

# Breeding and Evaluating for Landscape Performance and Fruitlessness in Mexican Petunia (*Ruellia*, Acanthaceae)

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**Abstract.** Mexican petunia (*Ruellia simplex* Wright) is a non-native plant that was introduced to Florida sometime in the 1940s and since then has naturalized in most of the state and in other southern states. Since 2007, we have developed at the University of Florida/Institute for Food and Agricultural Science in Gainesville the first *Ruellia* L. breeding program aiming to develop fruitless plants with different flower colors and growth habits that will not be invasive by seed dispersal. A combination of polyploidization and hybridization methods was used. In 2011, a total of 15 plants were selected and grown in southeastern, north-central, and northwestern Florida (Fort Pierce, Citra, and Quincy) using a randomized block design with three blocks and three plants per plot at each site. Plants were evaluated monthly for landscape performance, flowering, and fruiting. Two hybrids (R10-102 and R10-108) had outstanding potential as new fruitless cultivars for the plant industry having improved landscape performance and flowering.

*Ruellia* L. is one of the largest genera in the Acanthaceae and consists of ≈250 species of perennial herbs, subshrubs, and shrubs with mostly tropical and subtropical distribution (Ezcurra, 1993). Five species of *Ruellia* are native to Florida, and three non-native species are listed as naturalized in the state:

*R. blechum* L., *R. ciliatiflora* Hook, and *R. simplex* Wright (Wunderlin and Hansen, 2012). In past years, *R. simplex* (commonly known as Mexican petunia, Mexican bluebell, or Britton's petunia) was previously listed as *R. tweediana* Griseb. (or *R. brittoniana* Leonard). This species was introduced to Florida sometime before 1940 (Hupp et al., 2009) and since then has become a very popular landscape plant in the southern United States as a result of its prolific flowering and low-maintenance requirements (Gilman, 1999). Meanwhile, *Ruellia coerulea* Morong was considered restricted to subtropical South America and was considered closely related to North American species *R. malacosperma* Greenm. and *R. brittoniana* (Ezcurra, 1993). More recently, taxonomists have grouped both *R. tweediana* and *R. coerulea* under the name *R. simplex*. This is the name of a species of *Ruellia* described from Cuba in 1870, and because it is the oldest name recorded, it has taxonomic priority and reduces the latter names to synonyms (Ezcurra and Daniel, 2007).

Chromosome numbers of  $2n = 34$  for 27 taxa of *Ruellia* have been reported (Grant, 1955) as well as a few rare counts of  $n = 16$  and  $n = 18$  (Federov, 1969). Furthermore,

chromosome numbers in several *Ruellia* species from Central and North America were all found to be  $n = 17$ . Thus, this chromosome number appears to be widespread in this large and variable genus from various parts of the world (Daniel and Chuang, 1993; Daniel et al., 1984, 1990). *Ruellia simplex* (as *R. tweediana*) was also reported as  $2n = 34$  (Piovano and Bernadello, 1991).

*Ruellia simplex* is found in sunny areas on periodically inundated soils in the south of the United States, Mexico, the Antilles, western Bolivia, southwestern Brazil, Paraguay, Uruguay, and northeastern Argentina (Ezcurra and Daniel, 2007). *Ruellia simplex* has the ability to grow in a wide range of environmental conditions, from wetlands to almost xeric (Hupp et al., 2009). It produces on average 20.6 seeds per capsule with 98% to 100% germination rate under ideal conditions of 30 °C day and 20 °C night (Wilson and Mecca, 2003). Explosive dehiscence of the seed capsule results in seed dispersal distances from the parent plant of 2.5 to 3 m (Witzum and Schulgasser, 1995). Furthermore, *Ruellia* seeds turn mucilaginous and adhesive on contact with water and in this way can be dispersed by animals (Ezcurra, 1993). Consequently, *R. simplex* has naturalized in disturbed uplands and wetlands of seven southern U.S. states (Florida, Georgia, South Carolina, Alabama, Mississippi, Louisiana, and Texas) plus the Virgin Islands, Puerto Rico, and Hawaii (Kartesz, 2012; U.S. Department of Agriculture, Natural Resources Conservation Service, 2012).

In Florida, *R. simplex* has formed naturalized populations in 28 counties throughout the state (Wunderlin and Hansen, 2012) and is listed in 18 areas reserved for conservation in South Florida (Gann et al., 2008). Since 2001, the Florida Exotic Pest Plant Council (FLEPPC) has considered Mexican petunia as a Category I invasive plant, described as “plants that are altering native plant communities by displacing native species, changing community structures or ecological functions, or hybridizing with natives” (FLEPPC, 2011). The Institute for Food and Agricultural Science (IFAS) Assessment of the Status of Non-Native Plants in Florida's Natural Areas states that Mexican petunia is invasive and not recommended for use in the central and south parts of Florida. It is also not recommended in northern Florida unless its specified use is approved (IFAS Invasive Plant Working Group, 2011).

