New Opportunities for Breeding Allopolyploid Azaleodendrons

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Significance to Industry: Breeding and development of improved nursery crops depends on the potential for combining desirable traits among genetically diverse plants. However, hybrids between distantly related taxa (e.g., interspecific and intergeneric) often result in sterile progeny, thereby limiting breeding opportunities. By restoring fertility in these hybrids, we can greatly expand the potential for developing new hybrids. An induced allopolyploid form of the sterile azaleodendron, *Rhododendron* 'Fragrant Affinity' exhibited high pollen viability as shown by staining and germination assays. With restored fertility, this allopolyploid may now be utilized in breeding for more cold hardy, fragrant rhododendrons. This technique may have potential for restoring the fertility of other sterile hybrids within the genus.

Nature of Work: *Rhododendron* 'Fragrant Affinity' is a cold hardy (-26°C, -14.8°F), fragrant azaleodendron with semi-evergreen foliage that is believed to be an intersubgeneric hybrid between *R. catawbiense* and *R. viscosum* (Dr. Augie Kehr, personal communication). Due to its cold hardiness and fragrant lavender flowers, *R.* 'Fragrant Affinity' would be an asset in a breeding program; unfortunately, it is sterile.

Hybrid sterility often occurs due to improper chromosome pairing during gametogenesis. Doubling the chromosome compliment of sterile hybrids (allopolyploidy) provides a homologue for each chromosome with which to pair during meiosis and can allow for the development of fertile gametes (1). Oryzalin (Surflan® A.S., Dow AgroScience LLC, Indianapolis, Ind.) has been shown to be effective in producing fertile allopolyploids from wide hybrids such as *xChitalpa tashkentensis* (4).

Apical meristems of diploid *Rhododendron* 'Fragrant Affinity' were treated with a 150μM (super-saturated) oryzalin solution for 24-hours. Ploidy levels were determined using flow cytometry (Ploidy Analyser, Partec GmbH, Münster, Germany).

Pollen was collected from diploid and allopolyploid plants, dried overnight and frozen at 10.4°F (-12°C). Fertility was evaluated using pollen staining and germination assays as measures of pollen viability (5). Pollen from both diploid and allopolyploid plants was stained using 1% acetocarmine solution (2). Well-formed grains with at least one of the tetrads stained were scored as stained. The germination study was conducted using Brewbaker-Kwak media with 5% sucrose for eight-hours (3). Pollen with pollen tubes equal to or greater than

the diameter of the pollen grain was scored as germinated. Pollen analysis was conducted using a compound light microscope (Micromaster, Fischer Scientific, Pittsburgh, Penn.) under 100x and 400x magnification. Pollen staining and germination assays were conducted on ten sub-samples, of 100 pollen grains each, for both the diploid and allopolyploid plants. Data are presented as means and the standard error of the means.

Results and Discussion: The development of an allopolyploid *Rhododendron* 'Fragrant Affinity' was an effective technique for restoring male fertility. Although the diploid produced a high percentage of well-formed tetrads, the pollen grains were small and only had 8% staining (Table 1, Fig. 1). The allopolyploid generally produced larger, well-formed tetrad pollen that stained at 52%. No pollen from the diploid *R*. 'Fragrant Affinity' germinated but the allopolyploid pollen germinated at 22% (Table 1). In addition to *in vitro* testing, 144 flowers on the allopolyploid were self-pollinated and have produced seven capsules, which are currently developing.

Literature Cited:

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Table 1. Results from pollen staining and germination tests.

Ploidy Level	% Staining	% Germination
Diploid (2x)	8.3 ± 0.8*	0.0 ± 0.0
Allopolyploid (4x)	51.8 ± 4.8	21.6 ± 6.5

*Means (n=10) followed by ± 1 standard error of the mean.

Figure 1. Diploid pollen (left) and allopolyploid pollen (right) stained with 1% acetocarmine.