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Ruellia simplex R10-105-Q54 (‘Mayan Pink’)

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Ruellia (Acanthaceae) consists of ≈250 species of perennial herbs, subshrubs, and shrubs with mostly tropical and subtropical distribution. *Ruellia simplex* (commonly known as “Mexican Petunia” or “Mexican Bluebell”) is native to Mexico, the Antilles, western Bolivia, southwestern Brazil, Paraguay, Uruguay, and northeastern Argentina (Ezcurra and Daniel, 2007). 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). There are many accepted synonyms for *Ruellia simplex* (*R. tweediana*, *R. brittoniana*, *R. malacosperma*, *R. coerulea*) with the name *R. simplex* being the first documented, therefore having taxonomic priority (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). Consequently, this species 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 (U.S. Department of Agri-

culture, 2013). In Florida, *R. simplex* has formed naturalized populations in 29 counties throughout the state (Wunderlin and Hansen, 2013). 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 University of Florida (UF) 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 Florida (IFAS Invasive Plants Working Group, 2012).

There are tall (‘Purple Showers’, ‘Chi Chi’, and ‘Snow White’) and dwarf (Katie series) cultivars available of *Ruellia simplex* in purple, pink, and white flower colors. These cultivars are propagated vegetatively, and with the exception of ‘Purple Showers’, all set fruit and are potentially invasive, so their propagation and sales are not encouraged. Sales of Mexican petunia ‘Purple Showers’ in Florida were ranked third for herbaceous perennials after pentas and lantana (Ornamental Outlook, 2009). In 2012, we released two new fruitless cultivars of *R. simplex*, ‘Mayan Purple’ and ‘Mayan White’ (Freyre et al., 2012), that are currently commercially available. We have now released a new pink-flowered cultivar, R10-105-Q54.

Origin

R10-105-Q54 is an open-pollinated progeny from R10-105. R10-105 is a pink tetraploid hybrid obtained at the UF in Gainesville, FL (north-central Florida, lat. 29.6° N, long. 82.3° W, AHS heat zone 9, USDA hardiness zone 9a) from a cross between RU25-1 × ‘Chi Chi’ made in Sept. 2010. RU25-1 is a pink tetraploid plant, obtained as a vegetative propagule of RU3-25. RU3-25 was 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

on Dec. 2008 (as in Jones et al., 2008), and appeared to be chimeric diploid-tetraploid. *Ruellia simplex* plants produce adventitious shoots when they grow, so RU25-1 may have been derived from a stem cutting from one of these shoots, which would explain the loss of the ploidy chimera. 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) as described by Czarnecki and Deng (2009) using wild *R. simplex* (the naturalized and cultivated, non-improved form of the species) as a diploid control. R10-105 was selected after multilocation trials performed in 2011 at the Indian River Research and Education Center (IRREC) in Ft. Pierce, FL (southeastern Florida, lat. 27.4° N, long. 80.4° W, AHS heat zone 9 to 10, USDA hardiness zone 10a), at the Plant Science Research and Education Unit (PSREU) in Citra, FL (north-central Florida, lat. 29.4° N, long. 82.2° W, AHS heat zone 10, USDA hardiness zone 9a), and at the North Florida Research and Education Center in Quincy, FL (northwestern Florida, lat. 30.5° N, long. 84.6° W, AHS heat zone 9, USDA hardiness zone 8b) (American Horticultural Society, 1998; U.S. Department of Agriculture, 2013). R10-105 was submitted for cultivar release to the UF/IFAS Invasive Plants Task Force in Apr. 2012; however, it was rejected because it had some fruit and seed production.

Fruits were harvested from open pollination of R10-105 plants grown in the fields in Citra, Fort Pierce, and Quincy in 2011 and seed were germinated. A total of 142 progeny were obtained (42 plants originating from Citra, 19 plants from Fort Pierce, and 81 plants from Quincy). These plants were transplanted in a field in Citra in 2012, where they were grown interspersed with fertile wild *R. simplex* and ‘Chi Chi’ to ensure pollen availability for fruit production. By the end of the season, 29 plants were selected based on ornamental value and apparent lack of or very low fruiting, and they were propagated vegetatively and grown in a greenhouse in Gainesville. For the 2013 summer trial, 19 plants were selected for replicated field trials in Citra and pot trials in a poly house in Gainesville. Fifteen of the selected plants were also trialed in replicated field trials in Fort Pierce. R10-105-Q54 (which is a progeny numbered 54 from seed harvested in Quincy) was selected based on superior and consistent overall plant performance at all locations as well as very low fruit production.

