Daphne Research at the University of British Columbia: The Search for Fungal Resistance©

Andrew Riseman
University of British Columbia Botanical Garden and Centre for Plant Research, University of British Columbia, 6804 SW Marine Drive, Vancouver, BC, V6T 1Z4, Canada
E-mail: andrew.riseman@ubc.ca

A *Daphne* research program was initiated in 2000 at the University of British Columbia (UBC) designed to develop cultivars with improved commercial and garden performance. The overall appeal of daphne is based on many desirable characteristics, including its attractive foliage, variable plant habits, and flower colors, but most of all, its sweet fragrance or perfume. The most "popular" species among nursery producers and home gardeners is *Daphne cneorum* L. (rose daphne or garland flower). Both total sales of *D. cneorum*, as well as the number of nurseries producing stock, are increasing rapidly. In British Columbia, Canada, over 23 commercial growers are currently selling *D. cneorum* compared to only seven growers in 1992 (British Columbia Landscape and Nursery Association Buyer's Guide and Directory, 1992 and 2005).

Despite this increase in production and inherent appeal, daphne has acquired a poor reputation for long-term performance because of disease problems reported by both producers and consumers. Although reports on daphne disease in Canada are lacking, anecdotal evidence suggest significant losses are occurring and that these losses are primarily due to root pathogens. However, disease susceptibility appears to be species-specific, with some species highly susceptible while other species appear to be immune. Previously, pathology reports identified *Fusarium* Link ex Gray (Pataky, 1988), *Phytophthora cactorum* (Lebert & Cohn) Schröter (Linderman and Zeitoun, 1977), *P. nicotianae* var. *parasitica* (Breda de Haan), Tucker (Tompkins, 1951), and *Pythium* Pringsh. (Grand, 1985), plus several unidentified fungi, as possible causal agents of this problem. In 2001, symptoms of an undescribed daphne disease were reported in Vancouver, British Columbia. Typical symptoms were somewhat inconsistent with those in previous pathology reports on daphne in that these plants all had black lesions on the roots and died within 2 weeks following appearance of the first foliar symptoms. This disease, coined "Daphne Sudden Death Syndrome" (DSDS) or "Mad Daphne Disease" by gardening enthusiasts, kills plants quickly following the first foliar symptoms. Observations of DSDS-infected plants indicate the following progression of symptoms: (1) brown to black necrotic lesions on the roots, (2) leaf chlorosis leading to abscission, (3) whole plant stunting, and (4) stem collapse and plant death.

To identify the causal agent of DSDS, root tissue samples were collected from diseased and healthy plants (paired samples of diseased and healthy plants acquired from individual nurseries throughout the greater Vancouver region) of *D. cneorum* 'Ruby Glow' and included roots of various diameters, discoloration, and degrees of degradation. From diseased plants, the following fungi were isolated: *F. roseum* (Snyder & Hansen), *F. oxysporum* (Snyder & Hansen), *Trichoderma* sp. (Persoon ex Gray), *Aspergillus* sp. (Micheli ex Link), and *Thielaviopsis basicola* (syn. *Chalara elegans* Nag Raj et Kendrick) (Berk. et Br.) Ferr. However, only *T. basicola* was isolated from all diseased plants but was absent from healthy plants.

Thielaviopsis basicola is a widespread pathogen of several economically important plant species (Nag Raj and Kendrick, 1975). In Canada, this pathogen causes the disease "black root-rot" on crops such as carrot (*Daucus carota* L.) (Punja et al. 1992) and tobacco (*Nicotiana tabacum* L.) (Gayed, 1972; Stover, 1950a, 1950b), while also found on several ornamental species such as poinsettia [*Euphorbia pulcherrima* (Willd. ex Klotzsch) Graham] and petunia (*Petunia* hybrid Vilm.) (Punja et al., 1992). In all of these plant species, *Thielaviopsis*-infected plants produced shoots that were stunted and chlorotic, with roots having black lesions containing the characteristic spores of the fungus (Punja et al., 1992). These symptoms are consistent with those reported for DSDS. To test pathogenicity, a conidial suspension was applied to healthy roots of both 2-year-old nursery-grown and rooted in vitro-produced plantlets of *D. cneorum*. The following 0–5 rating for disease progression was used: 0 = healthy plant, no symptoms; 1 = less than five lesions on lateral roots, no lesions on tap root, no foliar symptoms; 2 = greater than five lesions on lateral roots, less than five lesions on tap root, no foliar symptoms; 3 = most lateral roots with lesions and some necrosis, greater than five lesions on tap root, five to ten chlorotic leaves; 4 = most lateral roots necrotic, greater than five lesions on tap root, most leaves chlorotic with some leaf abscission; 5 = plant is dead. These data were then used to create a composite value for Plant Disease Index (PDI).

