Developing Non-Invasive Callery Pears: Fertility and Reproductive Biology of Triploid Cytotypes

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Significance to Industry: *Pyrus calleryana* has become a popular landscape tree in the United States and is grown in USDA hardiness zones 5 to 8(9). It is valued for its abundance of white flowers, fall color, broad adaptability, and pest resistance. As a species, *P. calleryana* is not without problems and can be susceptible to fireblight, splitting and breakage of trunks and branches in older trees, and most recently the concern of invasiveness (1, 3). Birds readily eat the fruits and spread the seeds of the species into areas near where it is planted. In many areas, *P. calleryana* has naturalized in old fields and along highways. *Pyrus calleryana* is a diploid species ($2n = 2x = 34$) (4). Development of triploid plants could result in seedless, low fertility cultivars of *P. calleryana* and related hybrids that would be desirable as an alternative for those in the current landscape. Triploids typically have low fertility due to unbalanced chromosome segregation in meiosis. However, triploids can have limited fertility resulting from formation of apomictic embryos, unreduced gametes and the union of aneuploid gametes (3). The objective of this study was to evaluate fertility and reproductive pathways in selected triploids of *Pyrus calleryana*.

Nature of Work: A population of triploid *Pyrus calleryana* was established at the Mountain Horticulture Crops Research and Extension Center (MHCREC) in Mills River, NC. Fourteen triploids were selected from the population based on desirable traits including precociousness, heavy flowering, desirable forms, resistance to fire blight, and limited fruit set. Female fertility was determined based on fruit set (%), seeds per fruit, germination (%), and overall fertility expressed as the number of seedlings per flower from open pollinated trees. At flowering, three branches were selected on each tree and number of flowers were counted. Fruit was collected and seed were extracted and stratified for 90 days at 40°F (4°C) then moved to a greenhouse at 65-75°F (18-21°C) for 90 days to determine the number of viable seedlings. The experiment was completely randomized with three diploid and 14 triploid clones (Table 1).

Flow cytometry was used to determine the 2C genome sizes of parents and seedlings. Approximately 0.5 cm^2 of plant material was placed in a 55 mm plastic Petri dish, along with 0.5 cm^2 *Pisum sativum* L. 'Citrad', an internal standard with a known genome size of 2C = 8.75 pg. Four hundred µl of extraction buffer (CyStain UV Precise P Nuclei

Extraction Buffer, Partec, Munster, Germany) was added to the Petri dish. Tissue was chopped with a razor blade for 30 to 60 seconds and incubated for approximately 30 seconds (no more than five minutes). The suspension was then filtered through Partec 50 µm CellTrics disposable filter into a sample tube. Nuclei were stained with 1.6 mL 4',6-diamindino-2-phenylindole (DAPI). Nuclei were analyzed using a flow cytometer (PARTEC PAII, Partec) to determine the mean sample nuclei florescence relative to that of the internal standard. Approximately 5,000 nuclei were measured per sample. Genome sizes of samples were calculated as: mean florescence value of sample x nuclear DNA content of standard / mean fluorescence value of standard.

Results and Discussion: *Female Fertility.* Fruit set, seeds per fruit, germination, seedlings per flower, and relative fertility all varied considerably among individual triploid clones (Table 1). Relative female fertility represents the number of seedling germinated per flower relative to the most fertile diploid control and ranged from 0.00 to 73.00% among the triploids. Of the 14 triploids used in this research, five accessions had a relative fertility of <2%. Three accessions, H2008-047-008, H2008-048-010, and H2008-049-015, had no measurable female fertility (Table 1). These results demonstrate that the impact of triploidy on female fertility in Callery pear varies on a case-by-case basis and needs to be evaluated for individual clones.

