ABSTRACT

OATES, KELLY MARIE. Genetic Improvement of *Rudbeckia* and Evaluation of a New Triploid *Campsis* Cultivar. (Under the direction of Dr. Thomas G. Ranney.)

Experiments were conducted to evaluate fertility and morphology of induced polyploid and gamma irradiated *Rudbeckia spp.* and hybrids, determine the mode of inheritance of the tubular ray floret trait in *Rudbeckia subtomentosa*, and evaluate fertility and morphology of *Campsis* ×*tagliabuana* 'Chastity' - a triploid cultivar developed for use as a non-invasive landscape vine.

Rudbeckia spp. are valuable ornamental wildflowers and considerable opportunities for improvement in ornamental traits and disease resistance exist within the genus. Hybrids between *Rudbeckia subtomentosa* and *Rudbeckia hirta* 'Toto Gold' were generated and allopolyploids were artificially induced. Similarly, autotetraploids were generated from *R. subtomentosa* 'Henry Eilers'. Fertility and morphology of these lines were evaluated in a randomized complete block design with 12 replications. Compared with their diploid counterparts, autotetraploid lines of *R. subtomentosa* 'Henry Eilers' had similar internode lengths, plant heights, number of stems, flowering times (date at first anthesis), and fall and spring survival (100%); reduced number of inflorescences and male and female fertility; and increased inflorescence diameters. Compared with the diploid counterparts, allotetraploids of *R. subtomentosa* × *hirta* had similar internode lengths, reduced number of inflorescences, delayed flowering times, and increased pollen staining. Female fertility was not observed in diploid hybrids or allotetraploids. Plant height and number of stems either decreased or showed no change with induced allotetraploidy. Spring survival of diploid hybrid genotypes ranged from 0 to 82% and was not improved in the allotetraploid hybrids.

The effect of gamma radiation dose on in vitro survival and development, and ex vitro fertility, phenology, and morphology of tetraploid *Rudbeckia subtomentosa* 'Henry Eilers' was investigated. In vitro maintained organogenic callus was treated with gamma radiation (0, 5, 10, 20 or 40 Gy). Callus shoot regenerative capacity was significantly reduced by radiation dose two months following treatment. Callus survival had no response to dose two months after treatment, though shoot survival was significantly reduced with increasing dose. Plant height, average stem height, number of flowers, flower diameter, percent winter survival and pollen viability were all reduced with increased radiation dose. Date of first anthesis was delayed with increased radiation dose. Several off–type floral phenotypes were observed. Gamma radiation effectively reduced height of *R. subtomentosa* 'Henry Eilers', but flower morphology was often negatively affected with radiation treatments. Treatments of 5 - 10 Gy provided a desirable range of mutations while minimizing undesirable side effects.

The mode of inheritance of the tubular ray floret trait in *R. subtomentosa* 'Henry Eilers' was investigated. Crosses were made between wild type *R. subtomentosa* and *R.* 'Henry Eilers' to generate an F_1 population. Backcrosses were made with plants from each reciprocal F_1 population back to *R.* 'Henry Eilers' and the wild-type parent. An F_2 population was generated by intercrossing among full-sib F_1s. Chi-square goodness of fit tests were performed to assess likely genetic models. The tubular ray floret trait in *R. subtomentosa* 'Henry Eilers' appears to be a single recessive trait, with one major gene controlling the phenotype, though other minor genes or developmental factors can result in some partial expression.

Campsis ×*tagliabuana* ‘Chastity’ is a triploid cultivar developed at the Mountain Crop Improvement Lab, Mills River, NC. In order to evaluate fertility and morphological characteristics, 7 plants of *C.* ‘Chastity’, 6 diploid *C. radicans* cultivars, and 1 diploid *C. grandiflora* ‘Morning Calm’ (1 plant/replication per variety) were grown in the field in a completely randomized design. *Campsis* ‘Chastity’ differed morphologically from other commercial cultivars in several traits. Pollen germination , pollen staining, fruit set, and the average number of seeds/fruit were significantly reduced in *C.* ‘Chastity’ compared to the other diploids, thereby providing a highly infertile alternative to existing diploid cultivars.

Genetic Improvement of *Rudbeckia* and Evaluation of a New Triploid *Campsis* Cultivar

by
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APPROVED BY:

Dr. John Dole	Dr. Darren Touchell
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BIOGRAPHY

Kelly Oates was born in Greensboro, NC on July 29th, 1982 and raised there by her parents, Jill and Peter Tourtellot and Roger Oates. After graduating from Grimsley High School, she attended Virginia Tech where she studied Horticulture Science. Virginia Tech is a wonderful campus nestled in the Mountains of southwestern Virginia and her studies and friendships there were truly special to her. After graduating in 2004 with a degree in Horticulture and a minor in English Literature, Kelly moved to Oregon to work for Monrovia Nursery.

Kelly spent 4 years in Oregon and in that time it became home for her. Living in the Willamette valley, she was able to enjoy so many things the state has to offer, from the beautiful Pacific Ocean and the coastal mountain range, to the cascade mountain range, and the high desert in the eastern part of the state. Her love of nature and the outdoors blossomed while she lived there. Oregon is a very meaningful place to her and she is grateful for the time she was able to spend there. She moved to Ohio in 2008 and spent one year there before returning to North Carolina in the Fall of 2009 to begin her Master's degree.

In graduate school at NC State University, Kelly studied plant breeding under the direction of Dr. Thomas Ranney at the Mountain Crop Improvement Lab in Mills River, NC. Her graduate school career taught her so much about not only plant breeding, but her love of family, friends, and the Appalachian Mountains. Kelly is thankful to everyone who has helped her to arrive where she is today and looks forward to her future career and life endeavors. She finishes her degree faithfully trusting that God will lead her on the remainder of this vibrant and short journey that is life.

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Influence of Induced Polyploidy on Fertility and Morphology of *Rudbeckia* Species and Hybrids

(In the format appropriate for submission to HortScience)

Influence of Induced Polyploidy on Fertility and Morphology of *Rudbeckia* Species and Hybrids

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Influence of Induced Polyploidy on Fertility and Morphology of *Rudbeckia* Species and Hybrids

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***Abstract. Rudbeckia* spp. are adaptable and valuable ornamental wildflowers. Development of new cultivars of *Rudbeckia* spp. with improved commercial characteristics would be highly desirable. Interspecific hybridization and induced polyploidy may be avenues for improvement within the genus. The objective of this study was to evaluate fertility, morphology, phenology of flowering, and perennialness (overwintering survival) for lines of diploid and induced allotetraploids of *R. subtomentosa* × *hirta* and diploid and autotetraploids of *R. subtomentosa* 'Henry Eilers.' Polyploid lines were developed in vitro using oryzalin as a chromosome doubling agent. Plants were grown outside in a randomized complete block design with 12 replications. To evaluate male fertility, pollen was stained with a 1% acetocarmine solution and scored for viability. Compared with their diploid counterparts, autotetraploid lines of *R. subtomentosa* 'Henry Eilers' had similar internode lengths, plant heights, number of stems, flowering times (date at first anthesis), and fall and spring survival (100%), reduced number of inflorescences, and male and female fertility, and increased inflorescence diameters. Compared with the diploid counterparts, allotetraploids of *R. subtomentosa* × *hirta* had similar internode lengths, reduced number of inflorescences,**

delayed flowering times, and increased pollen staining. Neither the diploid hybrids or allotetraploids showed any female fertility. Plant height and number of stems either decreased or showed no change with induced allotetraploidy. Spring survival of diploid hybrid genotypes ranged from 0 to 82% and was not improved in the allotetraploid hybrids. Although induced polyploidy resulted in some desirable changes such as increases in inflorescence diameter in autotetraploids of *R. subtomentosa* 'Henry Eilers' and reduced height in some of the allotetraploid hybrids, these changes were often coupled with other undesirable changes such as reduced numbers of inflorescences and did not restore fertility in the interspecific hybrids. For a given genotype, some polyploidy lines varied significantly in certain morphological traits (e.g., plant height) indicating that somaclonal variation may have developed in vitro or that there were genomic or epigenetic changes associated with induced polyploidy.

The genus *Rudbeckia* consists of about 30 species endemic to North America (Armitage, 2008; Palmer et al., 2009). The genus includes annuals, biennials, and perennial species (Perdue, 1957) and is divided into two subgenera, *Rudbeckia* subg. *Macrocline* and *Rudbeckia* subg. *Rudbeckia* (Urbatsch et al., 2000). These two subgenera can be distinguished cytogenetically, with *R.* subg. *Macrocline* having a base chromosome number of 18 and *R.* subg. *Rudbeckia* having a base chromosome number of 19 (Urbatsch et al., 2000). Many of the commercially important species of *Rudbeckia* are from *R.* subg. *Rudbeckia*. Two of these species, *R. subtomentosa* and *R. hirta*, are commonly cultivated wildflowers and closely allied based on phylogeny (Urbatsch et al., 2000).

Rudbeckia subtomentosa is a durable, diploid perennial (hardy to USDA zone 5) well adapted to many environments, and has showy, yellow ray florets (Armitage, 2008). The tall stature of *R. subtomentosa* (2-3 m) limits its use within many cultivated landscapes. A reduction in height and an increase in the range of flower colors in *R. subtomentosa* would be highly desirable. *Rudbeckia subtomentosa* 'Henry Eilers' is a cultivar with showy tubular ray florets providing additional ornamental interest.

The annual species, *Rudbeckia hirta*, includes diploid and tetraploid cultivars with a diverse range of flower colors and forms. Cultivars of *R. hirta* range in mature height from 0.5 – 1.0 m with tetraploid cultivars typically having larger flowers and greater height (Hansen and Stahl, 1993; Palmer et al., 2009). However, *R. hirta* is short-lived and susceptible to certain diseases including cercospora leaf spot (*Cercospora* sp.) and rhizoctonia blight (*Rhizoctonia* sp.) (Fulcher et al., 2003; Harkess and Lyons, 1994).

Interspecific hybridization could be an avenue for improvement within *Rudbeckia*. Interspecific hybrids between *R. subtomentosa* and *R. hirta* may combine desirable traits from both species including moderate plant height, a range of flower colors and forms, disease resistance, and perennialness. Interspecific hybrids between these two species were created at the Mountain Crop Improvement Lab (Palmer et al, 2008), but, like many wide hybrids, appear to be infertile. Hybrid sterility may occur when chromosomes of different taxa are sufficiently different that pairing of chromosomes during meiosis fails (Hadley and Openshaw, 1980). The different chromosome sizes of *R. subtomentosa* and *R. hirta* may be creating a barrier to fertility in hybrids. Palmer et al. (2009) found 1Cx DNA content (DNA content of one complete set of chromosomes) to vary among *Rudbeckia* species by 320%.

Rudbeckia subtomentosa was found to have a 1Cx DNA content of 11.0 ± 0.1 pg while that of *R. hirta* ranged from 3.4 ± 0.2 pg to 4.0 ± 0.2 pg.

Ploidy manipulation may include the development of autotetraploids (containing multiple sets of chromosomes from a single species) or allotetraploids (containing multiple sets of chromosomes from different species) (Ranney, 2006). While the morphological response to induced autotetraploidy varies, it is often associated with an increase in flower size and vegetative tissues along with a decrease in internode lengths of the inflorescence (Horn, 2002). Autotetraploids may also have a slower growth rate compared to their diploid cytotypes (Chahal and Gosal, 2002). Allotetraploids often display phenotypes intermediate between the two parents (Horn, 2002). Doubling chromosomes in wide hybrids also creates a duplicate homologous chromosome set for each original parental set and can thereby restore disomic chromosome pairing and fertility (Contreras, et al., 2007; Olsen et al., 2006; Ranney, 2006).

This study was undertaken to evaluate fertility, morphology, phenology of flowering, and perennialness (overwintering survival) for lines of diploid and induced allotetraploids of *R. subtomentosa* × *hirta* and diploid and autotetraploids lines of *R. subtomentosa* 'Henry Eilers'.

Materials and Methods

In vitro polyploid induction. Allotetraploids of diploid interspecific hybrids of *R. subtomentosa* × *hirta* 'Toto Gold' and autotetraploids of *R. subtomentosa* 'Henry Eilers' were previously developed at the Mountain Crop Improvement Lab (Palmer, et al., 2008).

Briefly, shoot apices were treated in vitro with 30 µM oryzalin for 5 days resulting in multiple tetraploid lines of each genotype (Fig. 1). All polyploid lines and their diploid cytotypes were maintained in tissue culture by subculturing monthly to bimonthly on a DKW media (Driver and Kuniyuki, 1984) containing 2 µM 6-benzylaminopurine (BAP) and maintained under standard culture conditions [23 ± 2 °C and a 16 h photoperiod of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400-700 nm) provided by cool-white fluorescent lamps]. In Mar. 2010 microcuttings were placed onto a Murashige and Skoog (MS) media (Murashige and Skoog, 1962) with 5µM 1-napthyl acetic acid (NAA) to promote root initiation one week prior to being transferred into 72-cell trays containing a standard seedling mix.