Invasive species damage natural areas, alter ecosystem processes, displace native species, hybridize with natives, and/or support other potentially damaging plants, animals, and pathogens (Randall and Marinelli, 1996). Destruction of native ecosystems by invasive plants is an issue of worldwide concern with consensus that early eradication is critical. In the United States, an estimated 5000 plant species have escaped cultivation and are now established in natural ecosystems. The cost associated with invasive plant damage and control is estimated at nearly \$35 billion a year and plant invasions are increasing

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at a rate of 10% annually (Pimentel et al., 2005). Conventional and new biotechnologies such as use of wide hybridization, selection and breeding for double flowers, development of triploid plants, and use of induced mutations can be used to develop new, non-invasive cultivars (Ranney, 2004). New approaches include using genetic engineering to develop “supersterile” cultivars that are both male- and female-sterile (Li et al., 2004).

Currently there are tall (‘Purple Showers’, ‘Chi Chi’, and ‘Snow White’) and dwarf (‘Katie’ and ‘Southern Star’ series) cultivars available of *R. simplex* in purple, pink, and white flower colors. These cultivars are propagated clonally to maintain their identity with the exception of the ‘Southern Star’ plants, which are propagated by seed (PanAmerican Seed Co., Chicago IL). All known cultivars are fertile, set fruit and produce viable seed, and are potentially invasive with the exception of ‘Purple Showers’, which does not set fruit by open pollination (Wilson and Mecca, 2003). A survey conducted in 2002 including 946 active nurseries in Florida indicated that 15.9% of them grew or sold *R. simplex*, and the total reported annual sales for this plant was estimated at ≈\$12 million (Wirth et al., 2004). Sales of ‘Purple Showers’ in Florida were ranked third for herbaceous perennials after pentas and lantana (Ornamental Outlook, 2009).

New, sterile *Ruellia* cultivars with novel flower colors and growth habits have great potential for landscape use in Florida and other U.S. southern states as an alternative to the invasive wild *R. simplex* and fertile cultivars. Since 2007, we have developed at the University of Florida/IFAS in Gainesville the first *Ruellia* breeding program using a combination of polyploidization and hybridization techniques. The objective of this research is directed specifically toward creating fruitless cultivars for the landscape plant industry. Plants were evaluated in multilocation trials in Florida and selections were made for potential new cultivar releases.

## Materials and Methods

**Polyploidization.** Polyploidization experiments were performed at the University of

Florida in Gainesville between Sept. and Dec. 2008 using oryzalin on the apical meristem of seedlings as described (Jones et al., 2008). Seedlings obtained from self-pollination of *R. simplex* with four different flower colors were used: purple wild *R. simplex*, pink ‘Chi Chi’, white F<sub>2</sub> plant from the cross purple wild *R. simplex* × ‘Katie White’, and an accession with white flowers with a purple center, found at McKee Botanical Gardens, Vero Beach, FL, hereafter referred to as McKee. One seedling for each line was used as a control, and six seedlings were used for each dose (25 or 50 μM) and application frequency (one, two, three, or four times every 12 h) of oryzalin. Ploidy levels were determined on mature plants using flow cytometry as described by Czarnecki and Deng (2009) with wild *R. simplex* used as a diploid control. Plants with means for the relative DNA fluorescence curves approximately double in value than the diploid were considered tetraploid, and those with curves with means intermediate between diploid and tetraploid values were considered triploid.

**Hybridizations.** Hybridizations were performed with plants of different ploidy levels such as 4x × 2x and 2x × 4x, aiming to obtain sterile triploid plants. Additionally, 4x × 4x crosses were also performed. Between five and 10 hybridizations were performed for each cross. Fully expanded flower buds or recently opened flowers on the maternal parent plants were emasculated by removing the corolla and attached anthers. Immediately, the stigma was rubbed with the anthers of a flower from the paternal parent. The pollinated flower was then tagged with a plastic colored string. When the fruit developed, it was enclosed with an empty tea bag secured with a paper clip to prevent losing the seeds when the fruit ripened and dehisced.

**Preliminary evaluations.** Plants were initially evaluated in a greenhouse in Gainesville for growth habit, flowering, and lack of fruit formation. Some plants were propagated vegetatively and grown in field trials in Citra, FL, in the summers of 2009 and 2010, while simultaneously being maintained in the greenhouse. In Apr. 2011, 270 *Ruellia* clones were

transplanted to a field in Wimauma, FL, for a preliminary evaluation and visual selection process for landscape performance and flowering.

**Performance at multisite replicated trials.** Plants were trialed in three simultaneous field experiments conducted at the North Florida Research and Education Center in Quincy, FL, at the Plant Science Research and Education Unit in Citra, FL, and the Indian River Research and Education Center in Ft. Pierce, FL, corresponding to northwestern, north-central and southeastern Florida, respectively (Table 1). The experimental design used at each site was a randomized complete block with three blocks. Each plot consisted of three plants for each cultivar, hybrid, or mutant spaced 50 cm apart. At each experimental site, wild *R. simplex* and ‘Purple Showers’ were included as purple-flowered comparisons and ‘Chi Chi’ as a pink-flowered comparison. ‘Snow White’ and McKee were included as white-flowered and white with purple corolla tube comparisons, respectively, in Citra and Quincy but not in Fort Pierce as a result of space limitations.