Description of R10-105-Q54

Using flow cytometry, the mean of the fluorescent area for R10-105-Q54 was 1.7 times the value of the diploid (mean of 168 and 100, respectively) indicating that this plant could possibly be a triploid. Plants were ≈25 weeks of age when description of color for plant parts was determined based on comparison with the Royal Horticultural Society Color Chart [Royal Horticultural

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Society (RHS), 1995]. Measurements for plant height and width (average of two perpendicular widths) were taken on a total of three plants grown in full sun in the field in Citra and in Fort Pierce and three plants grown in 11.4-L pots in a 25% shade poly house in Gainesville. Five measurements of flower corolla diameter and leaf length and width were taken on plants grown in the field in Citra, poly house and greenhouse in Gainesville, and then averaged.

R10-105-Q54 has an upright clumping and dense growth habit, and on average, it is shorter and more compact than 'Chi Chi'. Average plant height in the field in Citra was 51 cm, similar to 'Chi Chi' with 48 cm. In Fort Pierce, average plant height for R10-105-Q54 was 50 cm, whereas 'Chi Chi' was taller with 77 cm. In the poly house, the average plant heights for R10-105-Q54 and 'Chi Chi' were 69 cm and 94 cm, respectively. Average plant widths in Citra for both plants were 53 cm and 62 cm, respectively, in Fort Pierce they were 46 cm and 59 cm, and in the poly house 64 cm and 72 cm.

Stems on R10-105-Q54 are green (RHS 146A) and round/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 143 B), 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 pubescent. The leaves are oppositely attached and are linear to lanceolate with an entire margin, narrowly acute apex, and an attenuate base. Average length of mature leaves for R10-105-Q54 was 14.3 ± 2.3 cm and average leaf width was 2 ± 0.5 cm compared with 'Chi Chi' with average leaf length and width of 15 ± 3.6 cm and 1.3 ± 0.6 cm, respectively. Leaves are green on the adaxial and abaxial (RHS 147A) sides of the lamina. Venation on the abaxial surface of the lamina is pinnate and prominently raised.

Flowers are actinomorphic and funnel form with five petals, four anthers, and one stigma. 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 last for 1 d, after which the corolla falls. The average flower diameter for R10-105-Q54 was 4.8 ± 0.3 cm, similar to flowers on 'Chi Chi' with 4.8 ± 0.4 cm. The petals are red purple (RHS 68B) with a darker red-purple tube (RHS 63A), whereas in 'Chi Chi', they are a lighter red-purple color (RHS 68D and RHS 64C) (Fig. 1). The petals in some flowers in R10-105-Q54 tend to reflex back slightly, whereas in 'Chi Chi', they remain more fully open.

Multilocation Replicated Trials

All plants were propagated at UF in Gainesville. Cuttings were taken from



Fig. 1. Flowers of 'Chi Chi' (left) and R10-105-Q54 (right) on plants grown for 16 weeks in a 25% shade poly house in Gainesville, FL.

greenhouse-grown plants on 10 Apr. 2013. Fifteen cuttings per line 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 3 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 2 weeks, during which they were fertilized with 150 ppm N₂ with Peters liquid fertilizer (20N–10P₂O₅–20K₂O; EverrisTM, Charleston, SC).

Plants were trialed in 2013 in two simultaneous field experiments conducted at the PSREU in Citra and at the IRREC in Ft. Pierce, FL. The fields were either fumigated or glyphosate was applied at least 3 weeks before planting, were rototilled, and rows were formed by raising 10 cm off the ground. In Citra, the rows were covered with silver plastic, whereas in Fort Pierce, beds were covered with semipermeable black plastic and mulch. Plants were distributed in a completely randomized block design with three replications and plants spaced 50 cm apart. In Citra, a total of 19 selections was transplanted on 21 May 2013, and in each replication, there were a total of six plants of 'Chi Chi' or wild *R. simplex* planted at regular intervals to ensure pollen donors for fruit production. In Fort Pierce, a total of 15 plants plus 'Chi Chi' were transplanted on 3 June 2013. Within 3 d after transplanting, each plant was top-dressed with ≈ 9 g of the controlled-release fertilizer Osmocote[®] (15N–9P₂O₅–12K₂O, 12–14 months, Southern formulation; The Scotts Company, Marysville, OH). Irrigation was through drip tapes under the rowcovers in Citra and on top of the plastic at Fort Pierce. Irrigation was supplied as needed at each location depending on the soil type and weather conditions (typically three times per week). In Citra it was possible to apply additional liquid fertilizer, which was done accordingly after soil test results.

