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Fruitless *Ruellia simplex* R10-102 ('Mayan Purple') and R10-108 ('Mayan White')

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Ruellia L. consists of ≈250 species of perennial herbs, subshrubs, and shrubs with mostly tropical and subtropical distribution and is one of the largest genera in the Acanthaceae (Ezcurra, 1983). *Ruellia simplex* Wright (commonly known as mexican petunia, mexican bluebell, or Britton's petunia) has low maintenance requirements and prolific flowering and has become a very popular landscape plant in the southern United States (Gilman, 1999) since its introduction to Florida sometime before 1940 (Hupp et al., 2009). There are many synonyms for *R. simplex* (*R. brittoniana* Leonard, *R. coerulea* Morong, *R. malacosperma* Greenm., and *R. tweediana* Griseb.) with the name *R. simplex* being the first documented, thus having taxonomic priority (Ezcurra and Daniel, 2007). A meiotic complement of $n = 17$ appears to be widespread in *Ruellia* (Daniel et al., 1984). *Ruellia simplex* (as *R. tweediana*) has also been reported as $2n = 34$ (Piovano and Bernadello, 1991).

Ruellia simplex has naturalized in disturbed uplands and wetlands of six southern U.S. states (from South Carolina west to Texas) plus the U.S. Virgin Islands, Puerto Rico, and Hawaii (Kartesz, 2012; U.S. Department of Agriculture, Natural Resources Conservation Service, 2012). Since 2001, the Florida Exotic Pest Plant Council 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" (Florida Exotic Pest Plant Council, 2011). The Institute for Food and Agricultural Science (IFAS) Assessment of the Status of Non-Native Plants in Florida's Natural Areas does not recommend use of mexican petunia in the central and southern parts of Florida, and in northern Florida, its specified use needs to be approved (IFAS, 2011).

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 clonally propagated, except for the 'Southern Star' series, which is propagated by seed (PanAmerican Seed Co., Chicago, IL). With the exception of 'Purple Showers', which does not set fruit by open pollination (Wilson and Mecca, 2003), all known cultivars set fruit and are potentially invasive. Sales of 'Purple Showers' in Florida were ranked third for herbaceous perennials after pentas and lantana (Ornamental Outlook, 2009). A survey conducted in 2002 including 946 active nurseries in Florida indicated that 15.9% of them grew or sold *R. simplex*, and total reported annual sales for this plant was estimated at ≈$12 million (Wirth et al., 2004).

Since 2007, we have developed the first *Ruellia* breeding program at the University of Florida (UF)/IFAS in Gainesville, FL, directed specifically toward creating new sterile cultivars for the landscape plant industry. To reduce the spread of *R. simplex* by seed, female sterility (and fruitlessness) or lack of seed viability is desired. The breeding lines of *Ruellia* described below were obtained by a combination of polyploidization using oryzalin and hybridization. They were selected in multilocation trials in Florida from a group of 15 different clones for superior landscape performance as compared with commercial cultivars and no fruiting (Freyre et al., 2012).

Origin

R10-102. This clone is a tetraploid seedling obtained at UF in Gainesville, FL, from a cross of RU36 × 26-1 made in Sept. 2010. RU36 is a purple-flowered, tetraploid South American accession of *R. simplex*. 26-1 is a pink-flowering tetraploid plant, obtained as a vegetative propagule from RU3-26, which is a diploid–tetraploid chimeric individual obtained by treating the apical meristem of a seedling of *R. simplex* 'Chi Chi' with three applications, every 12 h, of a 50-μM oryzalin solution in Dec. 2008. The ploidy levels of the breeding lines and species were determined by flow cytometrical analysis (Partec I, Germany) performed at UF Mid-Florida Research and Education Center, Apopka, FL, using wild *R. simplex* (the naturalized and cultivated, non-improved form of the species) as a diploid control.

R10-108. This clone is a tetraploid individual obtained in 2010 at UF in Gainesville, FL, from a cross of 69-1 × RU64 made in Sept. 2010. 69-1 is a tetraploid plant, obtained as a vegetative propagule from RU63-69. RU63-69 is a tetraploid white-flowered individual obtained by treating the apical meristem of a seedling of RU63 with three applications, every 12 h, of a 25-μM oryzalin solution in Dec. 2008. RU63 is a tall, diploid white-flowered plant obtained in 2008 as an F_2 from the cross of diploid purple-flowered wild *R. simplex* × diploid dwarf 'Katie White'. RU64 is a tetraploid white-flowered F_2 individual obtained from a cross between 'Snow White' × RUE1-1, a purple-flowered tetraploid South American accession of *R. simplex*.