Fifteen plants and five control cultivars were selected for the trials. Plants installed at all sites were propagated at the University of Florida in Gainesville. Cuttings were taken on the week of 4 Apr. 2011 with the exception of the cuttings for ‘Snow White’, which were taken 2 weeks later, when there were enough cuttings available. Twenty-seven cuttings per plant were stuck onto 128-cell cutting trays with Fafard 2P mix (Concord Fafard Inc., Agawam, MA; 60% Canadian peatmoss, 40% perlite) and placed under mist in a research greenhouse. After 2 weeks, rooted cuttings were transplanted into 10-cm Ellepots (Blackmore Co. Inc., Belleville, MI) and moved to an open-sided greenhouse for hardening. Plants were hardened for 3 weeks, during which they were fertilized at each irrigation with 150 ppm nitrogen with Peters liquid fertilizer (20N–4.4P–166K; Everris™, Charleston, SC).

When plants were 5 weeks old, they were distributed to each of the experimental sites and then transplanted to ground beds in full sun within 1 week. The fields were either

Table 1. Coordinates, heat and cold hardiness zones, soil type, and soil test results in May 2011 of three Florida sites where *Ruellia* plants were evaluated.

Site	Latitude/ longitude	Heat zone/ cold hardiness <sup>z</sup>	Soil type	ppm					pH
				NO <sup>3</sup> -N	Phosphorus	Potassium	Magnesium	Calcium	
Northwestern (Quincy) <sup>y</sup>	30.5° N 84.6° W	9/8b	Ruston series Fine loamy, siliceous, semiactive <sup>v</sup>	4.3	88	148	56	360	4.1
North Central (Citra) <sup>x</sup>	29.4° N 82.2° W	10/9a	Candler series Sand-forested, uncoated lamellic quartzipsamments hyperthermic	1.4	189	82	25	369	4.6
Southeastern (Fort Pierce) <sup>w</sup>	27.4° N 80.4° W	9–10/10a	Ankona series Sandy, siliceous, hyperthermic	6.6	92	75	117	590	5.8

<sup>z</sup>Heat zone map (American Horticulture Society, 1998) and Plant hardiness zone map (U.S. Department of Agriculture, 2011), respectively.

<sup>y</sup>North Florida Research and Education Center, Quincy (Gadsden County).

<sup>x</sup>Plant Science and Education Unit, Citra (Alachua County).

<sup>w</sup>Indian River Research and Education Center, Ft. Pierce (St. Lucie County).

<sup>v</sup>U.S. Department of Agriculture, Natural Resources Conservation Service (2012).

fumigated or glyphosate was applied at least 3 weeks before planting, then they were rototilled, and rows were formed and covered with black woven nursery groundcover (L & M Supply Co., Willacoochee, GA) held down by fabric pins and soil. In Fort Pierce, beds were raised 10 cm off the ground to facilitate drainage, whereas in Citra and Quincy, the beds were not raised. Transplanting was completed between 6 and 10 May 2011. Complete soil tests (Mehlich-III/H<sub>2</sub>O extraction; QAL, Panama City, FL) were performed for each site; each sample was a composite collected during transplant from three locations per site. Within 3 d after transplanting, each plant was top-dressed with ≈9 g of the controlled-release fertilizer Osmocote® (15N-39.6P-99.6K, 12-14 months, Southern formulation; Everris™). Irrigation was through drip tapes under the rowcovers in Citra and Quincy and on top of the covers at Fort Pierce. Irrigation was supplied as needed at each site depending on the soil type and weather conditions. At Citra additional liquid fertilizer was applied after subsequent soil test results performed every 8 weeks. Characteristics of the three evaluation sites are described in Table 1. Monthly average maximum and minimum temperatures, total rainfall, and solar radiation for the three evaluation sites and the 10-year average (recorded by Florida Automated Weather Network monitoring stations at each site) are shown in Figure 1.

Each plant was evaluated every 4 weeks, from Week 0 to 24, for landscape performance

with a scale from 1 to 5 where 1 = very poor quality, not acceptable, severe leaf necrosis or chlorosis, poor form; 2 = poor quality, not acceptable, large areas of necrosis or chlorosis, poor form; 3 = acceptable quality, somewhat desirable form and color; 4 = very good quality, very acceptable and desirable color and form; 5 = excellent quality, perfect condition, premium color and form. Flowering was rated on a 1 to 5 scale where 1 = no flowers or buds; 2 = buds but no open flowers; 3 = one to 10 open flowers; 4 = 11 to 20 open flowers; 5 = more than 21 open flowers per plant. Fruiting was rated on a 1 to 5 scale where 1 = more than 50 fruits per plant; 2 = 21 to 50 fruits; 3 = 11 to 20 fruits; 4 = one to 10 fruits; 5 = no fruits.

**Statistical analyses.** Data were analyzed using SAS PROC GLM with mean separation using Duncan's multiple range test at  $P = 0.005$  (SAS Institute, 2004).

