody plants including seven large-statured shade trees, eight smaller-statured flowering trees, two conifers, eight shrubs, fourteen deciduous azaleas, nine roses, and one vine. In more than 50 years of breeding effort, substantial cold-hardy germplasm pools have been developed for several landscape plant taxa. With this germplasm in hand, breeding efforts can be directed toward other objectives. The most notable efforts under the current project leadership have involved disease resistance. These are outlined below.

### **Deciduous Azalea Powdery Mildew**

The Woody Landscape Plant Breeding project at the University of Minnesota is probably best known for the development and release of thirteen cold hardy deciduous azalea cultivars, generically referred to as the 'Lights' series. The cultivars are extremely cold hardy with cultivar flower bud hardiness ranging from -42.8°C (-45°F) to -31.7°C (-25°F). They possess a palette of flower colors that range from white to deep orange but lack red; selection for which is a work in progress. Additionally, breeding efforts are also being directed toward other goals including disease resistance, prolonged flowering period, fragrance and reduced stature.

One of the few criticisms raised regarding the 'Lights' azaleas concerns their susceptibility to powdery mildew (PM) disease which is a common foliar disease of the genus *Rhododendron* (Basden and Helfer, 1995; Coyier, 1986; Kenyon, 1995). The disease can lead to early senescence, defoliation and may have an effect on plant hardiness and flower yield in the following season (Hall and Beales, 1998). Although PM resistant deciduous azalea cultivars and species exist, most of the cultivars named within the past 100 years are generally susceptible to PM, especially the Exbury hybrid group (Hardy, 1980). Identification of resistant germplasm that can be used in breeding programs would be an important step towards developing resistant cultivars.

Several reports of PM on deciduous azaleas exist in the literature (Ford, 1975; Hardy, 1980; Dufky, 1990; Cochran and Ellett, 1990; Basden and Helfer, 1995). Two studies, Ford (1975) and Cochran and Ellett (1990) report PM resistance/susceptibility of deciduous azaleas growing in the gardens at the Secret Arboretum in Wooster, Ohio, U.S.A. Interestingly, while most of the cultivars retained the same disease rating between the two studies, over one-third of the genotypes changed from a resistant to susceptible status or vice-versa.

In order to identify PM resistant cultivated deciduous azalea germplasm, we designed a field experiment to evaluate the resistance/susceptibility of 41 commercially available deciduous azalea genotypes to naturally occurring PM inoculum. The genotypes were replicated four times at each of two sites; the Horticultural Research Center and the Holden Arboretum's David G. Leach Research Station in, Excelsior, Minn. and Madison, Ohio U.S.A., respectively (Fig. 1). Data was collected from each site for three field seasons, 2002-2004. Disease incidence was scored on both the abaxial and adaxial leaf surfaces on a 1-5 scale where 1 signified no disease, 2 less than 25% of the leaf area infected, 3 less than 50% of the leaf area infected, 4 less than 75% of the leaf area infected and 5 greater than or equal to 75% of the leaf area infected with PM.

Symptoms of infection were varied, ranging from classical powdery mycelia on leaf tops and bottoms to purple/gray to brown lesions to necrotic lesions resembling a classic HR (host response). Because disease severity was more severe on the abaxial leaf surface in the field study, these scores were used in the analysis. It was found that the mean disease severity scores for the 41 genotypes in the study was 2.71, suggesting the collection was mostly susceptible. Six cultivars, June Flame, Fragrant Star, Garden Party, Millennium, Parade, and Popsicle showed no powdery mildew symptoms in either location during any of the three field seasons, suggesting resistant deciduous azalea cultivars exist. Another group of five genotypes had mean scores ranging from 1.05 - 1.13, 'Snowbird', 'Magic', 'Late Lady', 'Pink and Sweet', and 'Lollipop' and could be considered highly resistant. The remaining genotypes had disease severity scores ranging from 1.55 - 4.85. These were considered moderately to highly susceptible.