Description

To botanically characterize each breeding line, plants were propagated by cuttings and grown in a research greenhouse in Gainesville, FL. Plants were ≈20 weeks of age when description of color for plant parts was determined based on comparison with the Royal Horticultural Society Color Chart (1995). Measurements for plant height and width, flower corolla diameter, and leaf length and width were taken on plants that were 20 to 22 weeks in age on a total of 27 plants per breeding line or cultivar (three plants × three

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blocks × three sites) grown in Florida in 2011 and then averaged.

R10-102. This cultivar has an upright clumping and ascending growth habit (Fig. 1). Average plant height was 96 cm, whereas wild *R. simplex* was 67 cm high, and 'Purple Showers' was 102 cm. Average plant width was 100 cm, also significantly different from wild *R. simplex* at 61 cm and 'Purple Showers' at 92 cm. Stems are green (RHS 143A) and in between round and square. Stems can become woody near the base of mature plants, and rhizomes may form where conditions are favorable and resources are not limited. Nodes can exhibit swelling and are typically gray–purple (RHS 183B), but color can vary based on light exposure and fertility. The nodes and the midrib to approximately one-fourth up the leaf on the abaxial and adaxial surfaces are slightly tomentose. The leaves are oppositely attached and are linear to lanceolate with an entire margin, narrowly acute apex, and an attenuate base. Length of mature leaves ranges from 10.6 to 12.8 cm and leaf width from 1.6 to 2.0 cm. Comparatively, the leaf length for wild *R. simplex* ranges from 11 to 12.9 and leaf width from 0.7 to 0.8 cm, and in 'Purple Showers', leaf length ranges from 12 to 13.3 cm and width from 1.4 to 1.5 cm. In R10-102 leaves are green on the adaxial (RHS 139A) and abaxial (RHS 137C) side of the lamina, respectively. Venation on the abaxial surface of the lamina has the same color as the abaxial leaf surface, is pinnate, and prominently raised. Flowers are actinomorphic and funnel form with five petals, four anthers, and one stigma. The corolla diameter ranges from 5.4 to 6.7 cm. The flowers are pedunculate, complete-perfect. and borne from the axil either solitarily or in a several-flowered cyme. Glandular trichomes cover the sepal surface of unopened and open flowers. Flowers are violet (RHS 87A) with a darker violet (RHS 86A) corolla tube (Fig. 2) and last for 1 d, after which the corolla falls.

R10-108. This cultivar has a similar description as R10-102 with the following exceptions: the stems are all green (RHS 143A) with no purplish color at the nodes (Fig. 3). Average plant height was 88 cm, the same as for 'Snow White'. However, this breeding line had a different plant width at 84 cm compared with 67 cm for 'Snow White'. Length of mature leaves ranged from 9.2 to 13.2 cm and leaf width from 1.4 to 1.8 cm. Comparatively, leaf length for 'Snow White' ranged from 6.7 to 8.8 cm and leaf width from 1.8 to 2.2 cm. The flowers are white (RHS 155 C) (Fig. 4) and the corolla diameter ranges from 5.5 to 6.7 cm, whereas in 'Snow White', it ranges from 5.2 to 6 cm.