Reproductive Pathways. The mean 2C genome size of the three diploid cytotypes was 1.25 ± 0.05 (SEM) pg, whereas the mean for the fourteen triploid cytotypes was 1.88 ± 0.12 (SEM) pg (Table 1), thereby confirming their ploidy levels. The progeny from triploid maternal parents varied in genome size ranging from 1.35 to 3.11 pg with a mean of 1.70 pg (Fig. 1). The majority of the progeny from the maternal triploids had 2C genome sizes near a triploid level or between diploid and triploid levels which indicates that these parents are producing predominantly aneuploid gametes. Aneuploids typically have reduced fitness and fertility and can suffer from abnormal development (2). However, a few progeny from maternal triploids had genome sizes near diploid levels indicating that a generational reversion to a diploid cytotype may be possible in limited instances. Also, a limited number of progeny also had genome sizes greater than triploid, including near tetraploid and above, indicating fertilization from unreduced gametes from one or both of the parents (2).
This study documented that some triploid Callery pears display substantial reductions in fertility (as much as 100%) and that most of the seedlings derived from triploid maternal parents were abnormal aneuploids with the infrequent production of some apparent isoploids (2x and 4x). Selections of highly-infertile triploid cultivars should be a viable approach to reduce or eliminate self-sowing of Callery pears in the landscape.

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Table 1. Ploidy level, 2C genome size, fruit set, number of seeds per fruit, germination, number of seedlings per flower, and relative fertility based on diploid controls of selected *Pyrus calleryana* cytotypes.

Accession Number	Ploidy (x)	2C Genome Size[z]	Fruit Set (%)	Seeds/Fruit	Germination (%)	Seedlings/ Flower	Relative Fertility[x] %
H2008-047-008	3	1.75 ± 0.00[y]	0.30 ± 0.01	1.33 ± 4.53	0.00 ± 0.00	0.00 ± 0.00	0.00
H2008-048-010	3	1.95 ± 0.05	4.84 ± 0.14	1.36 ± 3.33	2.04 ± 0.11	0.00 ± 0.01	0.00
H2008-048-019	3	1.83 ± 0.00	32.90 ± 0.10	2.61 ± 0.57	12.32 ± 0.14	0.10 ± 0.12	13.05
H2008-048-046	3	1.92 ± 0.01	21.63 ± 0.17	2.53 ± 1.27	18.79 ± 0.16	0.12 ± 0.22	14.94
H2008-048-056	3	1.96 ± 0.02	33.94 ± 0.17	2.53 ± 1.27	19.89 ± 0.03	0.19 ± 0.08	23.21
H2008-049-009	3	1.86 ± 0.11	13.90 ± 0.09	2.24 ± 0.10	18.03 ± 0.09	0.06 ± 0.05	6.97
H2008-049-015	3	1.81 ± 0.02	0.45 ± 0.01	0.33 ± 1.13	33.33 ± 1.13	0.00 ± 0.15	0.00
H2008-049-047	3	1.87 ± 0.04	14.08 ± 0.04	1.98 ± 0.56	18.53 ± 0.15	0.05 ± 0.02	6.13
H2008-049-054	3	1.92 ± 0.04	11.74 ± 0.18	2.08 ± 2.59	10.48 ± 0.24	0.03 ± 0.09	4.22
H2008-049-066	3	1.84 ± 0.02	5.83 ± 0.05	2.95 ± 0.36	28.40 ± 0.13	0.05 ± 0.07	6.59
H2008-049-076	3	1.93 ± 0.01	45.68 ± 0.22	3.07 ± 0.34	20.66 ± 0.17	0.27 ± 0.13	33.63
H2008-049-118	3	1.89 ± 0.03	56.93 ± 0.11	3.16 ± 0.81	32.02 ± 0.07	0.58 ± 0.33	73.00
H2008-049-135	3	1.83 ± 0.00	16.06 ± 0.25	2.18 ± 0.32	3.56 ± 0.08	0.02 ± 0.03	1.91
H2008-049-145	3	1.93 ± 0.01	3.46 ± 0.07	2.05 ± 3.48	5.22 ± 0.11	0.01 ± 0.01	0.86
Diploid 1	2	1.26 ± 0.02	9.80 ± 0.07	3.89 ± 2.93	38.61 ± 0.11	0.13 ± 0.08	16.73
Diploid 2	2	1.22 ± 0.01	11.06 ± 0.11	3.24 ± 0.52	25.45 ± 0.49	0.13 ± 0.35	16.32
Diploid 3	2	1.23 ± 0.01	46.76 ± 0.22	3.16 ± 0.72	52.83 ± 0.15	0.80 ± 0.62	100.00

[z] 2C DNA values represent the mean value of four subsamples conducted for each taxon.
[y] Values are means ± 1 SEM.
[x] Calculated as (seedlings/flower)/(0.80), where 0.80 is the number of seedlings per flower of the most fertile diploid control.