Each plant was evaluated weekly in Citra (from Week 8 to Week 15) and every 4 weeks in Fort Pierce (from Week 2 to 14) 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 20 open flowers. Fruiting was rated on a 1 to 5 scale where 1 = more than 50 fruits; 2 = 20 to 50 fruits; 3 = 10 to 19 fruits; 4 = one to nine fruits; 5 = no fruits. At the last evaluation, flower diameter and plant height and average width (average of widths taken north-south and east-west) were measured for each plant. Plants that were leaning were propped up to get a full height measurement.

In addition, plants were also trialed in a poly house in Gainesville in 11.4-L pots. Plants were planted on 30 May 2013 and placed on three mesh benches, where they were spaced ≈ 30 cm apart for the first 9 weeks and 50 cm apart for the rest of the trial. There were three replications for each plant, distributed in a complete block design. For 'Chi Chi', there were four plants in each replication, and additionally, 16 plants of wild *R. simplex* were distributed evenly around the mesh benches as pollen donors for fruit production. The polypropylene greenhouse cover resulted in a 25% light reduction compared with outdoor light. All sides of the poly house were covered with shadecloth, and the east side was removed to allow pollinator entry. Plants were fertigated manually using 150 ppm 20N–10P₂O₅–20K₂O, and insecticide applications were done as needed to control aphids and white flies. Plants were evaluated weekly (from Week 8 to 15) using the evaluation scale mentioned. Data obtained from the two fields trials and the pot greenhouse trial were analyzed separately. In Citra and the poly house in Gainesville, one plant of 'Chi Chi' per replication was chosen for the evaluations so as to have a balanced set of data. The data over the different evaluation dates were averaged and then analyzed using SAS PROC GLM with mean separation using least significant difference_{0.05} and Tukey's Studentized range test (SAS Institute, 2004).

Performance Results

Results and rankings for 'Chi Chi' and R10-105-Q54 in Citra, Fort Pierce, and the poly house in Gainesville are shown in Table 1. Although 20 or 15 genotypes were evaluated, some of them had the same rating and, therefore, the same ranking for any one trait. In Citra, there were significant differences between plants only for fruiting with R10-105-Q54 ranking second in least fruiting, and 'Chi Chi' ranking sixth, which was the most fruiting. There was an abundance of pollinators observed in the field, so this evaluation of fruit production is representative of open pollination. Plant quality for R10-105-Q54 and 'Chi Chi' was not significantly different, and they ranked second and third, respectively. R10-105-Q54 had a denser growth habit, and 'Chi Chi' did not look as attractive when plants were covered with fruits (Fig. 2). R10-105-Q57, which ranked first in plant quality (data not shown), was not selected because it had copious fruiting. Flowering for R10-105-Q54 and 'Chi Chi' was not significantly different either, but 'Chi Chi' ranked first and R10-105-Q54 was second.

Table 1. Average plant quality, flowering and fruiting evaluations, and rankings for R10-105-Q54 and 'Chi Chi' in Citra, Fort Pierce, and a poly house in Gainesville.

	R10-105-Q54	Chi Chi
	Citra	
Plant quality ^z (ranking) ^w	4.3 (2) a ^u	4 (3) a
Flowering ^z (ranking) ^v	3.7 (2) a	4 (1) a
Fruiting ^x (ranking)	4.7 (2) a	2.7 (6) c
	Fort Pierce	
Plant quality (ranking) ^w	4.6 (1) a	3.3 (11) b
Flowering (ranking)	3 (6) ab	4 (1) a
Fruiting (ranking)	5 (1) a	3.1 (5) b
	Gainesville	
Plant quality (ranking) ^v	4.5 (3) ab	4.1 (7) abcd
Flowering (ranking)	2.8 (3) ab	3.9 (1) a
Fruiting (ranking)	5 (1) a	3.4 (2) b

^zScale 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.