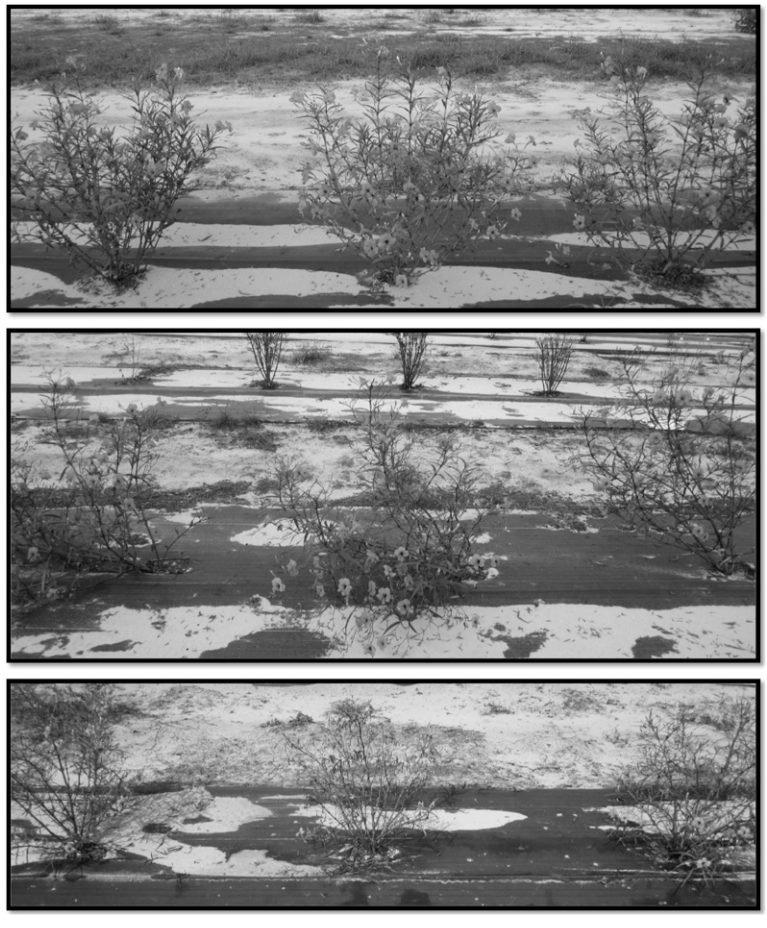

Fig. 1. Plots of three plants of R10-102, 'Purple Showers', and wild *R. simplex* (top, middle and bottom, respectively) on 29 Aug. 2011 (Week 16) at Citra, FL.

Multisite Replicated Evaluations of Plant Performance and Fertility

Plants were trialed in three simultaneous field experiments conducted at Quincy (northwestern Florida, lat. 30.5° N, long. 84.6° W, AHS heat zone 9, USDA hardiness zone 8b), at Citra (north–central Florida, lat. 29.4° N, long. 82.2° W, AHS heat zone 10, USDA hardiness zone 9a), and at Ft. Pierce (southeast Florida, lat. 27.4° N, long. 80.4° W, AHS heat zone 9 to 10, USDA hardiness zone 10a) (American Horticultural Society, 1998; U.S. Department of Agriculture, 2011). The experimental design was a randomized complete block with three blocks. Each plot consisted of three plants for each cultivar spaced 50 cm apart. At each experimental site, wild *R. simplex* (diploid) and 'Purple Showers' (tetraploid) were included as purple-flowered comparison lines. Because of field space limitations, 'Snow White' (tetraploid) was included as a white-flowered comparison line only in the two northern sites.

Details on plant propagation and installation at each site have been described elsewhere (Freyre et al., 2012). Briefly, 27 cuttings for each plant were clonally propagated, rooted in 2 weeks, and then transplanted into 10-cm Ellepots (Blackmore Co. Inc., Belleville, MI). They were moved to an open-sided greenhouse for hardening for 4 weeks, during which they were fertilized with 150 ppm nitrogen with Peters liquid fertilizer at each irrigation (20N–4.4P–166K; Everris™, Charleston, SC). When plants were 5 weeks old, they were distributed to each site and then transplanted to ground beds in full sun within 1 week. At each site, rows were formed and covered with black woven nursery groundcover (L & M Supply Co., Willacoochee, GA). Transplanting was completed between 6 and 10 May 2011. 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 tape under the rowcovers in the two northern sites and on top of the covers at the southeastern site. Irrigation was supplied as needed at each site depending on the soil type and weather conditions. In the north–central site, it was possible to use additional liquid fertilizer, which was applied after soil test results performed every 8 weeks (Mehlich-III/H_2O extraction; QAL, Panama City, FL).

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

Fig. 2. Flowers on *Ruellia* hybrid R10-102.

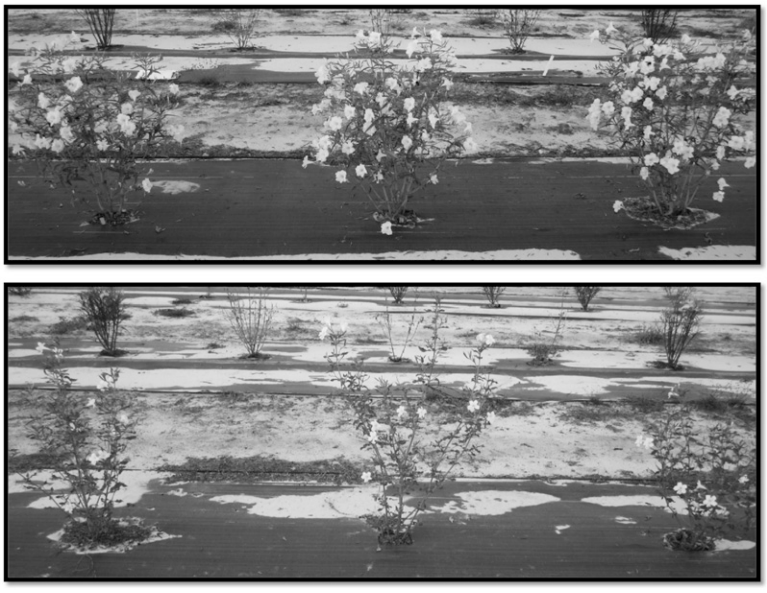

Fig. 3. Plots of three plants of R10-108 and *R. simplex* 'Snow White' (top and bottom, respectively) on 29 Aug. 2011 (Week 16) at Citra, FL.

