

## **Breeding *Ruellia* and Trialing for Sterility at the University of Florida<sup>©</sup>**

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### **INTRODUCTION**

*Ruellia* is one of the largest genera in the Acanthaceae, consisting of approximately 250 species of perennial herbs, subshrubs, and shrubs, which are found mostly in tropical and subtropical areas. There are many accepted synonyms for *Ruellia simplex* (*R. brittoniana*, *R. coerulea*, and *R. tweediana*) with the name *R. simplex* being the first documented, therefore having taxonomic priority. *Ruellia simplex* (“Mexican petunia”) is found in sunny areas on periodically inundated soils in Mexico, the Antilles, and southeastern South America (Ezcurra and Daniel, 2007). It was introduced to Florida sometime before 1940 (Hupp et al., 2009), and since then has become a very popular landscape plant in southern USA due to its high and continuous flowering and low maintenance requirements (Gilman, 1999). However, this introduced plant has escaped cultivation and become invasive in natural areas. For several years, ‘Purple Showers’ with tall habit and purple flowers was the only sterile commercial cultivar. Since 2007, the breeding objective at University of Florida (UF) has been to develop sterile cultivars with different flower colors such as pink, white, white with a purple corolla tube, and potentially different growth habits, such as, tall, semi-dwarf, and dwarf. Breeding approaches are ploidy manipulations and interspecific hybridizations.

### **MATERIALS AND METHODS**

#### **Breeding**

Polyploidization experiments were performed at UF in Gainesville in 2008 using oryzalin on the apical meristem of seedlings as described by Jones et al., 2008. Seedlings were treated at either of two doses (25 or 50  $\mu\text{M}$ ) and three application frequencies (1, 2, or 3 times every 12 h) of oryzalin. Ploidy levels were determined on mature plants using flow cytometry as described (Czarnecki and Deng, 2009). The treatments of three applications of 25 or 50  $\mu\text{M}$  oryzalin every 12 h were most successful in inducing polyploidy. A total of 15 tetraploid plants with different flower colors were obtained. Hybridizations were performed with plants of different ploidy levels, such as  $4x \times 2x$  and  $2x \times 4x$ , aiming to obtain sterile triploid plants.  $4x \times 4x$  crosses were also performed. A total of 495 *Ruellia* plants were obtained in 2010 and initially evaluated in the greenhouse for growth habit, flowering, and lack of fruit formation. Fifteen breeding lines and five controls were selected for the trials and propagated vegetatively.

#### **Multi-Site Replicated Field Trials 2011**

Plants were trialed in three simultaneous field experiments conducted at the North Florida Research and Education Center in Quincy, Florida (FL); at the Plant Science Research and Education Unit in Citra, Florida; and the Indian River Research and Education Center

in Ft. Pierce, FL (northwestern, north central, and southeastern Florida, respectively), as described by Freyre et al. (2012a).

The experimental design used was a randomized complete block with three blocks. Each plot consisted of three plants for each cultivar or breeding line, spaced 50 cm apart. Wild *R. simplex* (2x) and 'Purple Showers' (4x) were included as purple-flowered comparison lines, 'Chi Chi' (2x) as pink-flowered, 'Snow White' (4x) as white-flowered, and 'McKee' (2x) as white with purple corolla tube. Field rows were covered with black woven nursery ground cover and irrigation was supplied as needed with drip tapes at each site depending on the soil type and weather conditions.

Each plant was evaluated every 4 weeks, from May to October (24 weeks), for landscape performance with a scale from 1–5 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, peak landscape performance.

Flowering was rated on a 1-5 scale where:

- 1 = no flowers or buds;
- 2 = buds but no open flowers;
- 3 = 1-10 open flowers;
- 4 = 11-20 open flowers;
- 5 = more than 20 open flowers.

Fruiting was rated on a 1–5 scale where:

- 1 = more than 50 fruits;
- 2 = 21-50 fruits;
- 3 = 11-20 fruits;
- 4 = 1-10 fruits;
- 5 = no fruits.

### **Field Trials 2012**

Fruits were collected at the three field locations in 2011 from open pollination of a selected pink-flowered plant, R10-105. Seed was germinated obtaining 148 progeny, which were then trialed in Citra in similar conditions as previously described.

### **Multi-Site Replicated Trials 2013**

A total of 19 pink-flowered progeny were trialed in Citra using a randomized complete block design with three blocks and one plant per replication. Six plants of 'Chi Chi' or wild *R. simplex* were also included in each replication to ensure having fertile pollen donors for fruit formation. Field rows were covered with silver plastic and irrigation was supplied as needed with drip tape. Evaluations were conducted weekly using the rating system as previously described. This trial was also conducted in Fort Pierce using 14 progeny and one plant of 'Chi Chi' per replication, and evaluations were performed every 4 weeks. In addition, plants were also trialed in 11.4-L pots in a polyhouse in Gainesville, FL, which provided 25% shade. Four plants of 'Chi Chi' were included in each of the three blocks, and 16 plants of wild *R. simplex* were distributed evenly around the trial as pollen donors. Plants were fertigated manually and evaluations were performed weekly.

## **RESULTS AND DISCUSSION**

### **Field Trials 2011**

Average landscape performance for all lines was 3.6 for Fort Pierce, 3.5 for Citra, and 3.3 at Quincy. Three 4x plants with different flower colors were outstanding and better than their respective controls at all locations. White-flowered 10-108 and purple-flowered R10-102 had the best performance (4.4 and 4.3, respectively), and for pink-flowered

plants the semi-dwarf R10-105 was the best (4.0). Average flower ratings for all lines were 3.5 at Fort Pierce, 3.3 at Quincy, and 3.2 at Citra. R10-108 and R10-102 had high flowering (4.0 and 3.9, respectively), while R10-105 was medium (3.1). Average fruiting was highest in Fort Pierce and Quincy (4.0, respectively) and lower in Citra (4.2). Both R10-102 and R10-108 had no fruits, so their ratings were on average 5. R10-105 produced some fruits and its rating was on average 4.4.