In another set of experiments designed to develop a lower cost, high-throughput PM screening protocol, 27 cultivars, (of which 23 were in the previously described field study) were evaluated in growth chamber experiments. Four dormant liners of each of the 27 cultivars were planted in 9 cm pots containing 50:50 peat: perlite substrate and incubated under 16 h photoperiod; 92% relative humidity; 18°C day:16°C night conditions with capillary irrigation. Infected leaves from the source plants were brought into contact with the adaxial and abaxial leaf surfaces of the two youngest, fully expanded leaves on the two tallest stems of each trial plant to inoculate them. The inoculated plants

were scored approximately 30 days later, as described in the field experiment.

Unlike the field study, disease symptoms were more pronounced on the adaxial leaf surface. Adaxial scores were used for comparisons in the growth chamber studies. Although, all genotypes became infected and developed PM symptoms in the growth chamber, they differed significantly for levels of PM infection, indicating that variation in PM disease response was detectable in the growth chamber. Although the powdery mildew fungus infected all cultivars, Fragrant Star, Lemon Lights, Mandarin Lights, Millennium, Northern Hi-Lights, Parade, Snowbird and Tri-Lights were the most resistant with scores less than or equal to 2.0.

Growth chamber results were fair predictors of field performance with similar disease scores noted for several cultivars between the field and growth chamber experiments. 'Fragrant Star', 'Millennium', 'Northern Hi-Lights', 'Parade' and 'Snowbird' rated  $\leq 2$  in both experiments. Three cultivars, Cheerful Giant, Irene Koster and Yellow Cloud, were highly susceptible ( $\geq 4$ ) in both experiments. Several cultivars described as resistant in other studies proved susceptible in this growth chamber study. It was observed that young foliage was particularly susceptible to infection in the growth chamber conditions, presumably because of the optimal PM growth conditions created i.e., lack of free water on the adaxial leaf surface and temperature and humidity levels ideal for PM infection and development.

To evaluate a much larger spectrum of cultivars and species germplasm, a three year, five location evaluation of 112 deciduous azalea cultivars and 14 species was conducted at five botanical garden/arboreta locations. Powdery mildew severity was assessed between late August and early September in 2002, 2003 and 2004 on deciduous azaleas at the Dawes Arboretum, Newark, Ohio, the David R. Leach Research Station, Madison, Ohio, the Secrest Arboretum, Wooster, Ohio, the Holden Arboretum, Kirtland, Ohio, and the Minnesota Landscape Arboretum, Excelsior, Minn. (Fig. 1). Plants exposed to naturally-occurring inoculum were assessed for PM severity using the scale described above.

Similar to the replicated field trial described above, the overall mean scores for adaxial leaf surfaces were lower than the abaxial leaf ones. Overall, seventeen cultivars showed no powdery mildew symptoms at any of the sites over the three year period of evaluation. Similarly, five species, *R. arborescens*, *R. canadense*, *R. canescens*, *R. schlippenbachii*, and *R. vaseyi* showed no powdery mildew symptoms at any site over the course of the evaluation. As a group, the 11 North American species were more resistant, with a mean abaxial leaf rating of 1.24. The European species *R. luteum* and Asian species *R. molle* were more susceptible with mean disease scores of 2.7 and 4.1 respectively. These results suggest the high degree of powdery mildew susceptibility in many deciduous azalea cultivars such as the Knap Hill, Exbury, Girard, and Ilam types may be due to the extensive use of *R. luteum* and the *R. molle* species in these breeding programs.

### **Rose Black Spot**

In 1990 the project initiated a shrub rose breeding program. The goals of the program were to develop USDA Plant Hardiness Zone 3/4 hardy shrub roses with disease resistance, recurrent blooming and a full range of colors. Developing plants durably resistant to rose black spot (incited by the fungal organism *Diplocarpon rosae* Wolf) has proven difficult. Seedlings that appeared resistant to the disease in the early years of the selection/evaluation process later succumbed to the disease. This situation prompted a series of investigations into the nature of the fungal pathogen and resistance to the disease.