Fig. 4. Flowers on *Ruellia* hybrid R10-108.

but no open flowers; 3 = one to 10 open flowers; 4 = 11 to 20 open flowers; 5 = more than 20 open flowers. Fruiting was rated on a 1 to 5 scale where 1 = more than 50 fruits; 2 = 21 to 50 fruits; 3 = 11 to 20 fruits; 4 = one to 10 fruits; 5 = no fruits. At peak flowering on Week 16, the flower corolla diameter and the plant height and average width (average of widths taken north–south and east–west) were measured for each plant at each site. Plants that were leaning were propped up to get a full height measurement. Data were analyzed using SAS PROC GLM with mean separation using Duncan's multiple range test at $P = 0.005$ (SAS Institute, 2004).

Vegetative measurements. There were significant differences ($P < 0.005$) for average plant height, plant, width and flower size between sites, lines, and the interaction of site*line, whereas the differences between blocks were not significant. Both height and width were similar at Citra (north–central) and Quincy (northwestern) and larger than at Ft. Pierce (southeastern). For purple-flowered plants, the average height at Citra and Quincy was 94 and 93 cm, respectively, whereas at Ft. Pierce, it was 80 cm. At the three sites, 'Purple Showers' and R10-102 had similar heights and were taller than wild *R. simplex* (Fig. 5A). Average plant width was different at the three sites, at 96 cm at Citra, 87 cm at Quincy, and 73 cm at Ft. Pierce. At Citra and Quincy, R10-102 and 'Purple Showers' were wider than wild *R. simplex*, and at Ft. Pierce R10-102 was wider than 'Purple Showers' and wild *R. simplex* (Fig. 5B).

For white-flowered plants, the average height was 92 at Quincy, and plants were shorter at both Citra and Ft. Pierce at 87 and 86 cm, respectively. Plant width was 82 cm at both Ft. Pierce and Quincy compared with Citra at 70 cm. 'Snow White' and R10-108 had similar heights at both northern sites averaging 88 to 89 cm. However, R10-108 had a considerably wider growth habit and denser foliage than 'Snow White', which made it more attractive. At Citra the average width for R10-108 was 78 cm compared with 62 cm for 'Snow White'; at Ft. Pierce, it was 82 cm, whereas at Quincy, it was 94 for R10-108 and 73 cm for 'Snow White', respectively. By the end of the season, both 'Purple Showers' and 'Snow Showers' were prone to lodging.

There were also significant differences among sites, lines, and the location*line interaction for flower corolla diameter, but not between blocks. On average for all genotypes, flower corolla sizes were larger at Citra at 5.9 cm followed by Ft. Pierce and Quincy at 5.7 cm and 5 cm, respectively. Across the three sites, R10-102, R10-108, and 'Purple Showers' had larger flowers than wild *R. simplex* and 'Snow White'. At Ft. Pierce and Quincy, R10-102, R10-108, and 'Purple Showers' had flowers of similar size and larger than the flowers of wild *R. simplex* (Fig. 5C). This is not surprising because flowers of tetraploid plants are usually larger than those of diploid plants. At Citra, the flowers of R10-102 and R10-108 were of similar size and larger than those of 'Purple Showers', wild *R. simplex*, and 'Snow White'.

Performance. For all variables, there were significant differences among weeks, sites, lines, and all interactions, but not for blocks. Average landscape performance for the five lines at Citra and Ft. Pierce was 4.1, significantly different from the average rating at Quincy at 3.9. This may be because of different environmental conditions, a longer establishment period needed because of initial cooler temperatures, and a more rapid plant decline in Quincy, which was the most northern site. R10-102 had a higher landscape performance rating than 'Purple Showers' at all sites and than wild *R. simplex* at Citra and Ft. Pierce (Fig. 5D). White-flowered R10-108 had a higher landscape performance rating