## Results and Discussion

**Ploidy levels.** In 2009, a total of 14 tetraploid plants in four different flower colors was obtained. Successful polyploidization treatments were three or four applications of 25 μM oryzalin or one, two, three, or four applications of 50 μM oryzalin every 12 h. The treatment of two applications of 50 μM oryzalin every 12 h is considered optimal as a result of the ease of application. As expected, the flowers on tetraploid plants were larger than the original diploid plants

(Fig. 2). Interestingly, both 'Purple Showers' and 'Snow White' were found to be natural tetraploids as well as some accessions of South American *R. simplex*, previously considered *R. coerulea*. Our observations are that both the latter and 'Snow White' have leaves that are more lanceolate and lighter green than the other *R. simplex* genotypes; they have larger fruit capsules, seeds, and cotyledons; and they are also more susceptible to mite infestations in the greenhouse.

**Hybridizations.** In 2010 a total of 125 4x × 2x hybridizations was performed, and only 69 fruits (55%) were formed and bagged. Moreover, most of the fruits aborted before harvest, and only nine fruits (7%) could be harvested when ripe, indicating the effect of a triploid block. The use of embryo rescue could possibly be explored to obtain more triploid hybrids. A total of 96 seeds were obtained, most of which did not appear plump and normal, and subsequently only three seeds (3%) germinated. Interestingly, ploidy levels of the resulting hybrids were one diploid, one triploid, and one tetraploid (Table 2). This suggests that the diploid was produced by haploid parthenogenesis and that the tetraploid was the result of apomixis in the maternal parent or fertilization by 2n pollen. By pollen staining, we have observed that some genotypes, i.e., 'Chi Chi', produce 2n pollen (data not shown). Additionally, a total of 20 2x × 4x hybridizations was performed. Fruiting success was 70%, and

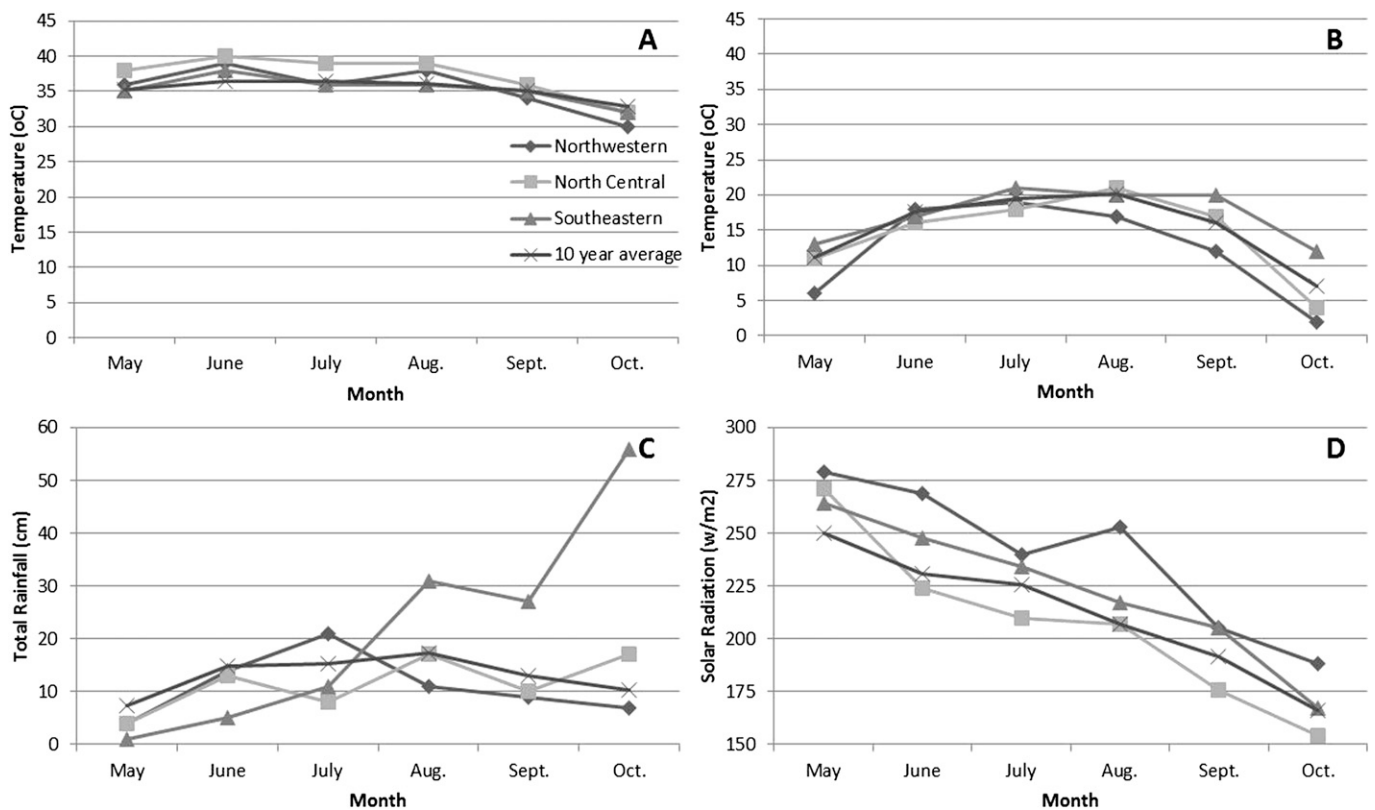


Fig. 1. Monthly average maximum daily temperature (A), minimum temperature (B), total rainfall (C), and daily solar radiation (D) from transplanting date (6 to 11 May 2011) to last evaluation date (22 Oct. 2011) at the northwestern (Quincy), north-central (Citra), and southeastern (Fort Pierce) Florida planting sites and the last 10-year average for the three sites.



most of the fruits could be harvested when ripe (65%). However, although a total of 219 seeds were obtained, most of them appeared shriveled and small and none germinated (Table 2). Some  $4x \times 4x$  hybridizations between genotypes with different morphology were also performed, a few of which were successful (data not shown).