The three selected breeding lines: purple-flowered R10-102, semi-dwarf pink R10-105, and white R10-108 were evaluated for female fertility by harvesting and germinating open pollinated fruits from the field, and by germinating seeds obtained from manual cross pollinations and self-pollinations in a greenhouse. Additionally, male fertility for each plant was determined by staining pollen grains with lactophenol cotton blue. It was estimated that R10-105 had 5% viable seeds per plant as compared to the invasive wild *R. simplex* and 6% as compared to female and male fertility than the existing commercial pink cultivar 'Chi Chi', and it was not approved for cultivar release by the UF/IFAS Invasive Plants Working Group. However, it was demonstrated that R10-102 and R10-108 are both female and male sterile. These lines were released as new cultivars 'Mayan Purple' and 'Mayan White', respectively, and were commercialized in 2013 (Freyre et al., 2012b).

### **Field Trials 2012**

A total of 29 pink-flowered open pollinated progeny from R10-105 were selected for further trials based on performance and apparent low or no fruiting. These plants were propagated vegetatively and grown in a greenhouse in Gainesville. Nineteen plants were selected for 2013 field trials.

### **Field and Potted Plant Trials 2013**

Data was averaged across dates and analyzed separately for each location. For Citra there were no significant differences between plants for plant quality or flower rating, however for fruiting there were significant differences between pink-flowered plants (ratings between 5 and 3.7) and 'Chi Chi' (2.7). In Fort Pierce the plant numbered R10-105-Q54 had the highest plant quality rating (4.6), while the other pink-flowered plants were significantly different (between 4.3 and 3.6) as was 'Chi Chi' (3.3). 'Chi Chi' had the highest flowering (4.0) while R10-105-Q54 was significantly different (3.0). R10-105-Q54 had no fruiting (5.0), while 'Chi Chi' was significantly different (3.1). Numerous pollinators were observed in the field so it is assumed that fruit formation was mostly due to open pollination. In the potted trials R10-105-Q54 had excellent quality and flowering (4.5 and 2.8) and 'Chi Chi' was not significantly different (4.1 and 3.9). In this trial no pollinators were observed so fruiting was due to selfing. 'Chi Chi' produced abundant fruits (3.4) while the other pink-flowered plants had no fruiting (5.0), with the exception of one plant that produced one fruit.

Overall, R10-105-Q54 was selected as the best performing pink-flowered plant in the trials that had the lowest fruiting. In Citra it was observed that this plant produced some fruits from open pollination (rating of 4.7) but they all seemed to abort prior to maturation. To confirm female fertility, 10 self-pollinations were performed in a greenhouse as well as 20 cross pollinations using either wild *R. simplex* or 'Chi Chi' as males. A few fruits were produced but they all aborted before maturation, with exception of one fruit which matured and dehisced naturally. This fruit contained 14 seeds but they did not germinate. Additionally, it was determined that R10-105-Q54 had only 10% pollen staining compared to wild *R. simplex* with 69%. Since it was demonstrated that R10-105-Q54 had extremely low to null fertility, it was approved for release as a new cultivar by the UF/IFAS Cultivar Release Committee and the Invasive Plants Working Group in October 2013. This line will be commercialized under the name 'Mayan Pink'.

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Fig. 1. 16-week old *Ruellia* plants in the field in north central Florida (Citra, FL) in 2011. *Ruellia* 'Purple Showers' (A), 'Mayan Purple' (B), 'Snow White' (C) and 'Mayan White' (D).

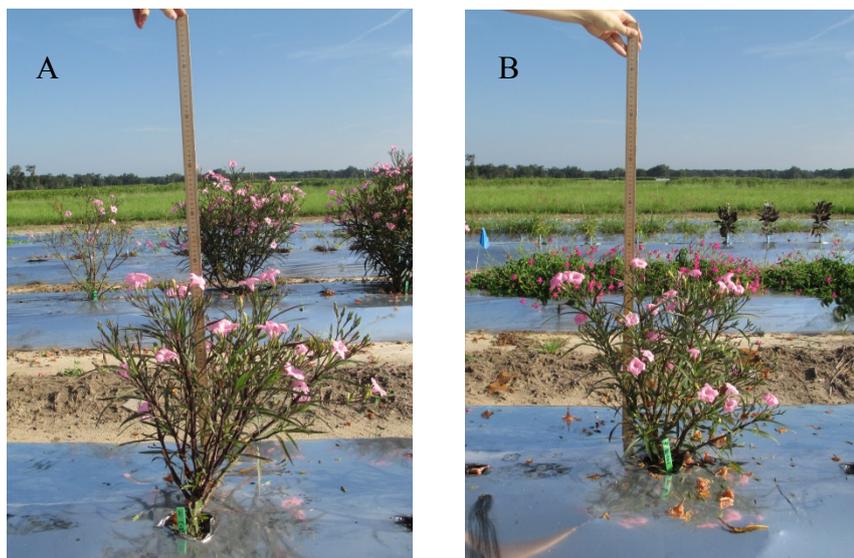


Fig. 2. Sixteen-week old *Ruellia* plants in the field in north central Florida (Citra, FL) in 2013. *Ruellia* 'Chi Chi' (A) and 'Mayan Pink' (B).