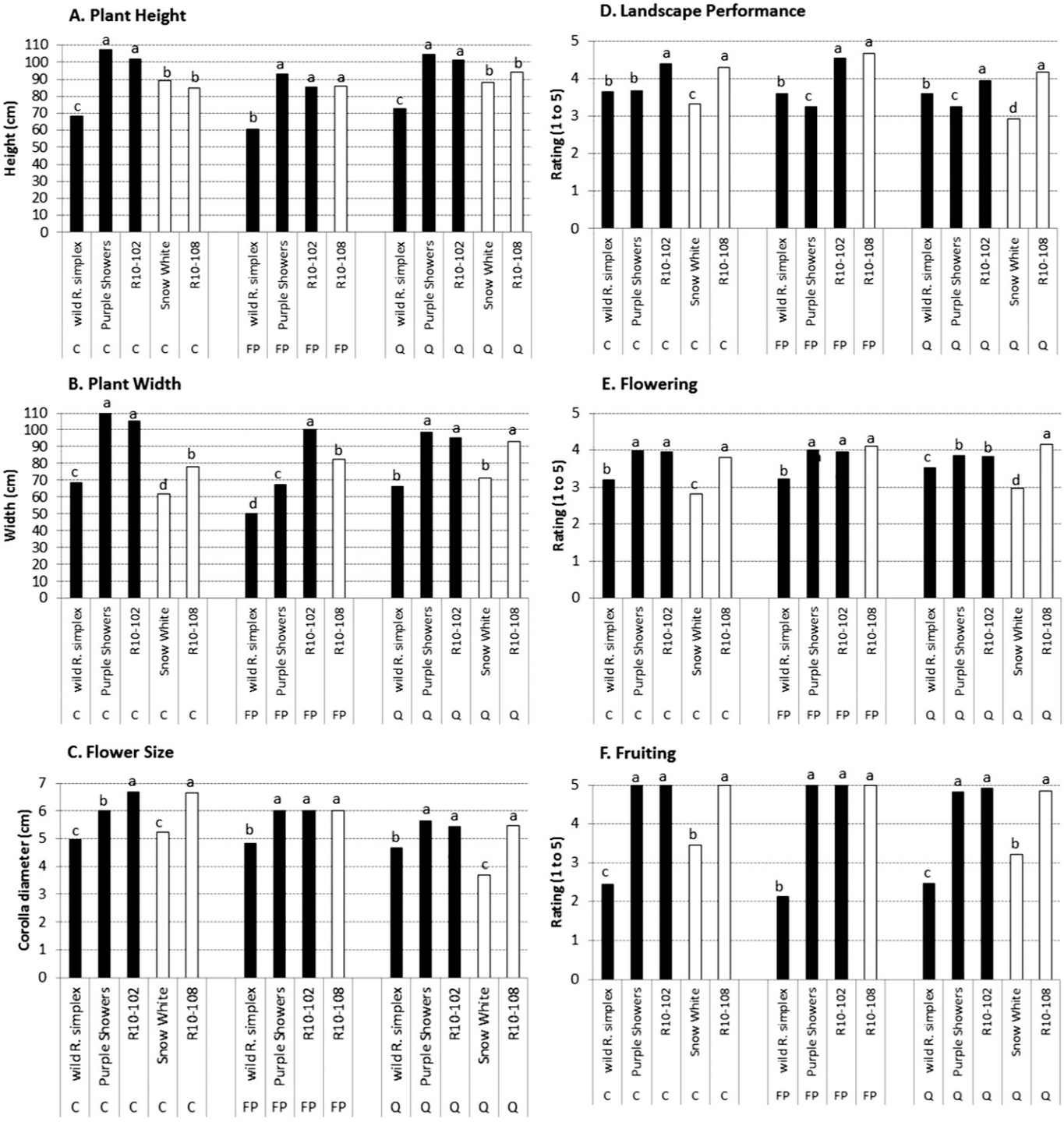

Fig. 5. (**A**) Plant height at Week 16 for wild *R. simplex*, three *Ruellia* cultivars, R10-102, and R10-108 at Citra (C, north–central) and Quincy (Q, northwestern). 'Snow White' is missing in Ft. Pierce (FP, southeastern). Each site is analyzed separately. For each site, the same letter over a bar indicates that the values are not significantly different ($P < 0.005$). Black bars are for purple-flowered plants and white for white-flowered plants. (**B**) Plant width at Week 16 for five *Ruellia* genotypes at three sites. (**C**) Flower size at Week 16 for five *Ruellia* genotypes at three sites. (**D**) Landscape performance averaged over 24 weeks for five *Ruellia* genotypes at three sites. Ratings range from 1 = poor landscape performance to 5 = excellent landscape performance. (**E**) Flowering averaged over 24 weeks for six *Ruellia* genotypes at three sites. Ratings range from 1 = no flowers to 5 = more than 20 flowers. (**F**) Fruiting averaged over 24 weeks for six *Ruellia* genotypes at three sites. Ratings range from 1 = more than 50 fruits to 5 = no fruits.

than 'Snow White' at Citra and Quincy. R10-108 had the highest rating of all lines evaluated at Ft. Pierce and Quincy, and at Citra, it was comparable to the best purple lines.