**Selection of plants for field trials.** In 2010 a total of 495 plants obtained either by polyploidization or hybridizations was evaluated under greenhouse conditions for vigor, flowering, and apparent fruitlessness or reduced fruiting. Subsequently, 270 plants were selected for a preliminary spring trial for field performance at Wimauma in Spring 2011 with one replication. Finally, a total of 15

plants were selected for replicated multi-location trials.

The ploidy levels, origin, or parentage of the 15 plants is shown in Table 3. The origin of these plants is varied. Some of the plants are tetraploid, obtained directly from oryzalin treatment. Other tetraploids were derived from  $4x \times 4x$  crosses combining the South American accessions (previously known as *R. coerulea*) by tetraploid *R. simplex* (R10-102, R10-107). There were only two triploid plants: R7-100 was derived from a  $4x \times 2x$  cross performed in 2007, and R10-100 was obtained in 2010, respectively. R10-100 is purple and was obtained from the cross  $4x$  white  $\times$   $2x$  pink indicating complex genetics of flower color. Some plants were  $F_2$  hybrids

(such as R8-100), and some had a more complex parentage (such as R10-108). Tetraploid R10-105 was obtained from a  $4x \times 2x$  cross using diploid 'Chi Chi' as a paternal parent, which either suggests apomixis on the maternal parent or that 'Chi Chi' produces functional  $2n$  pollen. R10-106 was obtained from a white  $4x \times$  pink  $2x$  cross, and it was white rather than the expected pink color, again indicative of apomixis on the maternal parent. The selected plants with white flowers with purple center were obtained directly by selection of McKee  $F_1$  progeny or from seedlings treated with oryzalin.

**Field performance.** All three sites had similar weather patterns and did not deviate significantly from the 10-year average, except for total rainfall at Fort Pierce, which was high during the months of August to October. There were highly significant differences ( $P < 0.0001$ ) for landscape performance, flowering, and fruiting for weeks, sites, plants, and all of the interactions, whereas the differences between blocks were not significant (data not shown). Average landscape performance for all plants was 3.3 at northwestern, 3.5 for north-central, and 3.6 for southeastern Florida (Table 4). This may be the result of different environmental conditions, a longer establishment period needed as a result of initial cooler temperatures, and a more rapid plant decline in the northernmost site as evidenced by the lower ratings from Weeks 16 to 24 (data not shown). Averaging over the three sites, the highest landscape performance rating was on Week 8 (4.0) followed by Week 12 (3.8) and Week 16 (3.5).



Fig. 2. Flower size and color of diploid (left) and tetraploid (right) *Ruellia* purple, pink, white, and white with purple corolla tube (clockwise from top left).

Table 2. Number of  $4x \times 2x$  and  $2x \times 4x$  hybridizations performed with *Ruellia* plants, number of fruits formed and harvested, number of seeds germinated, and number of  $2x$ ,  $3x$  and  $4x$  seedlings obtained.

Female parent ( $4x$ )	Male parent ( $2x$ )	No. hybridizations	No. fruits	No. fruits			No. seedlings obtained		
				harvested	No. seeds	No. seeds germinated	$2x$	$3x$	$4x$
Purple 1	Purple wild <i>R. simplex</i>	10	6	0					
Purple 1	White 3	10	6	0					
Purple 2	Purple wild	5	5	0					
Purple 2	White 3	9	1	0					
Pink 1	'Chi Chi'	5	1	0					
Pink 1	White 3	5	1	0					
Pink 2	'Chi Chi'	5	2	1	17	0			
Pink 2	White 3	5	0	0					
White/purple 1	White/purple	10	6	0					
White/purple 1	White 3	5	0	0					
White/purple 2	White/purple	8	4	0					
White/purple 2	White 3	5	5	1	3	0			
White 1	Purple wild <i>R. simplex</i>	5	2	0					
White 1	'Chi Chi'	5	4	1	14				
White 1	White/purple	5	3	0					
White 1	White 3	5	4	0					
White 2	Purple wild <i>R. simplex</i>	5	4	0					
White 2	'Chi Chi'	5	5	2	29	2			
White 2	White/purple	5	5	2	12	0	1	1	0
White 2	White 3	5	5	2	21	1	0	0	1
Total		125	69 (55%)	9 (7%)	96	3 (3%)			

Female parent ( $2x$ )	Male parent ( $4x$ )	No. hybridizations	No. fruits	No. fruits			No. seedlings obtained		
				harvested	No. seeds	No. seeds germinated	$2x$	$3x$	$4x$
Purple wild <i>R. simplex</i>	Purple 1	5	2	2	35	0			
'Chi Chi'	Pink 2	5	4	4	73	0			
White/purple	White/purple 1	5	4	3	43	0			
White 3	White 2	5	4	4	68	0			
Total		20	14 (70%)	13 (65%)	219	0			

Table 3. Characteristics and origin of 15 selected *Ruellia* plants and five controls included in the 2011 summer field trial at northwestern, north-central, and southeastern Florida.