Flow cytometry. Ploidy levels of all plants were confirmed using flow cytometry following Palmer et al. (2009). *Pisium sativum* 'Citrad' with a known DNA content of 2C= 8.75 pg was used as an internal standard (Greihuber et al., 2007). Approximately 2 mm^2 of young leaf tissue from both the sample and the standard were finely chopped with a razor blade in a petri dish containing 400 µL of extraction buffer (CyStain ultraviolet Precise P; Partec, Münster, Germany). The suspension was filtered through a 50 µm nylon mesh screen to remove plant debris. Nuclei were stained using 1.6 mL staining buffer containing 4', 6-diamidino-2-phenylindole (DAPI) (CyStain ultraviolet Precise P;Partec). Stained nuclei were analyzed with a flow cytometer (Partec PA-I Ploidy Analyzer or Partec PA II; Partec) to determine relative genome size. Ploidy level was determined by comparing the relative genome size of samples tested to published values for the diploid parents of the interspecific hybrids and *R.* 'Henry Eilers'(Palmer, et al., 2009).

Evaluation of morphological traits and perennialness. Plants were transferred from 72-cell trays to 0.80 L pots on 27 Apr. 2010. Plants were randomized on a greenhouse bench and allowed to grow for 3 weeks then moved outdoors under 50% shade for one week. On 27 May 2010, plants were transplanted to 5.7 L pots in a pine bark media (supplemented with 1.36 kg lime and 0.74 kg granular Micromax® micronutrients per cubic meter) and were placed on a gravel container pad, in full sun, in a randomized complete block design with 12 blocks with 1 plant (replication) from each of the thirteen taxa (see Fig. 1) per block. Each plant was top-dressed with approximately 24 g. of 5-6 month Osmocote® plus (15-9-12) slow release fertilizer. Additional plants of diploid *R. hirta* 'Toto Gold' and tetraploid *R. hirta* 'Cherry Brandy' were randomly interspersed as additional pollen donors. Other diploid seedlings of wild type *R. subtomentosa* were within 15 m on the same container pad, but not formally included in this experiment. The date of first anthesis was recorded for each plant in the experiment. Inflorescence diameter was measured at or after anthesis for 3 randomly selected inflorescences (subsamples) per plant. Internode length was determined on one randomly selected stem per plant and included 3 measurements: the second internode below the flower, the second internode from the base of the plant, and the fifth internode from the base of the plant. Plant height, number of flowers, and number of basal stems for all plants in the experiment were measured on 12 Aug. 2010. Other traits, such as petal and flower head morphology, were also observed and recorded.

To evaluate perennialness, survival was recorded in Sept. 2010 and May 2011. Plants were overwintered in containers in a heated (minimum temperature of 3˚C), polyethylene covered greenhouse, in a completely randomized design.

Evaluation of fertility. To evaluate male fertility, pollen was collected from newly opened florets from each plant between 10:30 AM and 11:30 AM. Pollen was placed on a glass slide and stained with 40 µL of acetocarmine (1%), covered with a coverslip, and allowed to sit for a minimum of 90 minutes. Pollen was observed the same day collected. Well-formed grains with a pink/red stain were scored as viable. Each sample consisted of a minimum of 200 grains. Due to variation in pollen availability, data was collected during both 2010 and 2011.

To evaluate female fertility, three randomly selected seedheads from each plant in the experimental block were collected on 28 Aug. 2010. Seedheads were air dried, cleaned and stored at 4˚C. Seeds were sown on 12 Dec. 2010 and placed into a dark cooler (4˚C) to stratify for one month. In January, 2011 seeds were removed from the cooler and placed in a greenhouse at 21 ˚C. Germination was recorded after 60 days.

Data were analyzed using analysis of variance (PROC GLM, SAS Version 9.1.3; SAS Institute Inc., Cary, NC) and means compared using Waller-Duncan K-ratio t Test, $P \leq 0.05$.

Results and Discussion

Ploidy confirmation. The autotetraploids of *R. subtomentosa* had a mean genome size of 2C = 41.80 ± 0.70 (SEM) pg, which is approximately twice that of the published genome size of diploid *R. subtomentosa*, 2C = 21.9 ± 0.17 (SEM) pg (Palmer et al., 2009). The allotetraploid hybrids had a mean genome size of 2C = 29.3 ± 0.36 (SEM) pg, which is

consistent with the expected genome size of allotetraploids between *R. subtomentosa* and *R. hirta*.

Morphological traits, date of first anthesis, and perennialness. Morphological responses to induced polyploidy varied by genotype and lines within genotype (Table 1). Plant height was reduced in some allopolyploid lines, increased in one, and there was no change in others including the autotetraploid lines. Number of stems either decreased or had no change in both auto and allotetraploid lines. Inflorescence diameter either increased, decreased, or remained unchanged for different allopolyploid lines and increased for the autotetraploid lines, while the number of inflorescences decreased for all induced polyploids. An increase in flower diameter is often associated with induced polyploids (Kehr, 1996; Zadoo, et al., 1975) and can be associated with a decrease in the total number of flowers (Machiewicz, 1965). The date of first anthesis was delayed for all allotetraploid lines, but was unchanged for the autotetraploid lines. Delayed flowering is a common response to induced polyploidy (Kehr, 1996), so it is not unexpected that the allotetraploid lines flowered later than their diploid cytotypes. There was no significant change in internode length for any of the polyploid lines in the experiment.

Variation in traits among different polyploid lines within a given genotype (e.g., genotype one and three) is surprising since they should be isogenic. This variation indicates that somaclonal variation may have developed in vitro or that there were genomic or epigenetic changes associated with induced polyploidy.

Somaclonal variation describes the genetic variability induced by culturing plants in vitro (Jain, 2001; Larkin and Scowcroft, 1981). Somaclonal variation has been investigated

in *Echinacea purpurea* (purple coneflower), a member of the Asteraceae and close relative of *Rudbeckia* (Chuang et al., 2010). Chuang et al. (2010) found a percentage (14 plants out of 1087 sampled), of off types can occur in *E. purpurea* as a result of somaclonal variation from primary regenerants derived from shoot organogenesis using leaf explants. In rice, Müller et al. (1990) found that plants regenerated from a single callus showed DNA polymorphisms as measured by RFLP analysis. Further, long-term in vitro culture can also result in somaclonal variation, but varies by taxa, culture conditions, and duration (Turner, et al., 2001). The diploid genotypes in our study were initiated through organogenesis from leaf tissues and all genotypes were maintained through several subculture cycles before and after polyploidy induction, increasing the potential for somaclonal variation. Additionally, chimerism as a result of somaclonal variation could have occurred, which would account for variability within tetraploid lines originating from the same diploid microcutting.

Novel gene expression can also result from wide hybridization (Hegarty et al., 2006; Wang, et al., 2006) and/or from genome duplication (Verhoevan et al., 2010) and has been documented in several genera (Adams and Wendel, 2005; Osborn, et al., 2003). Phenotypically, we did not observe any significant differences for any of the scored traits between the two autotetraploid lines, while we did observe variation among allopolyploid lines within a given genotype. Since these lines should be isogenic (within genotype), this suggests changes in gene expression associated with induced allopolyploidy. Causes for genomic and gene expression changes in polyploids have been reviewed extensively and proposed mechanisms include both genetic (sequence deletions, rearrangements, subfunctionalization, gene repression and dominance, novel activation, dosage effects) and

epigenetic factors (activation of transposons, DNA methylation) (Adams and Wendel, 2005; Chen and Ni, 2006; Osborne, et al., 2003). These types of changes may also be occurring in allopolyploid hybrids of *R. subtomentosa* × *hirta*.

In addition to the quantitative traits recorded, tubular or quilled ray florets were observed in some autotetraploids from genotype 3 (Figure 2). Neither parent or diploid hybrid for genotype 3 had a quilled petal phenotype. The appearance of the novel tubular ray florets has also occurred in induced tetraploids of *Tanacetum parthenium* (feverfew), another member of the Asteraceae (Majdi and Karimzadeh, 2010).

As expected, all plants from genotype 4 (*R.* 'Henry Eilers' and its autotetraploids) were perennial and survived at the end of the summer and through the winter (Figure 3). Although there was variable end-of-summer survival for hybrid genotypes, there was low overwinter survival (<25%), with the exception of genotype 1, where the diploid hybrid, H2008-128-004, had an 83% winter survival rate. End-of-summer data was included in the analysis to reflect the inability for some accessions to form a crown and spontaneously senesce at the end of the growing season, a trait associated with botanical annuals. Genotype 3 had a particularly low end-of-summer survival rate, with only 17%-25% of plants surviving by the end of the summer. Induced polyploidy significantly decreased overwinter survival in genotype 1 and significantly decreased fall survival in genotype 2. There was no other ploidy effect seen in any of the other genotypes.

Fertility. Pollen viability was increased for all allotetraploid lines compared to their diploid cytotypes and decreased for autotetraploids of *R.* 'Henry Eilers' (Table 2). From the seeds collected in 2010, no germination was observed for any of the diploid hybrids or

allotetraploids. From the twelve diploid *R*. 'Henry Eilers' plants sampled (36 flower-heads total), sixty-nine seeds germinated and all seedlings had a genome size consistent with diploid *R. subtomentosa*. Autotetraploid accession H2008-239-002 had nine seeds germinate and accession H2008-239-004 had four seeds germinate. All thirteen resulting seedlings from the autotetraploids had genome sizes, 31.39 ± 0.32, consistent with triploid *R. subtomentosa*. *Rudbeckia subtomentosa* and *R. hirta* are highly self incompatible, but can sometimes produce apomictic seeds via pseudogamy (Palmer, 2009). Based on the genome size of the offspring, it is likely that diploid wild type *R. subtomentosa* was the pollen parent. The two autotetraploids were of the same genotype and so the self-incompatibility mechanism in *R. subtomentosa* may have prevented these lines from intercrossing. No tetraploid of another genotype of *R. subtomentosa* was available at the time of the experiment which, along with self-incompatibility between the autotetraploid lines, could explain why no tetraploid seedlings were found. Female fertility, while low, was retained in the autopolyploids. The allotetraploids had pollen available from many sources, including other genotypes of allotetraploids, tetraploid *R. hirta* 'Cherry Brandy,' diploid *R. hirta* 'Toto Gold,' and diploid and tetraploid *R. subtomentosa*. Failure of any seeds to germinate suggests that female fertility was not restored in the allotetraploids of *R. subtomentosa* × *hirta*. In allotetraploids of *Alstroemeria aurea* × *A. caryophyllae*, Lu and Bridgen (1997) had similar results in which fertility was not restored, although pollen staining increased from 0% in the diploid hybrids to 12% in the allotetraploids. Cytological investigation of pollen mother cells revealed that while abnormal meiotic pairing occurred in many of the diploid hybrids examined, meiotic pairing was largely normal in the allotetraploids, suggesting other

mechanisms were involved in causing sterility in the *Alstroemeria aurea* × *A. caryophyllae* allopolyploids (Lu and Bridgen, 1997).

The effects of induced polyploidy on the fertility and morphology of allotetraploids and autotetraploids of *Rudbeckia spp*. were variable both between and within genotypes. Induced autotetraploids in *R. subtomentosa* 'Henry Eilers' displayed larger flowers, though the large stature of the plant is still restrictive and the reduced number of inflorescences is undesirable. While some desirable phenotypes were recovered in the allotetraploids, the inability to restore fertility coupled with the predominance of the shortlived-annual nature of these hybrids is undesirable. The higher survivability at both the end of summer and through winter of one hybrid accession suggests that larger F_1 populations between *R. subtomentosa* x *R. hirta* could yield more perennial types. Due to the lack of fertility of these F_1's and the inability to restore fertility in the allotetraploids, selection for new commercial cultivars would have to be made from F_1 populations.

Literature Cited

Adams, K. L. and J. F. Wendel. 2005. Novel patterns of gene expression in polyploid plants. Trends in Genetics 21: 539-543.

Armitage, A. M. 2008. Herbaceous perennial plants: A treatise on their identification, culture, and garden attributes. 3rd ed. Stipes Publishing, Champaign, IL. 3rd edition now out.

Chahal, G. S. and S.S. Gosal. 2002. Principles and procedures of plant breeding: biotechnological and conventional approaches. Alpha Science International, Ltd., Pangbourne, UK.

Chen, Z. J. and Z. Ni. 2006. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. BioEssays 28(3):240-252.

Chuang, S. J., C. L. Chen, J. J. Chen, and J. M. Sung. 2010. Using morphological diagnosis and molecular markers to assess the clonal fidelity of micropropagated *Echinacea purpurea* regenerants. Biologia Plantarum 54(3):539-542.

Contreras, R.N., T.G. Ranney, and S.P. Tallury. 2007. Reproductive behavior of diploid and allotetraploid *Rhododendron* L. 'Fragrant Affinity'. HortScience 42(1):31-34.

Driver, J. A. and A. H. Kuniyuki. 1984. In vitro propagation of paradox walnut rootstock. HortScience 19:507-509.