Flower ratings were similar in Ft. Pierce and Citra at 3.4 and 3.3, respectively, which was significantly different from Quincy at 3.1. This was probably because of a later start in flowering and a more rapid plant decline at Quincy. At Ft. Pierce and Citra flowering ratings were higher for 'Purple Showers', R10-102, and R10-108, whereas in Quincy, R10-108 had a higher rating than the other two genotypes (Fig. 5E). Both wild *R. simplex* and 'Snow White' had significantly less flowers.

There were no differences between sites for fruiting per plant with an average rating of 4.3 for Citra, 4.2 for Quincy, and 4.0 for Ft. Pierce. Wild *R. simplex* had an average fruiting rating of 3.2 and 'Snow White' 3.7 (Fig. 5F). Meanwhile 'Purple Showers' had an average rating of 4.8, and R10-108 and R10-102 both had 4.9. Although some

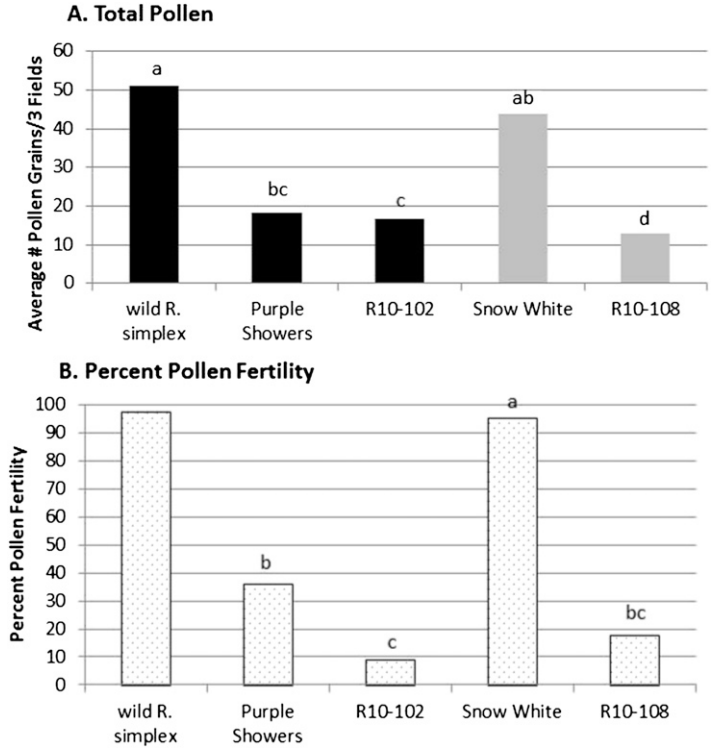

Fig. 6. (**A**) Total pollen grains counted for wild *R. simplex*, 'Purple Showers', 'Snow White', R10-102, and R10-108. Bars represent the average number of pollen grains for nine microscope fields at 10× from three flower samples. Purple-flowered lines are in black bars and white-flowered lines are in gray bars. (**B**) Percent of pollen grains considered fertile (stained normally) averaged for nine microscope fields at 10× from three flower samples for five *Ruellia* genotypes.

fruiting was recorded for these three clones, it was noted that the fruits aborted before maturity.

Female Fertility

Open-pollinated seed. Ten fruit per block (total 30 fruit, if available) for wild *R. simplex*, each control cultivar and hybrid, were enclosed with nylon media filter bags at each site (Aquatic Eco-systems, Inc., Apopka, FL). Capsules either dehisced inside the bags or mature fruit were removed from each plant and allowed to dehisce at room temperature. Cleaned seeds were gravity air-dried at 22 °C for 48 to 72 h before analysis. Total seeds per fruit were counted, and visually immature or damaged seeds (flattened or puckered seed, small, green or white instead of light to dark brown in color) were separated.

Seed germination for open-pollinated seed was performed at Ft. Pierce, FL, in Dec. 2011, following optimal germination conditions for *Ruellia* seeds (Wilson and Mecca, 2003). If produced, 100 normal seeds per line were germinated using two replications of 50 seeds. Seeds were placed in 10.9 × 10.9-cm transparent polystyrene germination boxes (Hoffman Manufacturing, Inc., Albany, OR) containing two sheets of germination paper (Hoffman Manufacturing Inc.) moistened with 15 mL deionized water. Germination boxes were placed in temperature- and light-controlled chambers equipped with cool-white fluorescent lamps (Model 818; Precision Scientific, Winchester, VA). Temperatures and photoperiod were administered by providing 12 h light at 30 °C (photosynthetic photon flux was 22 to 30 $mmol \cdot m^{-2} \cdot s^{-1}$ at shelf level) followed by 12 h dark at 20 °C, respectively. Germination of seed was monitored every 2 to 3 d for a period of 14 d. An additional 5 to 10 mL of deionized water was added to germination boxes as needed. A seed was considered germinated when radicle emergence was 2.0 mm or greater. Final germination percentage was determined per germination box, then the average final germination percentage (FGP) and SD were calculated.