Plant	Flower color	Ploidy	Maternal parent	Paternal parent	Origin/notes
Purple wild <i>R. simplex</i>	Purple	2x	Unknown	Unknown	Collected from naturalized populations in Fort Pierce, FL
'Purple Showers'	Purple	4x	Unknown	Unknown	Commercial cultivar, Riverview Farms, Seffner, FL
R7-100	Purple	3x	Purple South American <i>R. simplex</i> (4x)	Purple wild <i>R. simplex</i>	F <sub>1</sub> line bred in 2007
R10-100	Purple	3x	White oryzalin-treated <i>R. simplex</i> (4x)	'Chi Chi'	F <sub>1</sub> line bred in 2010
R10-101	Purple	4x	Pink oryzalin-treated <i>R. simplex</i> (4x)	White F <sub>2</sub> breeding line (4x)	F <sub>1</sub> line bred in 2010
R10-102	Purple	4x	Purple South American <i>R. simplex</i> (4x)	Pink oryzalin-treated <i>R. simplex</i> (4x)	F <sub>1</sub> line bred in 2010
'Chi Chi'	Pink	2x	Unknown	Unknown	Commercial cultivar, Grandiflora, Gainesville, FL
RU3-100	Pink	2x	Pink 'Chi Chi' self		2008 selection
R10-103	Pink	4x	Pink 'Chi Chi' self		Oryzalin-treated
R10-104	Pink	4x	Pink 'Chi Chi' self		Oryzalin-treated
R10-105	Pink	4x	Pink oryzalin-treated <i>R. simplex</i> (4x)	'Chi Chi'	F <sub>1</sub> line bred in 2010
'Snow White'	White	4x	Unknown	Unknown	Commercial cultivar, Grandiflora, Gainesville, FL
R8-100	White	2x	Purple wild <i>R. simplex</i>	'Katie White'	F <sub>2</sub> hybrid bred in 2008
R10-106	White	4x	White ('Snow White' × purple wild <i>R. simplex</i> ) F <sub>2</sub>	'Chi Chi'	F <sub>2</sub> hybrid bred in 2010
R10-107	White	8x	White ('Snow White' × purple South American <i>R. simplex</i> ) F <sub>1</sub> , oryzalin-treated	Pink oryzalin-treated <i>R. simplex</i> (4x)	F <sub>1</sub> hybrid bred in 2010
R10-108	White	4x	White (purple wild <i>R. simplex</i> × 'Katie White') F <sub>2</sub> , oryzalin-treated (4x)	White ('Snow White' × purple South American <i>R. simplex</i> ) F <sub>2</sub>	F <sub>2</sub> hybrid bred in 2010
McKee	White/purple center	2x	Unknown	Unknown	Collected at McKee Botanical Garden, Vero Beach, FL
R9-105	White/purple center	2x	McKee self		2008 selection
R10-110	White/purple center	4x	McKee self		Oryzalin-treated
R10-111	White/purple center	4x	McKee self		Oryzalin-treated

Table 4. Average landscape performance for 15 *Ruellia* selected plants and five controls in the 2011 summer field trial at three field sites in Florida.

Plant	Location			Overall ± SD
	Northwestern	North-central	Southeastern	
Wild <i>R. simplex</i>	3.6 <sup>c</sup>	3.7 cd	3.6 ef	3.6 ± 0.3
'Purple Showers'	3.3 d	3.7 cd	3.3 g	3.4 ± 0.3
R7-100	3.7 bc	3.1 fg	4.4 bc	3.7 ± 0.3
R10-100	2.8 efg	2.9 fg	3.1 gh	3.0 ± 0.3
R10-101	4.1 a	4.3 a	4.3 c	4.2 ± 0.3
R10-102	4.0 ab	4.4 a	4.5 ab	4.3 ± 0.3
'Chi Chi'	3.6 c	3.9 bc	3.6 ef	3.7 ± 0.3
RU3-100	2.9 efg	3.5 de	3.9 de	3.4 ± 0.3
R10-103	3.7 bc	4.2 ab	3.6 f	3.8 ± 0.3
R10-104	4.0 ab	4.2 ab	3.9 d	4.0 ± 0.3
R10-105	3.8 abc	4.4 a	3.8 def	4.0 ± 0.3
'Snow White'	2.9 defg	3.3 def	—	3.1 ± 0.3
R8-100	2.4 h	2.4 h	2.0 i	2.3 ± 0.3
R10-106	3.2 de	3.0 fg	3.3 g	3.2 ± 0.3
R10-107	3.1 def	3.0 fg	3.3 g	3.1 ± 0.3
R10-108	4.2 a	4.3 a	4.6 a	4.4 ± 0.3
McKee	2.7 fg	2.3 hi	—	2.5 ± 0.3
R9-105	2.9 efg	2.0 i	3.2 gh	3.0 ± 0.3
R10-110	2.6 gh	3.0 fg	2.9 h	2.9 ± 0.3
R10-111	2.8 fg	3.2 efg	3.1 gh	3.0 ± 0.3
Overall ± SD	3.3 ± 0.3	3.5 ± 0.3	3.6 ± 0.3	