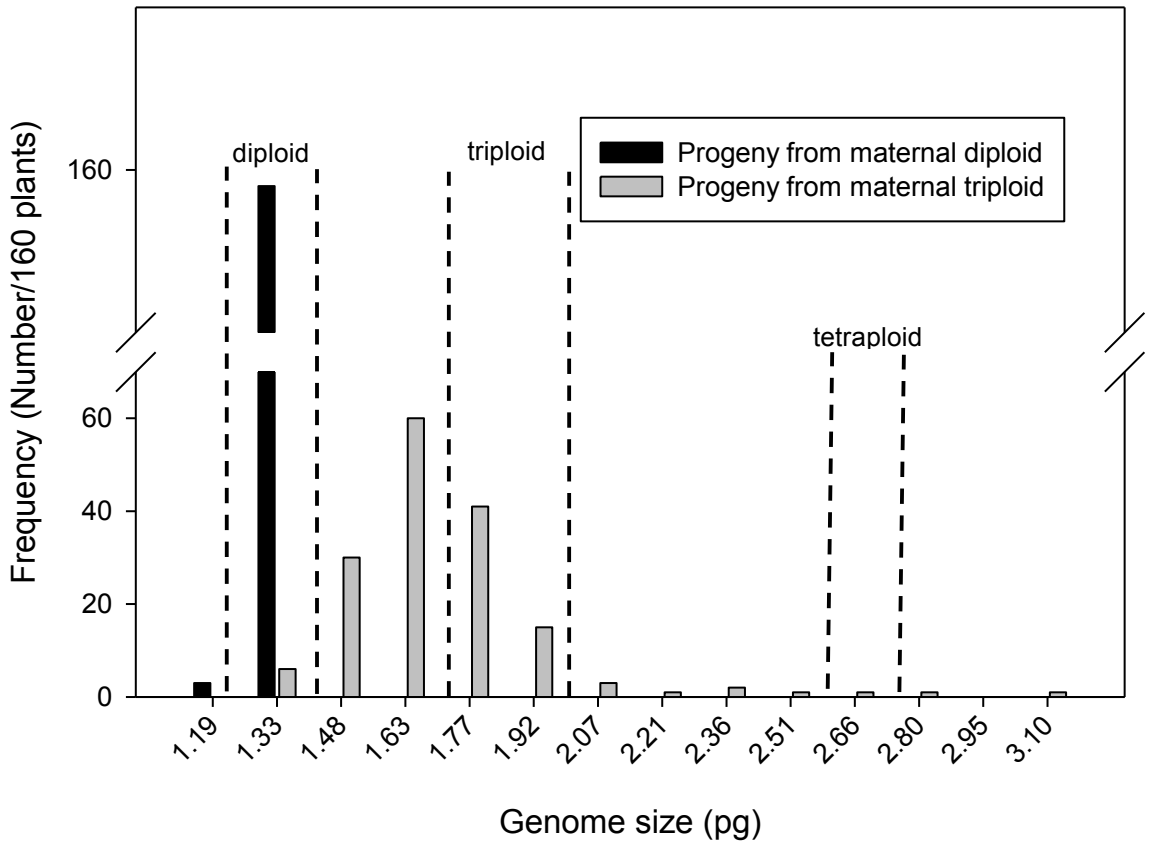

Figure 1. Frequency distribution of 2C genome sizes of seedlings derived from open pollinated diploid and triploid *Pyrus calleryana* cytotypes. Gate width for columns was based on a 95% prediction interval for diploid, triploid, and tetraploid parents calculated as the mean ± 1.96 (SEM)