Total number of fruits, normal seeds collected, and FGP (average and SD) for each site are shown (Table 1). The total number of seeds and germination percentages for all lines were lower at Ft. Pierce than at the other two sites, which was probably because of higher than average rainfall at Ft. Pierce during the harvesting period (Freyre et al., 2012). No mature fruits were found on 'Purple Showers' or on the breeding lines R10-102 and R10-108. On wild *R. simplex* 94% to 100% seeds were found to be normal; there were 11 to 15 normal seeds per fruit, and FGP ranged from 72% to 95%. On 'Snow White' between 92% and 94% of the seeds were considered normal; there were between 13 and 16 normal seeds per fruit, and their FGP was 94%.

Seed obtained from manual hybridizations. Female fertility was also evaluated by using diploid wild *R. simplex*, pink diploid cultivar Chi Chi, tetraploid cultivars Purple Showers and Snow White, and the hybrids R10-102 and R10-108 in a crossing block including self-pollinations. Plants were maintained in a greenhouse at UF, Gainesville, FL. Five manual hybridizations were performed as previously described (Freyre et al., 2012) for each cross combination. Fruiting percentage (FP) was determined per cultivar or breeding line. Total seeds were counted, and immature or damaged seeds were counted and separated. Normal seeds were sown in Dec. 2011, ≈1 to 2 cm deep in 20-row seeder trays (Landmark Plastics; 1331 Kelly, Akron, OH) using prewetted Fafard 2P mix. Seed trays were placed in a polycarbonate mist house (30% light irradiance) and received misting from 0800 HR to 1800 HR (5 s/30 min). Temperature was maintained between 18 and 24 °C. Data were collected every 2 to 3 d for a period of 14 d. A seed was considered germinated when the hypocotyl or cotyledons were visibly cracking the media surface. FGP was determined for each cultivar or hybrid.

FP, total number of seeds obtained, average number of seeds per fruit (AS/F), and FGP for each successful hybridization are shown (Table 2). The highest FP obtained was 60% for wild *R. simplex* (selfed) and for the cross of wild *R. simplex* × 'Chi Chi', which is an intraspecific hybridization at the diploid level. Both of these combinations had an AS/F of ≈20 and FGP of 99% to 100%. When 'Chi Chi' was selfed, the FP was lower at 40%, AS/F of 24, and FGP of 100%. The results of the cross 'Chi Chi' × wild *R. simplex* were almost identical to the previously mentioned reciprocal cross. 'Snow White' had only 20% FP when selfed. The hybridization 'Chi Chi' × 'Snow White' ($2x \times 4x$) resulted in two fruits that were aborted before reaching maturation. The reciprocal cross ($4x \times 2x$) was unsuccessful. The FP was only 20% for the cross wild *R. simplex* × 'Snow Showers' ($2x \times 4x$). This resulted in only one fruit with four seeds, all of which germinated. The majority of the cross combinations were not successful. 'Purple Showers' was not successful either as a female or male parent. Similarly, R10-102 and R10-108 were not successful either as female or male parents in these manual hybridizations, confirming their sterility.

Pollen Stainability

Flowers were collected on 7 Sept. 2011 from greenhouse-grown plants at UF, Gainesville, FL, from wild *R. simplex*, cultivars Chi Chi, Purple Showers, and Snow White and the hybrids R10-102 and R10-108 at 0900 HR, shortly after anthesis to ensure full anther dehiscence and minimal pollen loss. Three flowers for each line were evaluated. The corolla was carefully detached from each flower, and the four anthers were removed and placed in a 0.5-mL Eppendorf tube. Then 20 µL of lactophenol cotton blue was micropipetted per tube to stain the pollen grains for microscopy. The vials containing anthers and

Table 1. Open-pollinated seed obtained from harvesting 30 fruits for wild *R. simplex* and 'Snow White' at three sites.