Fulcher, Amy, W. C. Dunwell, and D. Wolfe. 2003. *Rudbeckia* taxa evaluation. Southern Nurserymen's Association Research Conference. 48:510-512.

Greilhuber, J. 1998. Intraspecific variation in genome size: A critical reassessment. Ann. Bot. 82:27–35.

Hadley, H. H. and S. J. Openshaw. 1980. Interspecific and intergeneric hybridization, p. 133-159. In: W. R. Fehr and H. H. Hadley (eds.). Hybridization of crop plants. Crop Sci. Soc. Amer., Madison, WI.

Hansen, R. and F. Stahl. 1993. Perennials and their garden habitats. Trans. Richard Ward. Timber Press, Portland, OR.

Harkess, R. L. and R. E. Lyons.1994. *Rudbeckia hirta* L.: a versatile north american wildflower. HortScience 29(3):134,227.

Hegarty, M. J., G. L. Barker, I. D. Wilson, R. J. Abbott, K. J. Edwards, and S. J. Hiscock. 2006. Transcriptome shock after interspecific hybridization in *Senecio* is ameliorated by genome duplication. Current Biology 16:1652-1659.

Horn, W. 2002. Breeding methods and breeding research, p. 47-83. In: Breeding for ornamentals: classical and molecular approaches. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Jain, S. Mohan. 2001. Tissue culture-derived variation in crop improvement. Euphytica 118:153-166.

Kehr, A. E. 1996. Polyploids in rhododendron breeding. Journal of the American Rhododendron Society. 50: 215-217.

Larkin, P. J. and W. R. Scowcroft. 1981. Somaclonal variation- a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60:197-214.

Lu, C. and M. P. Bridgen. 1997. Chromosome doubling and fertility study of *Alstroemeria aurea* × *A. caryophyllaea*. Euphytica 94:75-81.

Mackiewicz, H. O. 1965. Studies on di- and tetraploid alsike clover (*Trifolium hybridum* L.) part II, flower morphology and the problem of fertility. Genetica Polonica 6:41-77.

Majdi, M., G. Karimzadeh, M. A. Malboobi, R. Omidbaigi, and G. Mirzaghaderi. 2010. Induction of tetraploidy to feverfew (*Tanacetum parthenium* Schulz-Bip.): morphological, physiological, cytological, and phytochemical changes. HortScience 45:16-21.

Müller, E., P. T. H. Brown, S. Hartke, and H. Lörz. 1990. DNA variation in tissue-culture-derived rice plants. Theor. Appl. Genet 80:673-679.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15:473-497.

Olsen, R.T., T.G. Ranney, and Z. Viloria. 2006. Reproductive behavior of induced allotetraploid ×*Chitalpa* and in vitro embryo culture of polyploidy progeny. J. Amer. Soc. Hort. Sci. 131(6):716-724.

Osborn, T. C., J. C. Pires, J. A. Birchler, D. L. Auger, Z. J. Chen, H. Lee, L. Comai, A. Madlung, R. W. Doerge, V. Colot, and R. A. Martienssen. 2003. Understanding mechanisms of novel gene expression in polyploids. Trends in Genetics 19(3):141-147.

Palmer, I. E., D. H. Touchell, and T. G. Ranney. 2008. In-vitro polyploid induction of *Rudbeckia* spp. Southern Nurserymen's Association Research Conference 53: 186-189.

Palmer, I. E., Bir, R. E., Lynch, N. P., and Ranney, T. G. 2009. Crossability, cytogenetics, and reproductive pathways in *Rudbeckia* subgenus *Rudbeckia*. HortScience 44: 44-48.

Perdue, Jr., R. E. 1957. Synapsis of *Rudbeckia* subg. *Rudbeckia*. Rhodora 59: 293-299.

Ranney, T. G. 2006. Polyploid: from evolution to new plant development. Combined Proceedings of the International Plant Propagators Society 56: 137-142.

Turner, S., S. L. Krauss, E. Bunn, T. Senaratna, K. Dixon, B. Tan, and D. Touchell. 2001. Genetic fidelity and viability of *Anigozanthos viridis* following tissue culture, cold storage, and cryopreservation. Plant Science 161:1099-1106.

Urbatsch, L. E., Bruce G. Baldwin, and Michael J. Donoghue, 2000. Phylogeny of the coneflowers and relatives (heliantheae: asteraceae) based on nuclear rDNA internal

transcribed spacer(ITS) sequences and chloroplast DNA restriction site data. Systematic Botany 25(3): 539-565.

Verhoeven, K. J. F., P. J. van Dijk, and A. Biere. 2010. Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages. Molecular Ecology 19:315-324.

Wang, J., L. Tian, H. Lee, N. E. Wei, H. Jiang, B. Watson, A. Madlung, T. C. Osborn, R. W. Doerge, L. Comai, and Z. J. Chen. 2006. Genomewide nonadditive gene regulation in *Arabidopsis* allotetraploids. Genetics 172:507-517.

Zadoo, S. N., P. P. Roy, and T. N. Khoshoo. 1975. Cytogenetics of cultivated Bouganvilleas. V. induced tetraploidy and restoration of fertility in sterile cultivars. Euphytica 24: 517-524.

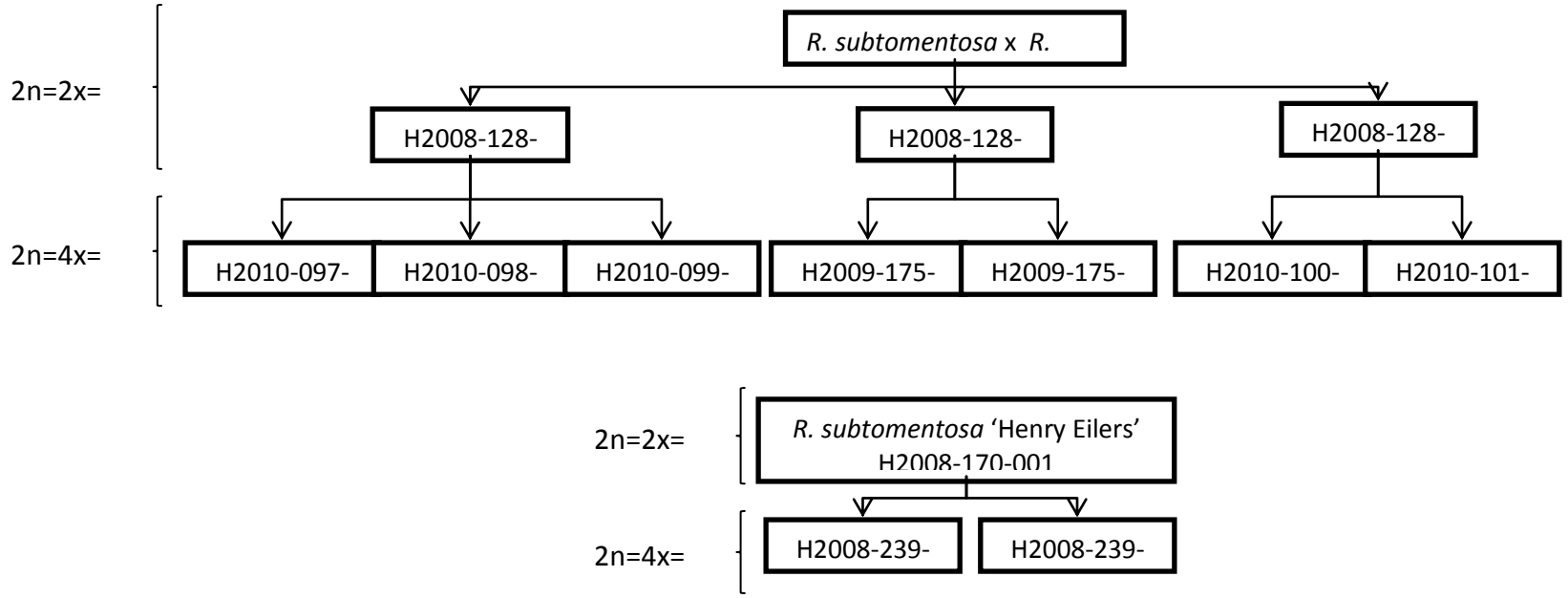

Figure 1. Origin and parentage of allotetraploid and autotetraploid *Rudbeckia* lines.

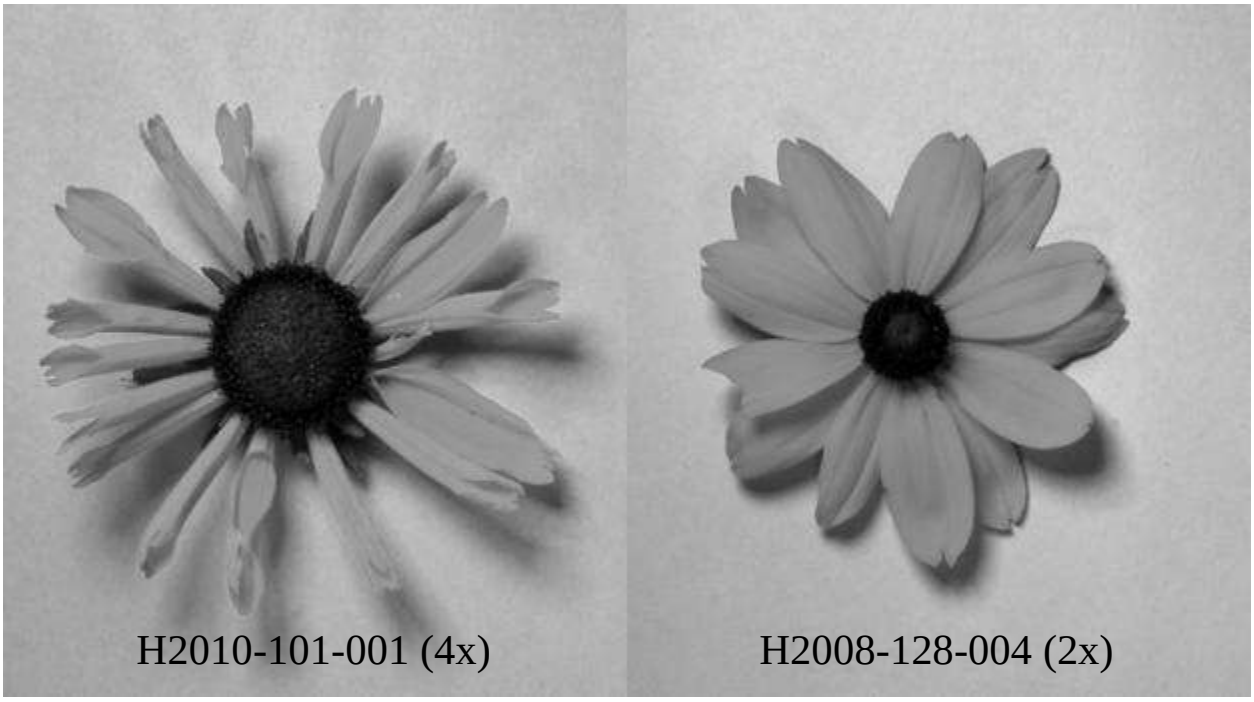

Figure 2. Novel quilled petal morphology in tetraploid clone compared to isogenic diploid clone.

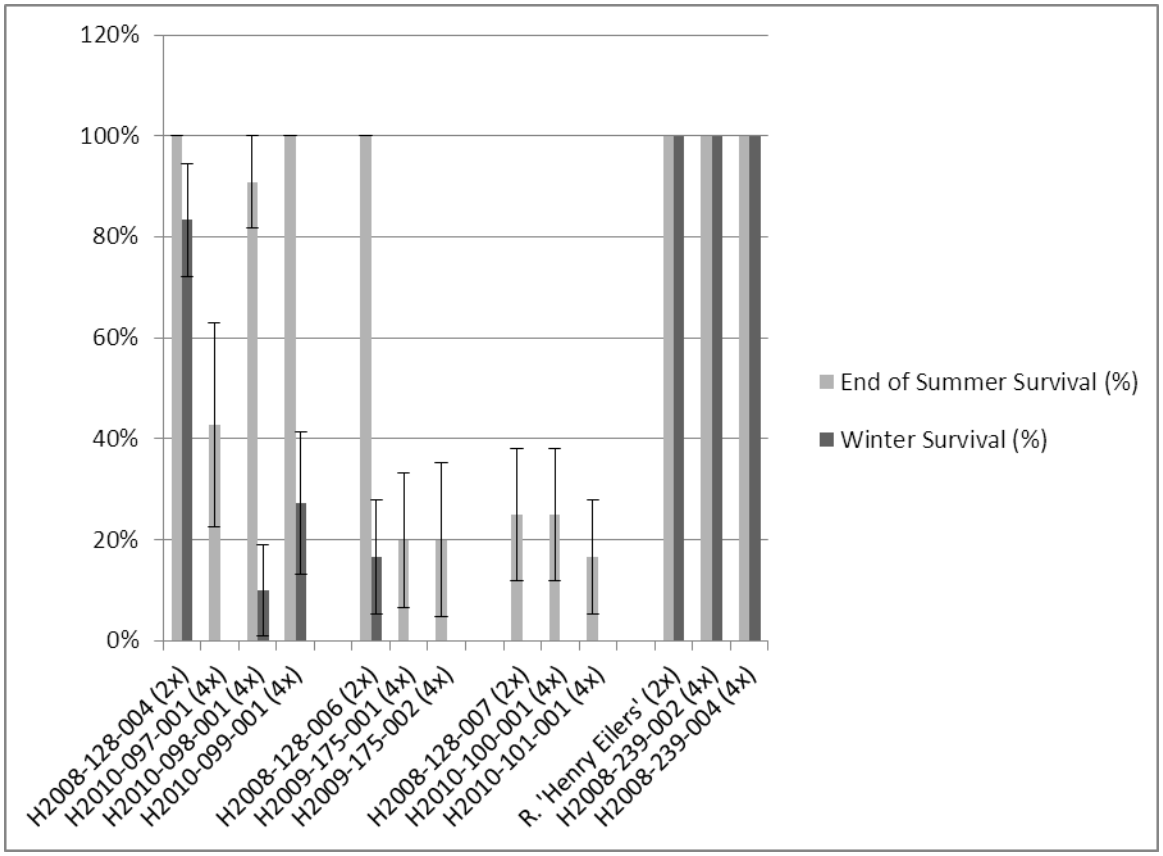

Figure 3. Percentage survival (± SEM) of diploid and allotetraploid lines of *R. subtomentosa* × *R. hirta* and diploid and autotetraploid lines of *R. subtomentosa* 'Henry Eilers' at the end of the summer (Sept. 2010) and the following spring (May 2011).