<sup>a</sup>Scale from 1 to 5 where 1 = very poor quality, not acceptable, severe leaf necrosis or chlorosis, poor form; 2 = poor quality, not acceptable, large areas of necrosis or chlorosis, poor form; 3 = acceptable quality, somewhat desirable form and color; 4 = very good quality, very acceptable and desirable color and form; 5 = excellent quality, perfect condition, premium color and form.

yLetters represent Duncan's multiple range test for mean comparisons by site at the *P* = 0.05 level.

By Weeks 20 and 24, some tall plants were lodging, had disease issues or less flowering, and more fruits, so ratings declined. The average and SD for landscape performance for the 15 plants and five controls at the three sites over the course of 24 weeks is shown in Table 4. For plants with purple flowers, R10-102 and R10-101 performed better than 'Purple Showers' and the purple wild *R. simplex*. Pinks R10-103, R10-104, and R10-105 all had better performance than 'Chi Chi'. White R10-108 had excellent performance at all sites, significantly better than 'Snow White'. All the plants with white corolla with purple center had low performances, although R9-105 and R10-111 were better than McKee.

Average flowering was similar at all sites, northwestern (3.2), north-central (3.3), and southeastern FL (3.5) (Table 5). Averaging over the three sites, flower ratings were highest on Weeks 12 and 16 (4.2) and then declined by Week 24 (3.2). For plants with purple flowers, higher flowering was seen for R7-100, 'Purple Showers', R10-102, and R10-101 (4.1, 3.9, 3.9, and 3.8, respectively). Interestingly, for triploid R7-100, this did not translate into a high landscape performance

as well (3.7). Of the pinks, diploid RU3-100 had the best flowering (3.8) followed by 'Chi Chi' and R10-105 (3.3 and 3.1, respectively). White R10-108 had excellent flowering (4.0), whereas all the plants with white corolla with a purple center were medium ( $\approx 3$ ).

Average fruiting was similar at all three sites, ranging from 3.9 to 4.2 (Table 6). For this trait the highest rating of 5 is for no fruits, which is considered the most desirable selection criteria. When transplanted, none of the plants had fruits; thus, the average fruit rating was 5, but by Week 4, some plants already had fruited and the rating declined to 4.5. Plants had more fruits and consequently

decreasing fruit ratings as the weeks progressed (3.3 on Week 24). All the selected purple plants were basically fruitless, comparable to 'Purple Showers' and significantly better than the invasive purple wild *R. simplex*. All the selected pink plants had significantly less fruiting than 'Chi Chi'; however, their fruiting ranged between 3.6 to 4.6. White R8-100 and R10-108 were fruitless, whereas 'Snow White' had an average fruiting of 3.3. All the selected plants with white corolla with a purple center had fruiting ranging from 3 to 4.1.

Overall, two selected plants were outstanding and have potential as new, tetraploid cultivars: purple-flowered R10-102 and white R10-108. They had better landscape

performance and flowering than the currently existing commercial cultivars Purple Showers and Snow White, respectively. These two plants combine *R. simplex* germplasm from South America (previously known as *R. coerulea*) and North America (previously known as *R. tweediana*) and have intermediate morphology between the two types. The different morphology and ploidy levels of these two types (2x in *R. tweediana* and 4x in *R. coerulea*) argue in favor of their identification as different species rather than the use of the all-encompassing *R. simplex* name.

Both R10-102 and R10-108 are fruitless, so they pose no threat of invasiveness by seed dispersal, and they are also male-sterile so will not pollinate other *Ruellia* plants. This is most likely the result of hybrid sterility when the two genomes from *R. coerulea* and *R. tweediana* are combined. Sterility in wide hybrids may be the result of lack of chromosome homology resulting in poor or no meiotic chromosome associations between genomes or it may be genic in nature (Stebbins, 1950). Because both R10-102 and R10-108 can be considered allotetraploids having two gene copies from each genome, their meiotic pairing should be normal. This suggests that the cause of the sterility is genic as described by Dobzhansky (1936) and as a result of incompatibility between genetic factors contributed by the two parents. This sterility barrier in the hybrids is further evidence that the former taxonomy, which separated *R. coerulea* and *R. tweediana* into different taxons, is more representative of the genetic relationship between the two species.

Table 5. Average flowering for 15 *Ruellia* selected plants and five controls in the 2011 summer field trial at three field sites in Florida.

