

Fertility of Neopolyploid *Rhododendron* and Occurrence of Unreduced Gametes in Triploid Cultivars

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Significance to Industry: Polyploidy, the condition of having multiple sets of chromosomes, has important implications for plant breeding and can influence ornamental characteristics, crossability, plant vigor, and gene expression. Polyploidy can also have a profound influence on reproductive biology, including fertility. The potential for utilizing polyploids in a breeding program is dependent upon fertility of specific taxa. A greater understanding of fertility mechanisms in polyploid *Rhododendron* and information on fertility of specific clones will better allow breeders to utilize polyploids in plant improvement programs, ultimately leading to the development of improved cultivars for the nursery industry.

Nature of Work: Fertility of newly developed polyploids (neopolyploids) can vary considerably and is influenced by their specific origins (5). Polyploids that arise from within a single species are referred to as autopolyploids. Autopolyploids may lack fertility due to the presence of multiple homologous chromosomes that can result in multivalent pairing and unequal segregation in meiosis (4). Polyploids that arise from hybrids between species are referred to as allopolyploids. Allopolyploids are often fertile due to nonrandom, disomic pairing between two distinct sets of chromosomes from the two parental species. In many cases, however, polyploids fall somewhere between an autopolyploid and an allopolyploid; where there is partial chromosome homology resulting in a combination of disomic and polysomic pairing, often referred to as segmental allopolyploids (4).

Moreover, fertility can also be affected by the number of chromosome sets. Many times, odd-ploidy cytotypes are found to be highly infertile, if not sterile (4). Such is the case with triploids within the genus *Rhododendron*. Infertility of triploids results from the fact that three sets of chromosomes cannot be divided evenly during meiosis, yielding unequal segregation of the chromosomes often resulting in aneuploid gametes or meiotic failure. Many triploids possess desirable ornamental characteristics in growth and flower morphology, yet the reproductive biology of triploids in *Rhododendron* is not well understood. Unreduced gametes may be associated with triploids (6) and can be utilized for breeding and as bridges for gene transfer between polyploid levels (4). The pollen structure in *Rhododendron* is typically a tetrad of grains that are tightly grouped at maturity; however, the structure of unreduced pollen is a mixture of larger dyads and monads. The objectives of this project were to: 1) evaluate the effect of increased ploidy level on pollen fertility of selected *Rhododendron* and 2) evaluate pollen fertility of naturally occurring triploids found in the genus.

Comparing fertility between ploidy levels. To study the influence of increased ploidy level on fertility, neopolyploids and their progenitor taxa were chosen, based on prior work by Jones et al. (2), to compare the pollen viabilities between ploidy levels within the same genotype. All pollen was collected at anthesis from plants at the Mountain Horticultural Crops Research Station, dried at 70°F (~21°C) for 24 hrs., and stored at -13°F (-25°C) until testing. Pollen was placed on glass microscope slides, and the grains were stained with 1% acetocarmine (w/v) for 15 minutes. Pollen grains that stained a distinct red-pink color were scored as viable (5). The tetrad nature of *Rhododendron* pollen required each individual grain in the tetrad to be analyzed. Each tetrad has the potential to contain four viable grains. The experimental design was a randomized complete block with ten replicates blocked by day over a time of one week. A minimum of 50 tetrads were randomly selected and analyzed per replicate. Pollen was observed at 300× using a light microscope (Nikon Eclipse 80i, Nikon, Melville, NY). Pollen viability percentages were calculated and the data were subjected to analysis of variance and pairwise means comparisons between ploidy levels for a given genotype (LSMEANS option, PROC GLM; SAS version 8.02, SAS Institute., Cary, N.C.; SAS Institute, 1988).

Triploid fertility. The presence of dyad and/or monad pollen grains was utilized to study the existence of unreduced gametes in confirmed triploid taxa (2). Pollen was collected at anthesis, and the frequency of viable, unreduced gametes in the triploid taxa was determined using pollen staining as described above and observed under a light microscope at 300×. The experimental design was a randomized complete block with 5 replicates blocked over time. At least 50 sporads (tetrads, dyads, or monads) were randomly selected and analyzed per replicate. Pollen was considered viable and unreduced if there was a well-stained monad or dyad and the pollen diameter was visibly larger (>120%) than normal. The frequency of unreduced gametes was determined using the equation (3):

$$\text{Unreduced pollen frequency} = [(2 \times \# \text{ of dyads}) + (\# \text{ of monads})] / (\# \text{ of total grains}).$$

Data were then subjected to analysis of variance and means compared using least significant differences (LSD) (PROC GLM; SAS version 8.02, SAS Institute., Cary, N.C.; SAS Institute, 1988).