Table 1. Influence of induced polyploidy on morphology and date of anthesis among allotetraploid lines of *R. subtomentosa* × *R. hirta* and autotetraploid lines of *R. subtomentosa* 'Henry Eilers'.

Accession/line	Geno-type	Cyto-type	Plant height (cm)	Internode length (mm)[z]	Number of stems[y]	Inflorescence diameter (mm)	Number of inflorescences	Date of anthesis[x]
				R. subtomentosa × *R. hirta*				
H2008-128-004	1	2x	76.7 a[w]	33.0 a	11.4 a	77.9 a	112.5 a	6/22/2010 c
H2010-097-001	1	4x	68.7 bc	36.1 a	9.6 b	69.6 b	46.4 c	6/27/2010 b
H2010-098-001	1	4x	65.4 c	37.1 a	11.7 a	73.7 ab	68.2 b	7/5/2010 a
H2010-099-001	1	4x	72.0 ab	40.7 a	9.4 b	71.9 ab	59.7 bc	7/6/2010 a
H2008-128-006	2	2x	76.0 a	36.5 a	12.0 a	83.7 a	71.8 a	6/13/2010 b
H2009-175-001	2	4x	69.7 b	38.8 a	8.6 b	73.2 b	40.1 b	7/8/2010 a
H2009-175-002	2	4x	69.3 b	42.5 a	6.9 b	72.1 b	43.5 b	7/4/2010 a
H2008-128-007	3	2x	62.2 b	30.1 a	13.3 a	60.5 b	114.4 a	6/23/2010 b
H2010-100-001	3	4x	71.4 a	35.9 a	7.8 b	67.4 a	32.8 b	7/4/2010 a
H2010-101-001	3	4x	67.6 ab	31.8 a	6.8 b	71.2 a	27.8 b	7/4/2010 a
				R. subtomentosa 'Henry Eilers'				
H2008-170-001	4	2x	120.1 a	51.1 a	6.6 a	86.9 b	60.4 a	7/27/2010 a
H2008-239-002	4	4x	120.1 a	54.5 a	4.8 a	100.5 a	40.8 b	7/31/2010 a
H2008-239-004	4	4x	115.0 a	49.0 a	5.5 a	95.8 a	42.6 b	7/27/2010 a

[z] Internode length represents an average of the 2nd and 5th internode from the base and 2nd internode below the inflorescence.

[y]Number of stems arising from the crown.

[x]Date anthers began dehiscing.

[w]Mean separation, within columns and genotype, by Waller-Duncan, $P<0.05$, n~12

Table 2. Pollen viability staining among diploid and allotetraploid lines of *R. subtomentosa* × *R. hirta* and diploid and autotetraploid lines of *R. subtomentosa* 'Henry Eilers'.

Accession/line	Genotype	Ploidy	2010 Pollen staining (%)[Z]	2011 Pollen staining (%)[Z]
R. subtomentosa × *hirta*				
H2008-128-004	1	2x	7.9 c	6.9 c
H2010-097-001	1	4x	42.0 b	33.1 b
H2010-098-001	1	4x	62.5 a	42.5 ab
H2010-099-001	1	4x	39.3 b	-
H2010-154-001	1	4x	-	42.8 a
H2008-128-006	2	2x	12.1 b	8.0 c
H2009-175-001	2	4x	31.8 a	31.2 b
H2009-175-002	2	4x	25.5 ab	23.6 b
H2009-175-006	2	4x	-	56.0 a
H2008-128-007	3[Y]	2x	5.1	-
H2010-100-001	3	4x	30.7	-
H2010-101-001	3	4x	15.3	27.2
R. subtomentosa 'Henry Eilers'				
H2008-170-001	4	2x	95.7 a	-
H2008-239-002	4	4x	70.4 b	-
H2008-236-004	4	4x	68.4 b	-

[Z] Mean separation, within columns for each genotype, using Waller-Duncan K-ratio t Test, $P \leq 0.05$, n~12.

[Y]No statistics available for genotype three due to low sample sizes in 2010 (n=1) and unavailability of accessions in 2011.

Chapter 2

Induced Variation in Tetraploid *Rudbeckia subtomentosa* ‘Henry Eilers’ Regenerated from Gamma-Irradiated Callus

(In the format appropriate for submission to HortScience)

Induced Variation in Tetraploid *Rudbeckia subtomentosa* 'Henry Eilers' Regenerated from Gamma-Irradiated Callus

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Subject Category: Breeding, Cultivars, Rootstocks, and Germplasm Resources

Induced Variation in Tetraploid *Rudbeckia subtomentosa* 'Henry Eilers' Regenerated from Gamma-Irradiated Callus

Additional index words. Asteraceae, autotetraploid, blackeyed Susan, coneflower, in vitro induced mutation, ornamental plants, plant breeding

Abstract. ***Rudbeckia subtomentosa*** **'Henry Eilers' is an adaptable and popular garden plant; however, reduction in height and novelty in flower color would be desirable. In vitro maintained organogenic callus of tetraploid *Rudbeckia subtomentosa* 'Henry Eilers' was treated with gamma radiation (0, 5, 10, 20 or 40 Gy) to generate novel characteristics. Callus shoot regenerative capacity had no initial response to radiation dose, but was significantly reduced by radiation dose when measured two months following treatment. Callus survival had no response to dose 2 months after treatment; shoot survival approximately 2 months after treatment was significantly reduced with increasing dose. Plant height, average stem height, number of flowers, flower diameter, percent winter survival and pollen viability were all reduced and date of first anthesis was delayed with increasing radiation dose. Several off type phenotypes were observed including increased apical splitting of the ray florets in several plants, one plant with green disc florets, one plant with decreased diameter of ray florets, one plant with concave ray florets, and several plants treated with 20 Gy had irregular ray florets that were not evenly spaced around the disc. Gamma radiation was an effective means for reducing the height of *R. subtomentosa* 'Henry**

Eilers', but flower morphology was often negatively affected with radiation treatments. Treatments of 5 - 10 Gy resulted in a desirable range of observed mutations.

The genus *Rudbeckia* (black-eyed Susan or coneflower) consists of about 30 species, all of which are native to North America (Armitage, 2008; Palmer et al, 2009). The genus includes annuals, biennials, and perennial species (Perdue, 1957). *Rudbeckia* is a durable and popular perennial with broad adaptability (Armitage, 2008). The Perennial Plant Association named *Rudbeckia fulgida* var. *sullivantii* 'Goldsturm' perennial plant of the year in 1999. However, *R.* 'Goldsturm' is susceptible to downy mildew (*Plasmopara halstedii*) and leaf spot (*Cercospora spp.)* that can severely stunt and disfigure foliage and plants (Fulcher, 2003; Hong, 2006). *Rudbeckia subtomentosa* 'Henry Eilers' is an alternative coneflower species with excellent disease resistance and unique flower form with tubular ray florets. However, as with most *R. subtomentosa,* this cultivar tends to be too tall (>1m) for many landscape uses. A reduction in height of these plants as well as novel traits in flower color or form would be desirable.

Induced mutations are commonly used in plant breeding to create genetic variability. Mutations can be induced by both physical and chemical agents (van Harten, 1998). Fast neutron and ionizing radiation are two methods of physically inducing mutations. Although fast neutrons have high relative biological effectiveness (i.e., get greater biological effects with lower doses), use of neutrons as a radiation source requires more specialized equipment, either a nuclear reactor or particle accelerator, both of which can produce considerable heat and can cause residual radioactivity on the plant material. Alternatively the use of ionizing

radiation (X-rays or gamma-rays) does not result in any residual radioactivity in the plant material (van Harten, 1998). Gamma radiation can be provided by a ^{60}Co source, which is more commonly available, inexpensive to operate, and produces minimal heat at low activity. Gamma radiation was first discovered in 1900 by P. Villard and has been identified since the 1950's as a radiation source for inducing mutations in plant material (van Harten, 1998; Sparrow, 1954).

Radiation can alter various characteristics including plant architecture, growth rate, flower and foliage color and shape, fruit characteristics, chemical composition, and resistance to diseases and pests (Broertjes, et al., 1988; Dao et al., 2006, van Harten, 1998). Radiation can affect plant height by reducing the number of nodes and the length of internodes (Gottschalk and Wolff, 1983). Radiation has been used as a tool to induce mutations for the development of commercial varieties in many crop species, particularly the forages, as well as in ornamental crops, such as *Chyrsanthemum* (Dao et al., 2006), *Dendranthemum grandiflorum* (Datta et al., 2001), *Nelumbo nucifera* (Arunyanart and Soontronyatara, 2002), *Rosa hybrida* (Arnold et al., 1998, Gupta and Shukla, 1971, van Harten, 1998) and many others.

Both seed and vegetative tissues can be used as tissue sources for mutation treatments. *Rudbeckia* 'Henry Eilers' is a vegetatively produced crop; to maintain desirable characters of the cultivar, such as the tubular ray florets, vegetative tissue was used for radiation treatment. Mutation treatment of vegetative tissue, such as buds or cuttings, often results in chimeras (Datta et al., 2001) due to the complexity and number of initial cells in the apical meristem and the potential for each cell to be effected differently. In vitro treatment of

embryogenic callus results in fewer chimeric mutations as plants are being regenerated from one or a few individual cells (McKersie and Brown, 1996).

To date there are limited reports of the use of gamma irradiation to induce mutations in ornamental species. The objective of this study was to evaluate survival, development, and phenotypic variation of tetraploid *R.* 'Henry Eilers' regenerated from gamma-irradiated embryogenic callus to better understand effects and optimal dosages.

Materials and Methods

In-vitro culture and gamma irradiation. Tetraploid *R.* 'Henry Eilers' were generated previously at the Mountain Crop Improvement Lab (Palmer, et al., 2008). Callus cultures of tetraploid *R.* *'*Henri Eilers*'* were initiated in Oct. 2009 on Murashige and Skoog (MS) basal salts and vitamins (Murashige and Skoog, 1962) supplemented with myoinositol at 0.1 $g \cdot L^{-1}$ MES monohydrate at 0.1 $g \cdot L^{-1}$ 5µM 6-benzylaminopurine (BAP) 0.625 µM 1-napthyl acetic acid (NAA), 30 $g \cdot L^{-1}$ sucrose, solidified with 7.5 $g \cdot L^{-1}$ agar and pH adjusted to 5.75 ± 0.03. Leaves from in vitro maintained microcuttings were used as explants. Callus tissue was plated onto fresh media one week before irradiation treatment (23 Nov. 2009). Gamma radiation at 0, 5, 10, 20, and 40 Gy, was applied to 6 plates per treatment, and 6 callus units (subsamples, approximately 0.5 cm^2) per plate. Current dose rate of gamma irradiator (^{60}Co source) is 4500 rads/hour. Radiation treatments were applied by exposing cultures to ^{60}Co for sufficient durations to accumulate the desired total dose.