*Diplocarpon rosae* is the most serious disease of garden roses worldwide. This fungus is obligate to the genus *Rosa* and does not infect any other plant taxa. Several physiological races of *D. rosae* have been described in the literature including seven races discovered in Mississippi, U.S. (Spencer and Wood, 1992a,b), four in Ontario, Canada (Svejda and Bolton, 1980), four in England (Yokoya et al., 2000), and five in Germany

(Debener et al., 1998). At the time these studies were initiated, the races described in England and Germany were the only ones known to be in long-term storage in a virulent state.

The fungus can be cultured on artificial culture media, but it is difficult to do so. Fungal hyphae grow slowly on culture media and more importantly, spores produced in culture quickly lose virulence. Cultures have been reported to begin to lose pathogenicity after six months at 23°C (Drewes-Alvarez, 1992). A number of experiments demonstrated refrigeration and freezing slows the decline (Palmer et al., 1996; Knight and Wheeler 1978; Yokoya et al., 2000; Leus, 2005). However, cryogenic storage appears to be the most effective method for long-term preservation of black spot isolates. Castledine et al. (1981) succeeded in freezing conidia for 24 hr in liquid nitrogen (-196°C) with no loss of viability.

As a first phase of the work with the black spot organism, the efficacy of long-term cryogenic storage of infected leaf pieces was tested according to the technique of Whitaker et al. (2007a). In short, isolates recovered from cryogenically preserved infected leaf pieces retained virulence for several months both in the gas phase of liquid nitrogen and at -80°C. These low-temperature treatments proved superior to storage at -20°C for three different isolates. After three years the infected leaf pieces stored above liquid nitrogen were still capable of producing infective conidia. To insure virulence the freezer-stored race isolates were cycled through a living host at two year intervals.

Using this long-term storage protocol to preserve the isolates, experiments were designed to determine the extent of rose black spot race diversity in eastern North America according to the method of Whitaker et al. (2007b). Essentially, rose leaves infected with *D. rosae* were collected from 17 locations across eastern North America. Fifty single-spore isolates of *D. rosae* were cultured from infected leaves on potato dextrose agar according to the methods of Whitaker et al. (2007a). One isolate was randomly chosen from each of 14 of the 17 eastern North American locations for inclusion in the race differentiation test. The differential array utilized in the race test was comprised of 12 genotypes including hybrid rose cultivars (*Rosa hybrida* L.), a breeding selection and a rose species, *Rosa spinosissima*. The genotypes were chosen to represent a wide range of genetic backgrounds and horticultural rose classes. Based on ability to infect the rose hosts, the results established at least three races of *D. rosae*, designated race A, B, and C are present in eastern North America. Subsequently, a fourth race isolated from *Rosa rugosa* 'Hansa' has been identified

More recent work has involved the identification of resistance genes in cultivated rose germplasm. Cultivars resistant to North American races A ('George Vancouver'), B ('Applejack'), and C ('Love and Peace™') were crossed to multiple susceptible parents. It was found that resistances to races A and C both segregated approximately 1:1 in multiple F<sub>1</sub> progenies, a ratio indicative of single dominant genes in simplex (Rrrr) configuration, assuming random chromosome assortment. In crosses between 'Folksinger' and 'Love and Peace™', progeny resistant to both races A and C were recovered, demonstrating gene pyramiding as an effective method for gaining broader black spot resistance in rose. Efforts to find DNA markers linked to these genes are currently underway.