Plant	Location			Overall $\pm$ SD
	Northwestern	North-central	Southeastern	
Wild <i>R. simplex</i>	3.5 <sup>z</sup> cdef <sup>y</sup>	3.2 cde	3.2 cd	3.3 $\pm$ 0.3
'Purple Showers'	3.9 bc	4.0 a	4.0 a	3.9 $\pm$ 0.3
R7-100	4.3 a	3.9 ab	4.2 a	4.1 $\pm$ 0.3
R10-100	3.3 defg	3.5 abc	3.8 ab	3.5 $\pm$ 0.3
R10-101	3.6 cde	3.8 ab	4.0 a	3.8 $\pm$ 0.3
R10-102	3.8 bc	4.0 a	4.0 a	3.9 $\pm$ 0.3
'Chi Chi'	3.2 efgh	3.4 bc	3.4 bcd	3.3 $\pm$ 0.3
RU3-100	3.6 cd	3.9 ab	4.1 a	3.8 $\pm$ 0.3
R10-103	2.6 jk	2.7 efg	3.1 de	2.8 $\pm$ 0.3
R10-104	2.5 k	2.7 efg	3.2 cd	2.8 $\pm$ 0.3
R10-105	3.0 ghij	3.2 cd	3.2 cd	3.1 $\pm$ 0.3
'Snow White'	3.0 ghij	2.8 defg	—	2.9 $\pm$ 0.3
R8-100	2.9 hijk	2.8 defg	2.6 e	2.8 $\pm$ 0.3
R10-106	3.4 def	3.1 cdef	3.3 cd	3.3 $\pm$ 0.3
R10-107	2.7 ejk	2.4 g	2.6 e	2.6 $\pm$ 0.3
R10-108	4.1 ab	3.8 ab	4.1 a	4.0 $\pm$ 0.3
McKee	3.1 fghi	2.6 fg	—	2.9 $\pm$ 0.3
R9-105	3.2 efgh	3.0 cdef	3.7 abc	3.4 $\pm$ 0.3
R10-110	2.8 hijk	2.6 fg	3.0 de	2.8 $\pm$ 0.3
R10-111	2.9 hijk	3.7 defg	3.0 de	2.9 $\pm$ 0.3
Overall $\pm$ SD	3.2 $\pm$ 0.3	3.3 $\pm$ 0.3	3.5 $\pm$ 0.3	

<sup>z</sup>Scale of 1 to 5 where 1 = no flowers or buds; 2 = buds but no open flowers; 3 = one to 10 open flowers; 4 = 11 to 20 open flowers; 5 = more than 20 open flowers per plant.

<sup>y</sup>Letters represent Duncan's multiple range test for mean comparisons by site at the  $P = 0.05$  level.

Table 6. Average fruiting for 15 *Ruellia* selected plants and five controls in the 2011 summer field trial at three field sites in Florida.

Plant	Location			Overall $\pm$ SD
	Northwestern	North-central	Southeastern	
Wild <i>R. simplex</i>	2.5 <sup>z</sup> f <sup>y</sup>	2.4 g	3.2 f	2.3 $\pm$ 0.2
'Purple Showers'	4.8 a	5.0 a	5.0 a	4.9 $\pm$ 0.2
R7-100	4.9 a	4.9 ab	5.0 a	4.9 $\pm$ 0.2
R10-100	4.9 a	5.0 a	5.0 a	5.0 $\pm$ 0.2
R10-101	4.9 a	5.0 a	5.0 a	5.0 $\pm$ 0.2
R10-102	4.9 a	5.0 a	5.0 a	5.0 $\pm$ 0.2
'Chi Chi'	2.6 f	2.7 g	2.0 f	2.4 $\pm$ 0.2
RU3-100	4.5 ab	4.6 abc	3.6 d	4.2 $\pm$ 0.2
R10-103	3.8 d	4.2 de	4.0 cd	4.0 $\pm$ 0.2
R10-104	3.8 d	4.3 cde	3.7 d	3.9 $\pm$ 0.2
R10-105	4.3 bc	4.6 bcd	4.2 bc	4.4 $\pm$ 0.2
'Snow White'	3.2 e	3.5 f	—	3.3 $\pm$ 0.2
R8-100	4.9 a	5.0 a	5.0 a	5.0 $\pm$ 0.2
R10-106	2.6 f	3.2 f	2.3 f	2.7 $\pm$ 0.2
R10-107	4.7 a	4.9 ab	4.4 b	4.7 $\pm$ 0.2
R10-108	4.8 a	5.0 a	5.0 a	5.0 $\pm$ 0.2
McKee	3.1 e	3.4 f	—	3.2 $\pm$ 0.2
R9-105	4.1 cd	3.4 f	3.6 d	3.8 $\pm$ 0.2
R10-110	3.7 d	3.9 e	3.3 e	3.6 $\pm$ 0.2
R10-111	3.8 d	4.1 e	3.0 e	3.6 $\pm$ 0.2
Overall $\pm$ SD	4.0 $\pm$ 0.2	4.2 $\pm$ 0.2	3.9 $\pm$ 0.2	

<sup>z</sup>Scale of 1 to 5 where 1 = more than 50 fruits; 2 = 21 to 50 fruits; 3 = 11 to 20 fruits; 4 = one to 10 fruits; 5 = no fruits per plant.

<sup>y</sup>Letters represent Duncan's multiple range test for mean comparisons by site at the  $P = 0.05$  level.

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