Cultures were irradiated on 30 Nov. 2009. Callus was kept in the dark at 23 ± 2 °C before and after treatment. Callus was transferred to fresh media on 18 Dec. 2009 (19 days

post treatment) and callus survival and number of shoot per callus were recorded. Shoot number per explant and number of dead shoots was again recorded on 29 Jan. 2010 (60 days post treatment) to determine if radiation treatment influenced callus survival and shoot regenerative capacity. A random sample of seven plants per original plate resulting in 42 plants per treatment were cultured onto a shoot regeneration media, DKW basal salts and vitamins (Driver and Kuniyuki, 1984) supplemented with myoinositol at 0.1 $g \cdot L^{-1}$ MES monohydrate at 0.1 $g \cdot L^{-1}$, 2µM BAP, 30 $g \cdot L^{-1}$ sucrose, solidified with 7.5 $g \cdot L^{-1}$ agar and pH adjusted to 5.75 ± 0.03, and maintained under standard culture conditions [23 ± 2 °C and a 16 h photoperiod of 30 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (400-700 nm) provided by cool-white fluorescent lamps] for three subculturing cycles (approx. 12 weeks). Survival of shoots in culture was recorded on 12 May 2010 and surviving microcuttings were placed onto a rooting media consisting of MS basal salts and vitamins supplemented with myoinositol at 0.1 $g \cdot L^{-1}$ MES monohydrate at 0.1 $g \cdot L^{-1}$, 5µM IBA, 30 $g \cdot L^{-1}$ sucrose, solidified with 7.5 $g \cdot L^{-1}$ agar and pH adjusted to 5.75 ± 0.03 and maintained under standard culture for one week. Microcuttings were then transferred ex vitro into 72-cell trays and maintained under mist in a completely randomized design. Plants were grown to size using standard nursery practices and then grown in the field in a completely randomized design with approximately n=40 per treatment, and overwintered for one season before data was collected.

Evaluation of morphological features and pollen viability. Plant height, number of flowers, diameter of the terminal flowers, diameter of the secondary flowers, date of first anthesis, winter survival, pollen viability, number of stems, number of nodes, and internode length data were collected on mature second-year M_1 plants, where the M_1 generation is the

generation receiving the treatment. Data was collected daily to capture the first date of anthesis, and data for all other traits was collected once all plants had reached anthesis (3 Aug. 2011 and 8 Aug. 2011).

To evaluate male fertility, pollen was collected from newly opened florets from each plant between 10:30 AM and 11:30 AM. Pollen was placed on a glass slide and stained with 40 µL of acetocarmine (1%), covered with a coverslip, and allowed to incubate for a minimum of 90 minutes at room temperature. Pollen was observed the same day collected. Well-formed grains with a pink/red stain were scored as viable. Each sample consisted of a minimum of 200 grains and was collected from a minimum of 3 flowers per plant.

Data were analyzed using regression analysis (PROC GLM, SAS Version 9.1.3; SAS Institute Inc., Cary, NC)

Results and Discussion

Callus survival and shoot regeneration. Callus survival and shoot regeneration showed no significant response to radiation dosage three weeks after treatment (data not shown). Further, callus survival was not significantly affected by radiation dose 2 months after treatment (29 Jan 2010) (Fig. 1). However, 2 months after treatment, callus shoot regeneration showed a significant quadratic response to radiation dose (Fig. 1) with shoot production declining with increased dosage and plateauing at <5 shoots per callus at dosages >20 Gy. By 12 May 2010, all regenerated shoots from the 40 Gy treatments had died and 67%, 100%, 100%, and 83% of the regenerated shoots from the 20, 10, 5, and 0 Gy treatments survived, respectively (Fig. 1). Similarly, Witjaksona and Litz (2004) found

radiation treatments influenced regenerative capacity of embryogenic callus of avocado (*Persea americana*). They found that while radiation dose did not affect the number of early stage somatic embryos, higher dosages (≥ 10 Gy) influenced the ability of somatic embryos to fully mature (Witjaksona and Litz, 2004). Charbaji and Nabulsi (1999) found low doses of radiation can increase in-vitro vigor in grapevine (*Vitis vinifera*).

Morphology, date of anthesis, winter survival, and pollen viability. Regression analysis showed that gamma radiation dose had a significant effect on plant height, number of flowers, winter survival, and pollen viability (Fig. 2). There was no dosage effect on the number of stems, number of nodes, or internode length (data not shown). Plant height, number of flowers, winter survival, and pollen viability all had a negative linear relationship with radiation dose (Fig. 2). Due to similarity of measurements, terminal and secondary flower measurement data was pooled. Flower diameter had a negative linear response to dose ($y = 86.12 – 0.75x$; $P = 0.002$, $r^2 = 0.10$) and means for 0, 5, 10, and 20 Gy treatments were 87.4, 81.5, 75.6, and 85.1 mm, respectively. Only 2 replicates of the 20 Gy treatment were flowering at the time data was collected and hence this data may not be representative. The date of first anthesis had a positive linear response to dose ($y = 7/22/11 +0.49x$, $P < 0.001$, $r^2 = 0.11$). The low r^2 value for all variables reflects the variability of our data; given the random nature of mutation treatments to DNA, this is not unexpected.

Similar to our study, a decrease in the total number of flowers, flower size, and plant height in response to radiation dose has also been observed in gamma-irradiated cuttings of *Chrysanthemum* (Dilta, et al., 2003). Reduced plant height is a common response to

radiation and numerous varieties of grains and crop species have been improved using mutation treatments (see van Harten, 1998 for review).

In our study, the reduced number of flowers and date of first anthesis also may be correlated as secondary flowers on *Rudbeckia* do not form until the terminal flower has been initiated. A delayed response in date of first anthesis may be interpreted as a delayed response in development, minimizing the time available for secondary flower formation. While mutations towards late blooming are common, they are generally undesirable in many agronomic crops and are often selected against in M_1 and M_2 mutagenic populations, (van Harten, 1998). In African marigold, (*Tagetes erecta*) bud-initiation, days to first color, and days to first bloom were all delayed with 200, 300, and 400 Gy treatments to seed (Singh et al., 2009). The number of flower heads per plant was also significantly reduced in African marigold with all radiation treatments (Singh et al., 2009).

Mutation treatments are often used to induce sterility. While we had only a moderate decline in pollen staining in response to radiation dose, a considerable (>71%) reduction in pollen viability in response to gamma irradiation has been observed in citrus (Bermejo et al., 2011).

Interestingly, we saw a reduction in winter survival with higher radiation dose. Cold tolerance is a trait often selected for in mutation experiments. For example, mutants with higher cold tolerance have been selected from radiation treatments in vitro in rice (*Oryza sativa*) (Rakotoarisoa et al., 2008). Further, X-ray's have been used to develop cold tolerant chrysanthemum (Huitema et al., 1991). Reduction in winter survival is generally undesirable and has not been addressed in most mutation studies.

We also observed unusual qualitative traits (Fig. 3), such as an effect on ray floret morphology in response to irradiation. Unusual phenotypes in the 5 Gy treatment included ray florets with increased apical splitting, cupping of the ray florets, and a decrease in the diameter of the ray floret. The 10 Gy treatment displayed some increased apical splitting of the ray and one mutant with green disc florets, as opposed to the black/brown color of the control. The 20 Gy treatment showed more severe apical splitting of the ray floret than that which was found in the lower treatments, and a more irregular presentation of ray florets around the disc head. Irregularity of the ray floret distribution around the disc has also been noted in gamma-irradiated *Chyrsanthemum* (Dao et al., 2006). Irregular ray floret morphology has also been noted in gamma irradiated Aster (*Aster novi-belgii*) cultivars (Kristiansen and Petersen, 2009) and African marigold (Singh et al., 2009). No change in flower color was observed in our study.

In some cases, mutations can result in dominant gene mutations that are apparent in M_1 phenotypes; this has been observed in maize, barley, and other crop species (van Harten, 1998). However, the majority of gene mutations occurring in plants as a result of induced mutations are typically recessive or quantitative (van Harten, 1998; Stadler, 1944). As such, phenotypes associated with the mutation may not be observed until later generations. Since *R. subtomentosa* is highly self infertile (Palmer et al., 2009), development of lines with homozygous, recessive mutations is difficult. However, crosses of plants with complimentary dominant mutations (e.g. reduced height), may further enhance those traits.

Treating callus with low levels of gamma radiation (5-10 Gy) resulted in relatively high in vitro and field survival while inducing a range of other mutations. Several accessions

with a reduced height and a desirable flower form were selected from the 5 Gy and 10 Gy treatments for further assessment of commercial traits. Higher doses resulted in increased and undesirable irregularity of the ray floret morphology. Development of larger populations of plants derived from 5-10 Gy treatments would be a promising approach to recover combinations of desirable mutations.

Literature Cited

Armitage, A.M. 2008. Herbaceous perennial plants: A treatise on their identification, culture, and garden attributes. 3rd ed. Stipes Publishing, Champaign, Il.

Arnold, N.P., N.N. Barthakur, and M. Tanguay. 1998. Mutagenic effects of acute gamma irradiation on miniature roses: target theory approach. HortScience 33:127-129.

Arunyanart, S. and S. Soontronyatara. 2002. Mutation induced by γ and X-ray irradiation in tissue culture lotus. Plant Cell, Tissue, and Organ Cult. 70:119-122.

Bermejo, A., J. Pardo, and A. Cano. 2011. Influence of gamma irradiation on seedless citrus production: pollen germination and fruit quality. Food and Nutr. Sci. 2:169-180.

Broertjes, C., A.M. van Harten, and C. Broertjes. 1988. Applied mutation breeding for vegetatively propagated crops. Elsevier, New York.

Charbaji, L. and I. Nabulsi. 1999. Effect of low doses of gamma irradiation on in vitro growth of grapevine. Plant Cell, Tissue and Organ Cult. 57:129-132.

Dao, T.B., P.D. Nguyen, Q.M. Do, T.H. Vu, T.L. Le, T.K.L. Nguyen, H.D. Nguyen, and X.L. Nguyen. 2006. In vitro mutagenesis of chrysanthemum for breeding. Plant Mutation Rpt. 1(2):26-27.

Datta, S.K., D. Chakrabarty, and A.K.A. Mandal. 2001. Gamma ray-induced genetic manipulations in flower colour and shape in *Dendranthemum grandiflorum* and their management through tissue culture. Plant Breeding 120:91-92.

Dilta, B. S., Y. D. Sharma, Y. C. Gupta, R. Bhalla, and B. P. Sharma. 2003. Effect of gamma-rays on vegetative and flowering parameters of chrysanthemum. J. of Ornamental Hort. 6:328-334.

Driver, J.A. and A.H. Kuniyuki. 1984. In vitro propagation of paradox walnut rootstock. HortScience 19:507-509.

Fulcher, A., W.C. Dunwell, and D. Wolfe. 2003. *Rudbeckia* taxa evaluation. Southern Nurserymen's Assoc. Res. Conf. 48:510-512.

Gottschalk, W. and G. Wolff. 1983. Induced mutations in plant breeding. Springer-Verlag, New York.

Hong, C.X. 2006. Downy mildew of *Rudbeckia fulgida* cv. Goldsturm by *Plasmopara halstedii* in Virginia. Plant Dis. 90:1461.

Huitema, J.B.M., W. Preil, and J. De Jong. 1991. Methods of selection of low-temperature tolerant mutants of *Chrysanthemum morifolium* Ramat. using irradiated cell suspension cultures. III. comparison of mutants selected with or without preselection in vitro at low-temperature. Plant Breeding 107:135-140.

Kristiansen, K. and K.K. Petersen. 2009. In vitro mutagenesis of *Aster novi-belgii* cultivars. Acta Hort. 836:207-214.

McKersie, Bryan D. and D. C. W. Brown. 1996. Somatic embryogenesis and artificial seeds in forage legumes. Seed Science Research. 6:109-126.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia plantarum. 15:473-497.

Palmer, I.E., D.H. Touchell, and T.G. Ranney. 2008. In-vitro polyploid induction of *Rudbeckia* spp. Southern Nurserymen's Assoc. Res. Conf. 53:186-189.

Palmer, I.E., R.E. Bir, N.P. Lynch, and T.G. Ranney. 2009. Crossability, cytogenetics, and reproductive pathways in *Rudbeckia* subgenus *Rudbeckia*. HortScience 44:44-48.

Perdue, Jr., R.E. 1957. Synopsis of *Rudbeckia* subg. *Rudbeckia*. Rhodora 59:293-299.

Rakotoarisoa N. V., A. Andrianjaka, F. J. Zapata-Arias, R. Afza, and C. Mba.2008. Inducing cold tolerance in Malagasy rice varieties IR 58614, Malady and Rojofotsy through in vitro mutagenesis. Plant Mutation Reports Vienna: Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency 2(1): 16-23.

Singh, V.N., B.K. Banerji, A.K. Dwivedi, and A.K. Verma. 2009. Effect of Gamma irradiation on African marigold (*Tagetes erecta* L.) cv. Pusa Narangi Gainda. J. Hort. Sci. 4(1):36-40.

Sparrow, A.H. 1954. Somatic mutations induced in plants by treatment with X and γ radiation. Caryologia. 6 (suppl.):1105.

Stradler, L.J. 1944. The effect of X-rays upon dominant mutation in maize. Proc. Natl. Acad. Sci. 30:123-128.

van Harten, A.M. 1998. Mutation breeding, theory and practical applications. Cambridge Univ. Press, Cambridge.

Witjaksono and R.E. Litz. 2004. Effect of gamma irradiation on embryogenic avocado cultures and somatic embryo development. Plant Cell, Tissue and Organ Cult. 77:139-147.