Four weeks post-treatment, all nursery-grown plants inoculated with *T. basicola* developed symptoms consistent with DSDS (PDI = 3.5), while all other plants, either inoculated with the other isolates or the control plants, remained symptomless and healthy (PDI = 0). In vitro inoculated plants displayed the same pattern of disease occurrence among the isolates as expressed on the nursery-grown plants. However, *T. basicola* induced symptoms in significantly less time (< 2 weeks) on these plantlets than on the nursery plants. *Thielaviopsis basicola* was successfully re-isolated and re-identified from all inoculated plants confirming its ability to induce DSDS (Noshad, et al., 2006).

Once the causal agent for the disease was identified, we initiated a germplasm screen to assess individual species' resistance to DSDS. Terminal cuttings from 32 *Daphne* taxa (container-grown) were harvested at two time periods (mid-July and mid-August) and treated as follows: flower buds and lower leaves were removed to give a clear stem to stick; cuttings were given a single shallow wound and soaked in a solution of Physan 20™ (1 teaspoon per U.S.A. gal) for 60 sec and allowed to dry; cuttings were dipped in Stim Root #2 Rooting Powder™ (0.4% IBA) and then direct stuck in $2^{1}/_{4}$ inch Premo™ pots filled with propagation grade perlite, peat, granite grit #2, and pumice (double screened to remove fine particles) (10 : 8 : 6 : 1, by volume) and amended with dolomite lime (65AG at 900 g/yd^3) and Micromax™ (400 g/yd^3). The flats were placed under mist with bottom heat set at 22 °C. The cuttings were checked weekly for rooting and removed from mist on an individual basis as they rooted. Rooted cuttings were placed under Vispore™ (a synthetic shade material) and misted twice daily by hand for 1 week, following which they were transferred to an open bench in the greenhouse and fed with Excel Cal-Mag™ 15N–5P–15K at 100 ppm total nitrogen. The rooted cuttings were transferred to a polyhouse in the fall where they were allowed to go dormant but kept frost-free. They were repotted in the spring with medium composed of peat, Turface™ MVP, granite grit #2, soil (screened and pasteurized), and pumice (8 : 8 : 6 : 4 : 1, by volume) amended with dolomite lime (65AG at 670 g·m^{-3}), Osmocote™ 18N–6P–12K (2150 g·m^{-3}), and

Psi Matric™ (wetting agent). Four months following potting, plants were subjected to the pathogen challenge as described above.

Rooting percentages varied by species and cutting time, with some species rooting easily regardless of time [*D. collina*, *D.* × *eschmannii*, *D. genkwa* (large flowered form), *D. laureola*, *D.* 'Lawrence Crocker', *D.* × *manteniana*, and *D.* × *rollsdorfii* 'Wilhelm Schacht'], some species rooting best in early summer [*D. bholua*, *D. cneorum*, *D. genkwa* (Hackenberry Group) *D. tangutica* Retusa Group, (syn. *D. retusa*), *D. tangutica*, and *D.* × *thauma*], some species rooting best in later summer (*D. circassica* and *D. odora*), and some species that were difficult to root regardless of time (*D. longilobata* and *D.* × *napolitana*).

Following the pathenogenicity test protocols described above, all propagated species were screened for disease resistance using *T. basicola*. The pathogen challenge revealed significant differences among the species for resistance. In general, the three most susceptible species were *D. cneorum* (PDI = 64), *D. pontica* (PDI=60), and *D.* × *antensiana* (PDI = 57) while the three most resistant species were *D. mezereum* (PDI = 10), *D. caucasica* (PDI = 9), and *D. tangutica* Retusa Group/*D. tangutica* (PDI = 0).

Future research will characterize disease development in both susceptible and resistant species, with special attention paid to the very early infection events. Once characterized, anatomical, morphological, and biochemical traits associated with disease resistance will be identified and their underlying mechanism(s) described. Ultimately, these "resistance" mechanisms will be manipulated so they can be incorporated into commercial cultivars.

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