### **Dogwood Canker**

The most commonly used dogwood trees in the landscaping industry, *Cornus florida* L. (Flowering dogwood) and *C. kousa* (Buerger ex Miq.) Hance (Kousa dogwood), are not hardy in USDA Plant Hardiness Zone 4 and colder locations (Dirr, 1998). A closely related species, the Pagoda dogwood (*C. alternifolia* L.) is a species native to much of the upper Midwestern U.S. with a range extending from northern Minnesota to the Florida panhandle. The primary limitation to Pagoda dogwood being widely utilized as a flowering dogwood alternative in the upper Midwest is its relatively short-lived duration in the landscape. Pagoda dogwood is susceptible to a canker disease that either kills the tree or reduces it to a shrubby stump sprout. The disease, golden

canker, is incited by the fungal organism, *Cryptodiaporthe corni* (Redlin and Rossman, 1991). Although the fungus was first described in 1874, little is known about its dissemination and infection of *C. alternifolia* and its growth and reproduction in or on the host. The disease cycle has not been investigated. The timing and mode of disease progression following initial infection within a plant is unknown, although the fungus is considered an endophyte and an opportunistic pathogen of *C. alternifolia* (Sinclair and Lyon, 2005). To date, no attempt to systematically identify resistant Pagoda dogwood seedlings has been undertaken.

To determine the timing of spore dissemination, a standard spore trapping approach was used. Vaseline coated microscope slides were suspended from stakes placed at four cardinal ordinates around the perimeter of *C. corni* infected trees. The slides were checked weekly between April 15 and November 30 for the presence of ascospores and/or conidia using a 400x light microscope. An attempt will be made to identify a relationship between peak incidence of spores and weekly minimum/maximum temperature and weekly humidity.

In another set of experiments designed to determine the timing of plant infection in the season, the above mentioned spore trapping sites were utilized. Three year old containerized plants, confirmed to be *C. corni*-free, were placed beside *C. corni*-infected and symptomatic Pagoda dogwood plants in four sites. Each set (4 plants at each of the four sites) were set out at mid-month (April through October) and removed in one month to be replaced by the next set. All exposed plants were maintained in the greenhouse for monthly monitoring of symptom development and for isolation of the pathogen from symptomatic tissues at the end of the experiment (12 months from exposure).

To gain insight as to how initial infections occur, containerized plants will be inoculated in greenhouse experiments to determine whether natural openings or wounds into the bark are necessary. A spore suspension ( $10^3$  spores  $\text{ml}^{-1}$ ) will be applied to run-off on 2-yr-old container plants with leaf scar wounds, bark abrasion damage, xylem-penetrating wounds and plants with no wounds. The experiment will be conducted in the spring (just after leaf flush) and fall (just prior to leaf abscission). The plants will be maintained at seasonally adjusted temperatures and symptom development (i.e. bark necrosis, foliage death, and shoot dieback) will be monitored up to one year after treatment. Because *C. corni* may infect without apparent symptom development, isolations will be made from the plants to test for presence of the fungus regardless of outward symptoms.

The influence of environmental stress on disease development will be determined. The role of freeze stress will be evaluated by freezing branch tips of uninfected plants in the greenhouse using liquid nitrogen followed by a spray inoculation. Disease levels for Pagoda dogwood trees will be determined in both managed and natural landscapes. Furthermore, an assessment of the effects soil type, soil fertility level and relative amount of light, e.g. full-, partial-sun, or shaded on disease incidence and severity will be made.

It is our hope that an understanding of the timing and conditions conducive to pathogen infection and disease progression will allow for the development of best management practices for prevention and control of golden canker disease on Pagoda dogwood in the nursery and the landscape. Work is in progress to develop a high-throughput screening process for selecting Pagoda dogwood seedlings resistant to isolates of *Cryptodiaporthe corni*. Identification of golden canker resistant Pagoda dogwood seedlings would lead to the development of a canker resistant, named Pagoda dogwood cultivar(s).

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## Figures



Fig. 1. U.S. map depicting locations of the field plots for the deciduous azalea powdery mildew field trials. 1) Horticultural Research Center, Excelsior, Minn. and 2) David G. Leach Research Station, Madison, Ohio and the botanical gardens and arboreta where the survey of deciduous azalea powdery mildew occurrence were conducted 1) Minnesota Landscape Arboretum, Excelsior, Minn., 2) as above, 3) The Holden Arboretum, Kirtland, Ohio, 4) The Seacrest Arboretum, Wooster, Ohio, and 5) The Dawes Arboretum, Newark, Ohio.