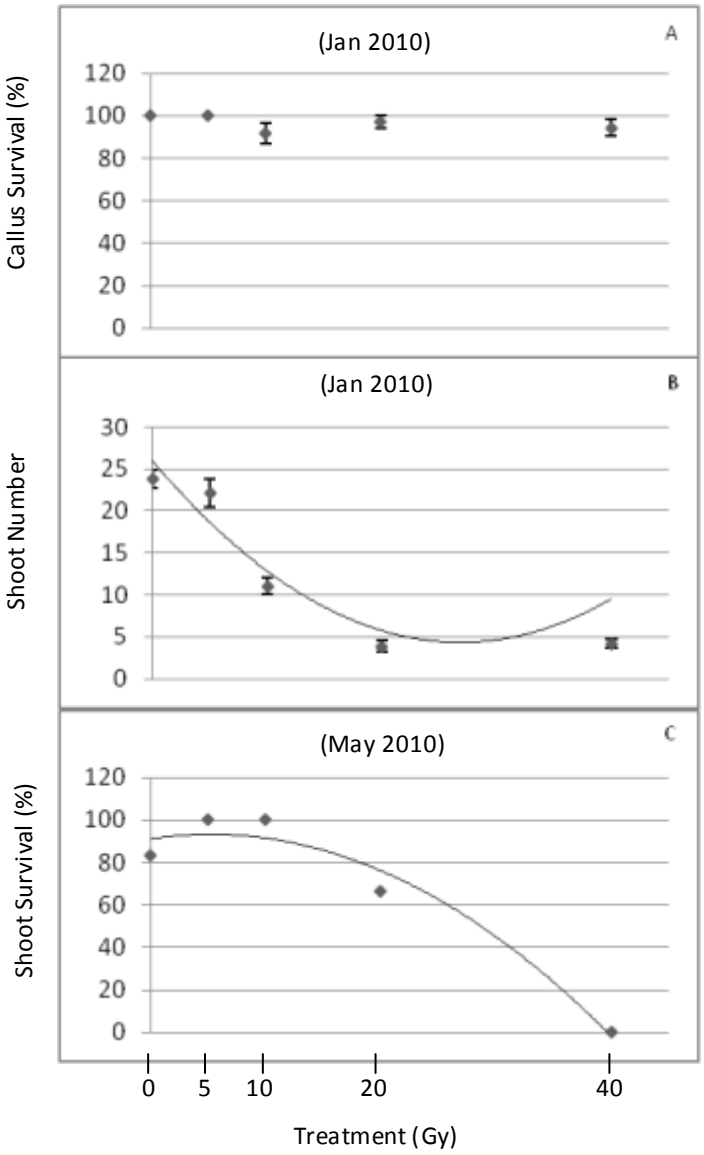

Figure 1. In-vitro callus survival, shoot counts, and shoot survival of tetraploid *R.* 'Henry Eilers' treated with gamma radiation (means ± SEM). A) Callus survival (1/29/2010) B) Number of shoots per callus (1/29/10) by treatment; $y=25.95 -1.61x + 0.03x^2$ ($P<.0001$, $r^2=0.88$). C) Shoot survival (5/12/2010) $y=0.91 + 0.008x - 0.0008x^2$.

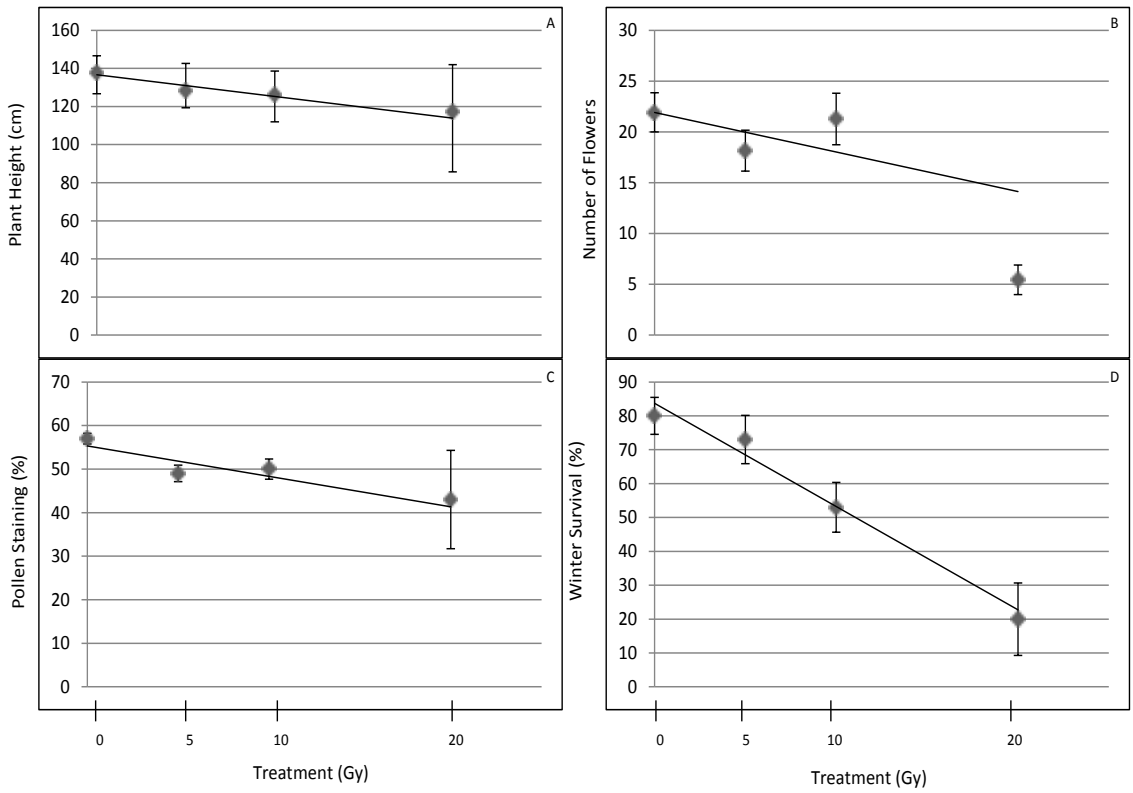

Figure 2. Response of selected traits to radiation dose (means ± SEM). Solid line represents linear regression. A) Plant height had a negative-linear relationship to radiation dose, y=136.67 – 1.14x (P=0.01, r^2 = 0.06). B) Number of flowers had a negative-linear relationship to radiation dose, y=21.92- 0.39x (P=0.04, r^2 =0.04). C) Pollen staining had a negative-linear response to radiation dose, y=0.55 -.007x (P=0.001, r^2 =.10). D) Winter survival had a negative-linear relationship with radiation dose, y= 0.84 – 0.03x (P<0.0001, r^2 =0.15).

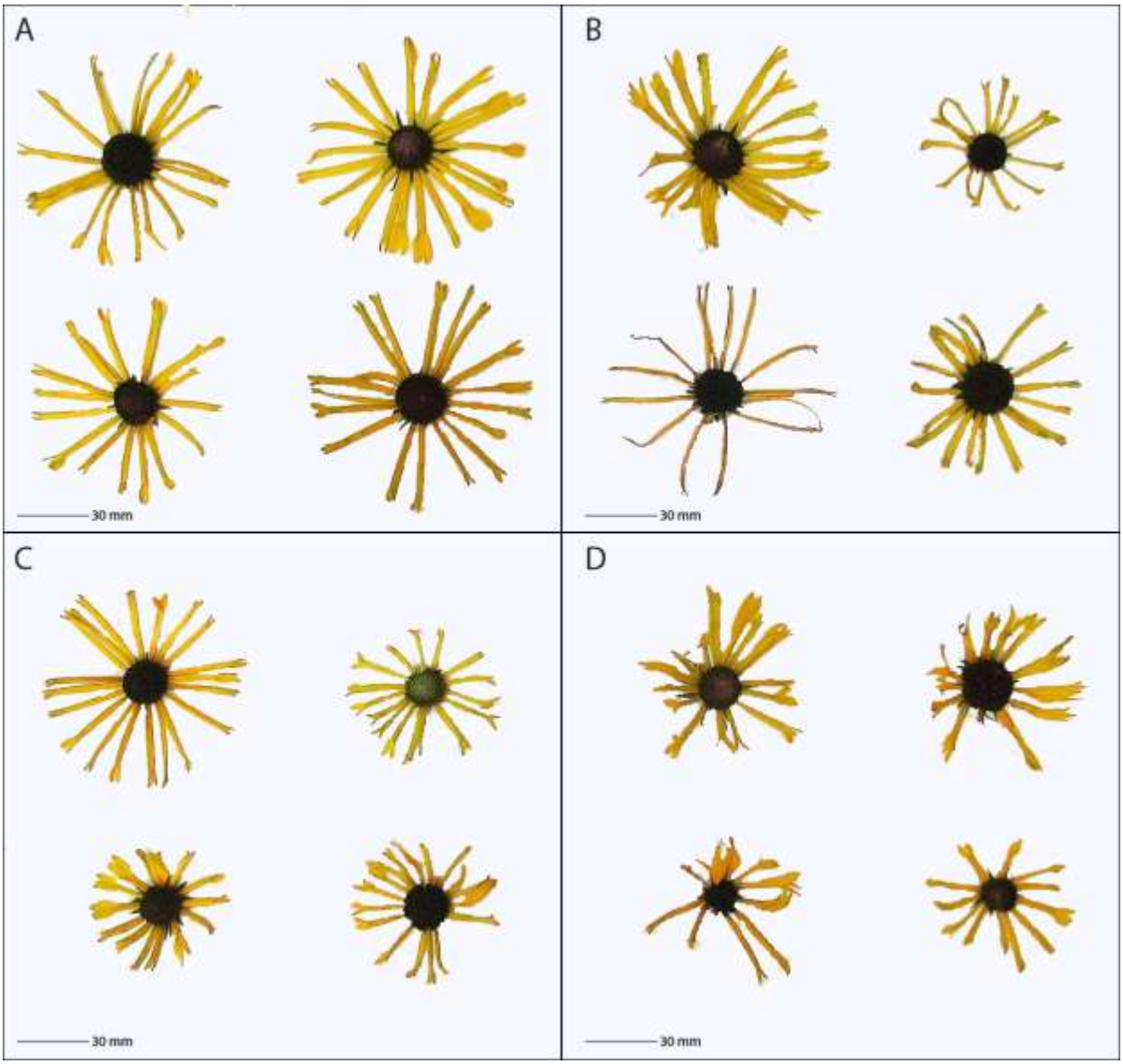

Figure 3. Off phenotypes from treatments and control. A. Normal phenotype B. Off-types from 5 Gy treatment C. Off-types from 10 Gy treatment D. Off-types from 20 Gy treatment

Chapter 3

Inheritance of the Tubular Ray Floret Trait in *Rudbeckia subtomentosa* ‘Henry Eilers’

(In the format appropriate for submission to HortScience)

Inheritance of the Tubular Ray Floret Trait in *Rudbeckia subtomentosa* 'Henry Eilers'

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Subject Category: Breeding, Cultivars, Rootstocks, and Germplasm Resources

Inheritance of the tubular ray floret trait in *Rudbeckia subtomentosa* 'Henry Eilers'

Additional index words. Asteraceae, blackeyed Susan , capitulum traits, coneflower, corolla symmetry, flower types

Abstract. **The mode of inheritance of the tubular ray floret trait in *R. subtomentosa* 'Henry Eilers' was investigated. An F_1 population was generated between wild-type *R. subtomentosa* and *R.* 'Henry Eilers'. Backcrosses were performed between plants from each reciprocal F_1 population back to *R.* 'Henry Eilers' and the wild-type parent. An F_2 population was generated by intercrossing among full-sib F_1s (diploid *R. subtomentosa* have been shown to be highly self infertile). Progeny from each population were characterized and chi-square goodness of fit tests were performed to assess likely genetic models. The tubular ray floret trait in *R. subtomentosa* 'Henry Eilers' appears to be a single recessive trait, with one major gene controlling the phenotype, though other minor genes or developmental factors may contribute to some partial expression.**

Rudbeckia spp. are widely grown ornamental wildflowers commonly found in both natural and cultivated landscapes. There are approximately 30 species (Armitage, 2008) of Rudbeckia and all are native to North America. *Rudbeckia subtomentosa* is a perennial species with good disease resistance, cold tolerance, and adaptability to many soil types.

Rudbeckia subtomentosa has showy yellow flowers that first appear in mid July in USDA hardiness zone 6b and continue flowering till frost.

Rudbeckia is a member of the Asteraceae and has the typical composite inflorescence which is a highly modified head or capitulum, that we will refer to as a "flower". The flowers of *Rudbeckia* are classified as radiate, having fertile disc florets in the center of the head surrounded by ray florets on the outer edge of the flower. The disc florets are actinomorphic with short radially symmetric adnate petals. The ray florets are typically zygomorphic (bilaterally symmetrical) with a flat, ligulate corolla tube held out from the inflorescence. The cultivar, *R. subtomentosa* 'Henry Eilers' is unique in that its ray flowers have corollas which are tubular and appear to be "quilled". *R.* 'Henry Eilers' was found alongside a stream bank in southern Montgomery County, Illinois by the nurseryman, Henry Eilers (Klingaman, 2005). It has since become a popular and common commercially available cultivar. There are many opportunities for improvement within *Rudbeckia* and the introgression of the tubular ray floret trait into other phenotypes would be desirable.