	Citra		Fort Pierce		Quincy	
	NS/TS[z] (%)	FGP[y] (% ± SD)	NS/TS	FGP (% ± SD)	NS/TS	FGP (% ± SD)
Wild *R. simplex*	405/432 (94%)	93 ± 7	343/343 (100%)	72 ± 3	462/488 (95%)	95 ± 4
'Snow White' mexican petunia	484/513 (94%)	94 ± 6	Not included in the study		375/408 (92%)	94 ± 0
'Purple Showers' mexican petunia	0[x]		0		0	
R10-102	0		0		0	
R10-108	0		0		0	

[z]NS/TS = normal seed/total seed.
[y]FGP = final germination percentage (average and SD) of two replications in the germination trial.
[x]No fruits could be harvested for 'Purple Showers', R10-102, or R10-108.

Table 2. Manual hybridization crossing block with wild *R. simplex*, three *Ruellia* cultivars, and two breeding lines.[z]

Female[z] / Male	Wild *R. simplex*	'Snow White'	'Chi Chi'	'Purple Showers'	R10-102	R10-108
Wild *R. simplex* (2*x*)	FP 60%[y] TS 68 AS/F 23 FGP 99%	FP 20% TS 19 AS/F 19 FGP 0%	FP 40% TS 28 AS/F 15 FGP 97%	0	0	0
'Snow White' (4*x*)	0	FP 20% (and 1 aborted fruit) TS 4 AS/F 4 FGP 100%	1 aborted fruit	0	0	0
'Chi Chi' (2*x*)	FP 60% TS 57 AS/F 19 FGP 100%	2 aborted fruits	FP 40% TS 47 AS/F 24 FGP 100%	0	0	0
'Purple Showers' (4*x*)	0	0	0	0	0	0
R10-102 (4*x*)	0	0	0	0	0	0
R10-108 (4*x*)	0	0	0	0	0	0

[z]Maternal parents are presented in the rows and paternal parents in the columns.
[y]Five manual hybridizations were made for each combination.
FP = fruiting percentage; TS = total seeds; AS/F = average number of seeds per fruit; FGP = final germination percentage.

stain were agitated manually for 10 min. Then the 20 µL of stain containing pollen was micropipetted onto individual microscope slides and spread using coverslips. The slides were examined using a light stereoscope at 10× magnification (Leica DM1000; Leica Inc., Allendale, NJ) after 30 min to allow full intercalation of stain to cytoplasm. A digital microscope camera (Retiga 2000R; Qimaging, Surrey, British Columbia, Canada) was used to take photographs of three predetermined fields on each replicate slide for a total of nine fields per plant. The number of pollen grains per field was counted and totaled for each slide. The viability of individual pollen grains is considered consistent with the amount of active, stainable cytoplasm. Normal, viable pollen is nearly opaque with a rich dark blue color. Sterile, non-viable pollen is only partially stained or remains mostly unstained with a translucent, light blue color and is typically smaller in size. Stained pollen grains of smaller size and abnormally shaped grains were also considered sterile. Data were analyzed using SAS PROC GLM with mean separation using least significant difference$_{0.05}$ and Duncan's multiple range test.

There were significant differences for total pollen grains and percentage stainable pollen between lines but not between replications. A representative microscope slide for each plant with stained pollen grains is shown (Fig. 5). Diploid wild *R. simplex* had pollen grains at ≈70 µm in diameter compared with ≈90 µm for tetraploid 'Snow Showers'. Both wild *R. simplex* and 'Snow White' had a high number of total pollen counted and pollen stainability (98% and 96%, respectively) (Fig. 6A–B). 'Purple Showers' had 36% pollen stainability; however, this cultivar was not effective as a male parent when used in manual pollinations. R10-102 had a low amount of pollen and a very low percentage of stainable pollen grains (9%); however, it also produced a small percentage (4%) of pollen ≈110 µm in diameter, which could possibly be 2*n*. R10-108 also had low total pollen and percentage stainable pollen (18%).

Conclusions

The two selected hybrids, purple-flowered R10-102 and white R10-108, have better landscape performance, growth habit, and flowering than the wild-type *Ruellia* and the currently existing commercial cultivars Purple Showers and Snow White. Furthermore, it has been demonstrated that R10-102 and R10-108 are fruitless, have very low pollen viability, and were not effective as male parents and therefore will not pose an invasive threat by seed dispersal. Both hybrids were approved for cultivar release by the UF/IFAS Invasive Plants Working Group and the UF/IFAS Cultivar Release Committee.

Availability

U.S. patents were applied for R10-102 and R10-108 in 2012. These plants will be marketed under the names 'Mayan Purple' and 'Mayan White', respectively. Information about plant material, licensing, and propagation agreements can be obtained from the Florida Foundation Seed Producers, Inc., P.O. Box 309, Greenwood, FL 32443.

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