Results and Discussion: Comparing fertility between ploidy levels. Pollen grains were readily apparent as being stained or unstained. Pollen viabilities for all taxa ranged from 1.5 – 63.8 % (Table 1). There was a significant effect of ploidy level ($P < 0.001$), genotype ($P < 0.0001$), and a ploidy-genotype interaction ($P < 0.0001$) on fertility. The significant interaction indicated the effect of polyploidy on fertility depended on genotype. The results demonstrated that fertility of polyploid *Rhododendron* can be highly variable and that neopolyploids may have enhanced or reduced fertility depending on the genotype. The effect of polyploidy on fertility most likely results from the level of homology among the chromosome sets and subsequent impacts on chromosome pairing during meiosis. Individuals of autopolyploid origin generally displayed reduced fertility compared to their progenitors. The induced tetraploid form of *R. fortunei* is a prime example of low fertility associated with an induced autopolyploid.

Reduced pollen fertility of the octoploid, *R.* 'Fragrant Star', is likely the result of the plant's autoallopolyploid background in which polysomic pairing most likely occurs among the 4 homologs from each of the parental species. Increased fertility was observed in polyploids of probable allopolyploid background most likely resulting from disomic pairing of chromosomes at meiosis. The potential to enhance fertility through polyploid induction is evident in the case of *R.* 'Fragrant Affinity'. The diploid form is the result of a wide inter-subgeneric cross and exhibits little to no fertility. Through the creation of an allopolyploid from the diploid progenitor cultivar, male and female fertility were effectively restored (1). In cases where no effect of increased ploidy on fertility was observed, the complex hybrids and induced tetraploids most likely functioned as segmental allopolyploids with moderately high fertility regardless of ploidy level.

Triploid fertility. Viable dyad and monad grains were observed in pollen samples from triploid taxa, ranging from 0.2 to 5.3% (Table 2), indicating the presence of unreduced pollen. The increased size of unreduced pollen diameter was also clearly evident. There was a significant effect ($P < 0.0001$) of taxa on the percentage of unreduced pollen. *Rhododendron* 'Red Wing' had the highest percentage of unreduced pollen at 5.3%, followed by *R.* 'Hallelujah' at 2.9%, while the remaining taxa were similar with less than 1.1% unreduced gametes. In studies of *Vaccinium* spp., also in the *Ericaceae*, significant differences in frequencies of unreduced pollen among taxa have also been reported (3). The production of unreduced pollen by triploid taxa indicated the potential for utilizing certain taxa in breeding programs. The greater frequency of unreduced pollen found in *R.* 'Red Wing' and 'Hallelujah' may allow for successful hybridizations given adequate numbers of pollinations.

Overall, the influence of polyploidy on fertility in *Rhododendron* is highly variable and appears to be influenced by the ploidy level, degree of homology among chromosomes, and in the case of triploids, the frequency of unreduced gamete formation. A greater understanding of fertility mechanisms in polyploid *Rhododendron* and information on fertility of specific clones will allow breeders to better utilize polyploids in plant improvement programs.

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Table 1. Pollen viability of polyploid *Rhododendron* and progenitor taxa.

Taxa	Genotype	Ploidy Level	Viability (%) ¹	Contrast ²
'Nova Zembla'	1	2x	33.4 ± 1.8	NS ³
'Super Nova'	1	4x	30.4 ± 2.5	
'Vulcan'	2	2x	48.0 ± 1.3	NS
'Vulcan Tetra'	2	4x	46.5 ± 6.4	
'Snowbird'	3	4x	63.8 ± 3.5	P<0.0001
'Fragrant Star'	3	8x	16.4 ± 1.5	
'Fragrant Affinity'	4	2x	1.5 ± 0.2	P<0.0001
'Fragrant Affinity Tetra'	4	4x	19.9 ± 2.3	
<i>R. fortunei</i>	5	2x	47.4 ± 4.0	P<0.0001
<i>R. fortunei</i> 'Tetra'	5	4x	7.7 ± 1.8	
'PJM'	6	2x	31.6 ± 1.4	P<0.0001
'Northern Starburst'	6	4x	47.0 ± 1.3	
'The Honourable Jean Marie de Montague'	7	2x	28.7 ± 2.8	P<0.0001
'Briggs Red Star'	7	4x	11.0 ± 1.1	
'Weston's Aglo'	8	2x	19.6 ± 0.9	P<0.0001
'Bubblenum'	8	4x	58.8 ± 3.2	

¹Values represent means ± SEM for 10 replications.

²Contrast represents LSD_{0.05} mean separations between common (highlighted) genotypes of different ploidy levels.

³NS = Not significant.

Table 2. Percent unreduced gametes in selected triploid *Rhododendron* taxa.

Taxa	Viable Unreduced Gametes (%) ¹
'Hallelujah'	2.87 ± 0.55 B
'Red Wing'	5.31 ± 0.81 A
'Taurus'	1.09 ± 0.13 C
'White Ruffles'	0.65 ± 0.19 C
Azaleodendron 94-28/2	0.45 ± 0.19 C
Azaleodendron 94-28/3	0.98 ± 0.27 C
Azaleodendron 94-28/7	0.60 ± 0.23 C
Azaleodendron 94-28/14	0.23 ± 0.01 C

¹Values represent means ± SEM. Means followed by a different letter are significantly different at P<0.05.