Inheritance of this trait has been investigated in sunflower (*Helianthus annuus*) where it was found to be a single recessive gene (Berti, et al., 2005, Fick, 1976). In *Chrysanthemum morifolium*, DeJong and Drennan (1984) found that crosses between ligulate and tubular corolla types produced offspring with varying ratios of tubular:intermediate:ligulate phenotypes, depending on the parents used. The objective of this study was to determine the mode of inheritance of the tubular ray floret trait in *R. subtomentosa*.

Materials and Methods

During Oct. 2009 through Dec. 2009, reciprocal crosses between a wild-type *R. subtomentosa* and *R.* 'Henry Eilers' were completed to generate F_1 progeny. Seeds from these reciprocal crosses were sown in February 2010. Sixteen F_1 seedlings were generated: 9 from the female *R.* 'Henry Eilers' and 7 from the female wild-type. Between June 2010 and July 2010, backcrosses were made with plants from each reciprocal F_1 population back to *R.* 'Henry Eilers' and the wild-type parent. Diploid *R. subtomentosa* have been shown to be highly self infertile (Palmer, 2009), therefore rather than self the F_1s, we generated a (pseudo) F_2 population by intercrossing among full-sib F_1s. Flowers were not emasculated.

Seed heads were collected once mature and allowed to dry. Seeds were separated from chafe and were moist stratified (5˚C) for one month in the dark and then moved to a greenhouse to germinate. Seedlings were transplanted into nursery containers and grown using standard nursery practices.

Three flowers each from individual plants in the F_1, F_2, BC_{HE} (Backcross to *R.* 'Henry Eilers') and BC_{WT} (Backcross to wild-type) populations were characterized as either being fully quilled or as having no or only some partially quilled florets. Chi-square analysis was performed to determine goodness of fit of the observed ratios of wild-type: quilled types to a theoretical monogenic control of the phenotype.

Results and Discussion

All plants in the F_1 progeny exhibited a wild-type phenotype (Table 1). Seven of the 16 plants in the F_1, including plants from both maternal parents, displayed some flowers with

a few partially tubular ray florets. However the majority of the flowers had normal, wild-type rays, so these plants were classified as wild-type (Fig.1). For the F_2 population, the distribution was 104 wild-type: 31 tubular and fit a ratio of 3:1 ($\chi^2 = 0.30$; P = 0.58) (Table 1). The distribution of BC_{WT} was 62 wild-type: 4 tubular. The 4 plants with the tubular phenotype were from a cross where *R*. 'Henry Eilers' was the female parent. Although highly self infertile, *R. subtomentosa* can self at a low percentage (Palmer et al., 2009). The small number of seedlings with the tubular phenotype suggests they may have been the result of self-pollination and so they were removed from analysis to give an observed ratio in the BC_{WT} generation of 62 wild-type: 0 tubular ($\chi^2 = 0$; P = 1) (Table 1). The distribution of BC_{HE} was 27 wild-type: 8 tubular with an expected ratio of 1:1 ($\chi^2 = 14.66$; P = 0.0) (Table 1). The small population size of the BC_{HE} seedlings (35) may be providing a poor representation of the true ratio. More seedlings are currently being evaluated. We observed some plants, in all populations, displaying partial quilling on several rays; these were not fully tubular, and so were categorized with the wild-type. The tubular ray floret trait in *R. subtomentosa* 'Henry Eilers' appears to be a single recessive trait, with one major gene controlling the phenotype, though other minor genes or developmental factors can result in some partial expression. We propose the gene symbol *he* (Henry Eilers) for the recessive allele producing the consistent tubular ray florets (*he/he*) found in the cultivar Henry Eilers. Furthermore, we would recommend using the designation *Rudbeckia subtomentosa* forma tubuliflora to identify this phenotype.

These results are consistent with results seen in sunflower where the tubular ray floret trait (*turf*) was a single recessive mutation (Berti, et al., 2005; Fick, 1976). In *Senecio*

vulgaris (Asteraceae), Kim et al. (2008) identified two genes (*RAY1* and *RAY2*) that control floral symmetry. In *Senecio*, as in *Rudbeckia*, disc florets are radially symmetric and ray florets are bilaterally symmetric. *RAY1* and *RAY2* are tightly linked genes and no recombinants were observed in a large F_2 population (Kim et al., 2008). Interestingly, transgenic plants expressing the N allele (non-radiate) of *RAY2* in plants with a radiate head background (radiate alleles at the *RAY1* and *RAY2* locus) had a tubular ray floret phenotype. Due to the tight linkage between *RAY1* and *RAY2*, it would be uncommon for naturally occurring populations to recombine to express radiate alleles at one locus (*RAY1*) and not the other (*RAY2*), and so it is unlikely that this trait would occur naturally with any regularity. Primers used to perform genotyping and linkage analysis of *RAY1* and *RAY2* in *Senecio* could be used in our populations to further elucidate the genetic control of the tubular ray floret phenotype in *Rudbeckia subtomentosa* and determine if it were under the same genetic control as *Senecio*. The finding the tubular petal trait found in *R.* 'Henry Eilers' is controlled largely by a single recessive gene indicate that this trait can be easily combined or introgressed with other desirable traits or phenotypes.

Literature Cited

Armitage, A.M. 2008. Herbaceous perennial plants: A treatise on their identification, culture, and garden attributes. 3rd ed. Stipes Publishing, Champaign, Il.

Berti, F., M. Fambrini, M. Turi, D. Bertini, C. Pugliesi. 2005. Mutations of corolla symmetry affect carpel and stamen development in *Helianthus annuus*. Can. J. Bot. 83:1065-1072.

DeJong, J. and D. L. Drennan. 1984. Genetic analysis in *Chrysanthemum morifolium*. II. Flower doubleness and ray floret corolla splitting. Euphytica 33:465-470.

Fick, G. N. 1976. Genetics of floral color and morphology in sunflowers. J. Hered. 67:227-230.

Kim, Minsung, Min-long Cui, Pilar Cubas, Amanda Gillies, Karen Lee, Mark A. Chapman, Richard J. Abbott, and Enrico Coen. 2008. Regulatory genes control a key morphological and ecological trait transferred between species. Science. 322: 1116-1119.

Laitinen, R.A.E., S. Broholm, V.A. Albera, T.H. Teeri, P. Elomaa. 2006. Patterns of MADS-box gene expression mark flower-type development in *Gerbera hybrida* (Asteraceae). BMC Plant Biol. 6(11).

Fig. 1. Segregating phenotypes in the F_2 A) Wild-type R. subtomentosa B) A wild-type displaying some tubular ray florets C) A fully "quilled" phenotype with all tubular ray florets

Table 1. Segregation of ray floret morphology in populations derived from crosses between *R.* 'Henry Eilers' (tubular rays) and wild-type *R. subtomentosa*

Family	Observed distribution (no. plants)			Expected ratio Tubular ray : wild-type	X^2	df	*P*
	Tubular ray	Wild-type	Total				
F_1	0	16	16	0 : 1	0.000	-	1.00
F_2	31	104	135	1 : 3	0.299	1	0.58
BC_{HE}	8	27	35	1 : 1	14.66	1	0.00
BC_{WT}	0	62	62	0 : 1	0.000	-	1.00

Chapter 4

Campsis ×*tagliabuana* ‘Chastity’: A Highly Infertile Triploid Trumpetvine for use as a Non-Invasive Landscape Vine

(In the format appropriate for submission to HortScience)

Campsis ×*tagliabuana* 'Chastity': A Highly Infertile Triploid Trumpetvine for use as a Non-Invasive Landscape Vine

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Subject Category: Cultivar and Germplasm Releases

Campsis ×tagliabuana 'Chastity': A Highly Infertile Triploid Trumpetvine for use as a Non-Invasive Landscape Vine

Additional index words. Bignoniaceae, cytology, fertility, invasive, pollen viability, polyploidy, seedless, sterile, trumpetcreeper

Campsis Lour. (Bignoniaceae) is commonly known as trumpetvine or trumpet creeper. The genus contains only two species, *C. radicans* (L.) Seem. and *C. grandiflora* K. Schum. and their interspecific hybrid, *Campsis ×tagliabuana* (Vis.) Rehder (Huxley et al., 1992) . *Campsis radicans* is native throughout eastern North America and is often seen growing along fencerows, telephone poles, and embankments. It is a robust climbing vine (reaching up to 6 m) that actively suckers from the base (Anderson, 1933). The showy trumpet shaped flowers form on current year's growth from mid-June through October with flower colors of different cultivars including yellow, orange, and red. *Campsis grandiflora* is also an aggressive grower with larger and more open funnel shaped flowers than *C. radicans*, typically with apricot/orange flowers. *Campsis ×tagliabuana* has intermediate characteristics between the two parental species. While *Campsis* is widely adaptable, with a long bloom period and showy display of flowers, it seeds prolifically, grows aggressively, and is considered weedy in many areas. The highly infertile, *C.* 'Chastity' was created to minimize

the reseeding potential of *Campsis*, while maintaining the prolific flowering nature of the genus.

Origin

Campsis 'Chastity' was developed at the Mountain Crops Improvement Lab (MCI) in 2005 and resulted from a controlled cross of *C.* ×*tagliabuana* 'Kudian' (PP13,139) Indian Summer® ($2n = 2x$) × *C.* ×*tagliabuana* H2000-034-001 (oryzalin treated, open pollinated seedling from *C. grandiflora* 'Morning Calm', $2n = 4x$). Due to the existence of a triploid block, fruit of interploid *Campsis* crosses typically abort before maturing (Ranney, per. obs.). To circumvent this barrier, an in vitro embryo rescue technique was used to develop a triploid. Briefly, embryo sacs were collected 6 weeks after pollination and cultured on Shenck and Hilberandt (SH) (Shenck and Hilberandt, 1972) media supplemented with 20 $g \cdot l^{-1}$ sucrose. After two weeks, embryos were transferred to a SH media supplemented with 20 $g \cdot l^{-1}$ sucrose and 1µM giberillic acid (GA). Germinated embryos were then transferred to SH media supplemented with 20 $g \cdot l^{-1}$ sucrose and 4µM GA. After one month, a single surviving seedling was transferred onto Murashige and Skoog (MS) (Murashige and Skoog, 1962) media supplemented with 20 $g \cdot l^{-1}$ sucrose and transplanted ex vitro 6 weeks later.

Description

To evaluate fertility and morphological characteristics, 7 plants of *C.* 'Chastity', 6 diploid *C. radicans* varieties, and 1 diploid *C. grandiflora* 'Morning Calm' (1

plant/replication per variety) were grown in the field in a completely randomized design at Mountain Horticultural Crops Research Station, Mills River, NC.

Campsis 'Chastity' has a vigorous vining growth habit. The flowers are tubular to funnelform, with adnate petals dividing into 5 lobes at the distal end of the corolla in reference to the petiole (Figure 1). The exterior of the corolla tube is orange (RHS 26B) and the interior of the corolla tube is yellow-orange (RHS 22B) to red (RHS 46A) with red (RHS 46A) striping (Royal Horticultural Society, 2001). The corolla lobes are orange (RHS 26B) to red (RHS 46A). The mean length of the corolla of *C.* 'Chastity' was 53 mm, which was similar to that of *C. grandiflora* 'Morning Calm' and *C.* × *tagliabuana* 'Kudian', but smaller than that of *C. radicans* 'Flamenco' (Table 1). Corolla width was measured twice, once at the proximal portion of the corolla closest to the petiole (minimum corolla width) and again at the distal portion of the corolla farthest from the petiole (maximum corolla width). The minimum corolla length of *C.* 'Chastity' was 10 mm, which was intermediate between *C. grandiflora* 'Morning Calm', 17 mm, and *C.* × *tagliabuana* 'Kudian' and *C. radicans* 'Flamenco', 6 mm and 5 mm, respectively (Table 1). The maximum corolla width was again intermediate between *C. grandiflora* 'Morning Calm' and *C.* × *tagliabuana* 'Kudian', although not significantly different from *C. radicans* 'Flamenco' (Table 1).

Leaves of *Campsis* are odd pinnately compound with 9-13 leaflets. Each leaflet is ovate with an attenuated apex and a coarsely dentate margin. Leaf morphology was taken by randomly collecting a minimum of 5 fully expanded, mature leaves from each variety assessed. The mean leaf length, leaf width, leaflet length, and leaflet width of *C.* 'Chastity' were significantly larger than all other varieties measured, except in the measurements of leaf

width and leaflet length, where *C.* 'Chastity' was not significantly different from *C. grandiflora* 'Morning Calm' (Table 1). Induced polyploidy is known to cause gigas effects in many crops, and the increase in leaf size of *C.* 'Chastity' could be associated with the higher ploidy of *C.* 'Chastity' ($2n = 3x$).

Male and female fertility was significantly reduced in *C.* 'Chastity' as assessed by pollen staining, pollen germination, and controlled crosses (Tables 2 and 3). Pollen staining was performed by collecting fresh pollen from each accession, applying pollen to a microscope slide, adding one drop of 1% acetocarmine stain, and then covering with a coverslip. Stained pollen was left at room temperature for a minimum of 90 minutes prior to visualizing. Pink, well-formed pollen grains were scored as stained. *Campsis* 'Chastity' had 41.6% stained pollen compared to 95.9%, 99.7%, and 99.9% of *C. grandiflora* 'Morning Calm', *C.* × *tagliabuana* 'Kudian', and *C. radicans* 'Flamenco', respectively (Table 2). Pollen germination was performed using a hanging drop method. Fresh pollen from at least 3 individual flowers of each accession (Table 2) was placed into a sterile 60mm x 15mm polystyrene petri dish. Approximately 500 µL of liquid Brewbaker and Kwack media (Brewbaker and Kwack, 1963) supplemented with 10g/L sucrose was added to the dish and the solution was mixed by gently pipetting until a homogenous solution was attained. To prepare the slides for hanging drop, the broadest end of a pasteur pipette was pressed into white petroleum USP (Petroleum Jelly) and then applied to a clean microscope slide to form a liberal ring of petroleum jelly. Approximately 36 mg (two drops) of the pollen solution was placed in the center of the petroleum jelly ring. The slide was then inverted and placed in a humidity chamber at room temperature. After an approximately 6 hour incubation time,

hanging drop slides were removed from the humidity chamber. The ring of petroleum jelly was carefully removed, a glass coverslip was applied, and pollen was scored for germination under 150× magnification. Only grains with a pollen tube greater than or equal to the diameter of the pollen grain were scored as germinated. *Campsis* 'Chastity' had only a 1% germination rate, while *C. grandiflora* 'Morning Calm', *C.* × *tagliabuana* 'Kudian', and *C. radicans* 'Flamenco' had germination rates of 44.9%, 43.4%, and 75.9%, respectively (Table 2). Male fertility was also assessed by performing controlled crosses on 8 to 24 flowers on each of the diploid *Campsis radicans* cultivars with pollen from either *C.* 'Chastity' or bulked pollen from diploid cultivars. Fruit set for *C.* 'Chastity' was 0% compared to 65% for the controls (Table 3).

To assess female fertility, controlled crosses were performed on 23 to 55 flowers on each plant. Pollen from diploid taxa was collected and bulked daily and used for hand pollinations. The percentage of fruit set per pollinated flowers and mean number of seeds per fruit were recorded. *Campsis* 'Chastity' had a fruit set of 1.0% which was significantly reduced from the 65.0% of *C. radicans* (Table 3). The mean number of seeds per fruit was also significantly reduced from 467 in *C. radicans* to 101 in *C.* 'Chastity' (Table 3). Viability of these seeds is currently being investigated.

To confirm ploidy of *C.* 'Chastity', flow cytometry and cytology of root tip cells were performed. *Pisium sativum* 'Citrad', with a known DNA content of 2C= 8.75 pg, was used as an internal standard (Greihuber et al., 2007). Approximately 2 mm^2 of young leaf tissue or petal tissue from the sample and leaf tissue from the standard were finely chopped with a razor blade in a petri dish containing 400 µL of extraction buffer (CyStain ultraviolet Precise

P; Partec, Münster, Germany). The suspension was filtered through a 50 µm nylon mesh screen to remove plant debris. Nuclei were stained using 1.6 mL staining buffer containing 4', 6-diamidino-2-phenylindole (DAPI) (CyStain ultraviolet Precise P;Partec). Stained nuclei were analyzed with a flow cytometer (Partec PA-I Ploidy Analyzer; Partec) to determine relative genome size. Ploidy level was determined by comparing the relative genome size of *C.* 'Chastity' with that of *C.* ×*tagliabuana* 'Madame Galen.' *Campsis* 'Chastity' had a relative genome size of 1.74 pg, which is approximately 1.5 times that of *C.* 'Madame Galen' with a relative genome size of 1.20 pg (Table 4). Cytology was performed as another means to confirm ploidy. Root tips from rooted cuttings of *C.* 'Chastity' and *C.* ×*tagliabuana* 'Madame Galen' were collected at 9:00 AM and placed into a mitotic inhibitor (2mM 8-hydroxylqinine + 70 mg/L cyclohexamide) and maintained at room temperature under dark conditions. After 3 hours, the root tips remained in the dark and in this solution and were placed into cold incubation at approximately 5˚C for 3 additional hours. Roots were then rinsed three times with cold distilled water, patted dry, and placed into a 3 part 95% Ethanol:1 part Propionic Acid solution. Roots were incubated at room temperature in this solution for approximately 16 hours. After this incubation period, root tips were rinsed three times with 70% ethanol and placed in a glass vial containing 70% ethanol as a storage solution and maintained ~5˚C until ready for use. To perform cytology on previously prepared root tips, 4 ml of a hydrolyzing solution of 3 parts 95% ethanol:1part 12N HCl was prepared. Root tips were then placed into the hydrolyzing solution for approximately 2 minutes and then were transferred to a modified carbol fushcin stain (Kao, 1975) for at least 5 minutes and not more than 30 minutes. The root tip was excised and moved to a clean

microscope slide and covered with one drop of modified carbol fuschin. The coverslip was used to gently squash the root tissue. Chromosomes were then viewed under oil immersion at 1500× magnification. *Campsis* 'Chastity' has approximately $2n = 3x = 60$ and *C.* ×*tagliabuana* 'Madame Galen' has approximately $2n = 2x = 40$. This confirms the flow cytometry data that *C.* 'Chastity' is a triploid. Previous studies (Bowden, 1945) reported diploid *C.* ×*tagliabuana* 'Madame Galen' to have a chromosome number of $2n = 2x = 40$.

Reduction in fertility can reduce invasive potential. The ability for *C.* 'Chastity' to propagate vegetatively will still pose a problem for local spread; however its invasive potential is vastly reduced by the 99.6% reduction in seed set.

Campsis 'Chastity' is a promising new, nearly sterile, variety of *Campsis* with reduced invasive potential. It has an extremely long bloom period with showy orange-red blossoms. It can be propagated easily from hard and soft wood cuttings. Rooted cuttings of *C.* 'Chastity' flowered the following year.

Availability

Plants of *C.* 'Chastity' have been distributed to commercial nurseries. Propagation material may also be available from the MCI Lab upon request.

Literature Cited

Anderson, E. 1933. Trumpet creepers. Arnold Arboretum of Harvard Univ. Bul. Popular Info. 4(1):1-5.

Bowden, W.M. 1945. A list of chromosome numbers in higher plants I. Acanthaceae to Myrtaceae. Amer. J. Bot. 32(2) 81-92.

Brewbaker, J.L. and B.H. Kwack. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Amer. J. Bot. 50 (9):859-865.

Greilhuber, J. 1998. Intraspecific variation in genome size: A critical reassessment. Ann. Bot. 82:27–35.

Huxley, A., M. Griffiths, and M. Levy. 1992. The New Royal Hort. Soc. dictionary of gardening. Macmillan, London, UK.

Kao, K.N. 1975. A chromosomal staining method for cultured cells, p. 63-64. In: O.L. Gambourg and L.R. Wetter (eds.). Plant tissue culture methods. NRC Canada, Saskatoon, Canada.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Royal Horticultural Society. 2001. RHS Colour Chart. RHS, London, UK.

Schenk, R.U. and A.C. Hildebrandt. 1972. Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. Can. J. Bot. 50: 199-204.

Figure 1. Flowers of *Campsis* ×*tagliabuana* ‘Chastity’

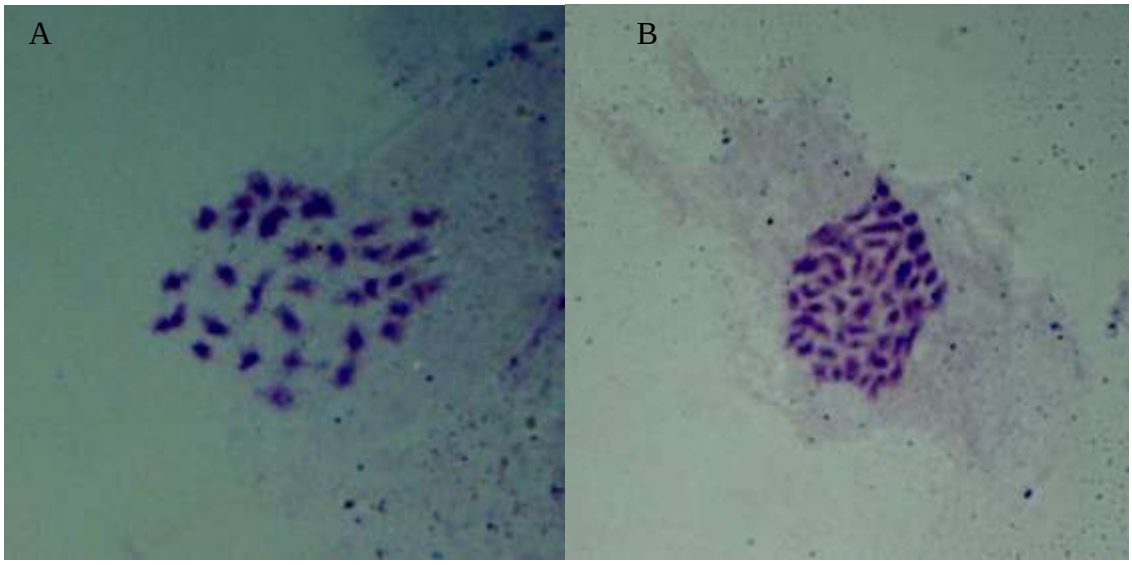

Figure 2. Photomicrograph of root tip cell of select *Campsis.* A) *C.* × *tagliabuana* 'Madame Galen', $2n = 2x = 40$; B) *C.* 'Chastity', $2n = 3x = 60$

Table 1. Morphology of selected *Campsis* cultivars

	Accession			
	C. 'Chastity'	*C. grandiflora* 'Morning Calm'	*C.* × *tagliabuana* 'Kudian'	*C. radicans* 'Flamenco'
Leaf length[z]	396 ± 19.98[y] a	319 ± 18.56 b	309 ± 21.02 bc	262 ± 8.08 c
Leaf width	159 ± 13.90 a	143 ± 6.26 ab	127 ± 6.08 b	162 ± 9.01 a
Leaflet length	88 ± 7.10 a	82 ± 3.05 ab	70 ± 3.18 c	72 ± 4.37 bc
Leaflet width	49 ± 3.97 a	40 ± 1.84 b	37 ± 2.36 bc	32 ± 2.41 c
Corolla length	53 ± 1.15 b	50 ± 1.82 b	50 ± 1.89 b	62 ± 1.20 a
Minimum corolla width	10 ± 0.41 b	17 ± 0.99 a	6 ± 0.23 c	5 ± 0.18 c
Maximum corolla width	47 ± 2.35 b	84 ± 4.92 a	35 ± 0.84 c	44 ± 1.87 bc

[z] Mean ± SEM; All measurements in mm

[y] Means followed by the same letter in the same row are not significantly different (Fisher's Least Significant Difference test, $P \leq 0.01$)

Table 2. Male fertility of selected *Campsis* cultivars

Accession	Pollen Germination (%)	Pollen Staining (%)
C. 'Chastity'	1.0 ± 0.01 c[z]	41.6 ± 0.024 b
C. grandiflora 'Morning Calm'	44.9 ± 0.10 b	95.9 ± 0.004 a
C. ×*tagliabuana* 'Kudian'	43.4 ± 0.04 b	99.7 ± 0.003 a
C. radicans 'Flamenco'	75.9 ± 0.04 a	99.9 ± 0.001 a

[z] Mean ± SEM; means followed by the same letter in the same column are not significantly different (Fisher's Least Significant Difference test, $P \leq 0.05$)

Table 3. Fertility of *C.* 'Chastity' and *C. radicans*

Taxa	Ploidy	Percentage fruit set (diploid pollen donor)	Percentage fruit set (triploid pollen donor)	Average number of seeds/fruit
C. 'Chastity'	3x	1 ± 1.0 b[z]	-	101 ± 9.0 b
C. radicans	2x	65 ± 6.6 a	0.0 ± 0.0	467 ± 47.2 a

[z] Mean ± SEM; means followed by the same letter in the same column are not significantly different (Fisher's Least Significant Difference test, $P \leq 0.05$)

Table 4. Relative genome sizes and ploidy of *C.* 'Chastity' and *C.* x *tagliabuana* 'Madame Galen'

Accession	Relative 2C genome size [mean ± SE(pg)]	Ploidy
C. 'Chastity'	1.74 ± 0.011	2*n* = 3*x* =54
C. x *tagliabuana* 'Madame Galen'	1.20 ± 0.003	2*n* = 2*x* = 36

[z] Mean ± SEM; means followed by the same letter in the same column are not significantly different (Fisher's Least Significant Difference test, $P \leq 0.05$)