^yScale from 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.

^xScale from 1 to 5 where 1 = more than 50 fruits; 2 = 20 to 50 fruits; 3 = 10 to 19 fruits; 4 = one to nine fruits; 5 = no fruits.

^uRanking out of 20 plants evaluated.

^vRanking out of 15 plants evaluated.

^wMean with the same letter are not significantly different at $P = 0.05$ using Tukey's Studentized range test. Comparisons are within sites.

In Fort Pierce, there were significant differences between plants for plant quality, flowering, and fruiting (Table 1). R10-105-Q54 and 'Chi Chi' ranked first and 11th for plant quality out of the 15 plants evaluated, sixth and first for flowering, and first and fifth for fruiting, respectively. A few fruits were observed in R10-105-Q54 after the completion of the study (after 15 weeks). In the poly house in Gainesville, there were significant differences between plants and replications for plant quality and flowering. The third and most southerly replication had better plant quality and more flowering, which can be explained as a result of more light exposure. Plant quality for R10-105-Q54 and 'Chi Chi' was not significantly different, and they ranked third and seventh, respectively. Plants of 'Chi Chi' were less full than those of R10-105-Q54 (Fig. 3). Flowering was significantly different and more abundant for 'Chi Chi', which ranked first, whereas R10-105-Q54 ranked third. For fruiting, there were significant differences between

plants. Only 'Chi Chi' produced abundant fruits, ranking second, whereas R10-105-Q54 had no fruit production. Only one fruit was harvested from one of the other 19 pink plants in the trial.

Determination of Fertility

Female fertility. Fruit production was assessed in the poly house in Gainesville, FL, where plants were protected from rain and strong winds. Very few pollinators were observed inside this poly house so it is assumed that fruit production resulted from selfing rather than open pollination. One plant of 'Chi Chi' in each replication was used to estimate its fruit production. All fruits observed during a period of 60 d (from 8 July to 6 Sept. 2013) were covered with drawstring mesh bags and harvested every other day when they were dark brown or after fruit dehiscence. Additionally, seed was counted in a total of 20 fruits to obtain the average seed production per fruit. 'Chi Chi' produced



Fig. 2. A representative plant of 'Chi Chi' (left) and R10-105-Q54 (right) on 6 Sept. 2013 (Week 15) at Citra, FL.



Fig. 3. Potted plants of 'Chi Chi' (left) and R10-105-Q54 (right) on 23 Aug. 2013 (Week 13) grown in a 25% shade poly house in Gainesville, FL.

120 ± 20 fruits per plant. Average seed production per fruit was estimated at 18; therefore, total seed production per plant was estimated at 2154. With an 82% seed germination rate (as estimated by Freyre, 2012, unpublished data), potential progeny production by 'Chi Chi' was estimated at 1766 seedlings produced per plant by selfing in a period of 60 d. In contrast, R10-105-Q54 had no fruit production by selfing in the poly house.

As mentioned, a few fruits were observed on plants of R10-105-Q54 in Citra during the study; therefore, its fruiting rating was 4.5 rather than 5. However, it was observed that most fruits seemed to abort before ripening and no seeds were harvested during the trial. Two fruits were harvested from open pollination of R10-105-Q54 in Citra in 2012 obtaining 13 seeds, but none of them germinated (Freyre, 2012, unpublished data). To confirm female sterility, greenhouse-grown plants of R10-105-Q54 were used to perform 20 manual crosses using 'Chi Chi' or wild *R. simplex*. Ten crosses were performed on 15 Aug. 2013 and the remaining 10 crosses on 17 Sept. 2013. When 'Chi Chi' was used as a male, a total of 10 fruits formed, and nine of them aborted before maturation. The remaining fruit dehisced at maturation, and it contained 14 abnormal seeds, which did not germinate. Using wild *R. simplex* as a male, seven fruit formed and also aborted before maturation.

Male fertility. To assess male fertility, pollen staining from R10-105-Q54 was compared with that of fertile 'Chi Chi' and wild *Ruellia simplex*. Five flowers were collected for each greenhouse-grown plant. After carefully detaching the corolla, the four anthers were removed and placed in 0.5-mL Eppendorf tubes. Lactophenol cotton blue was micropipetted at 30 µL per tube to stain pollen granules for microscopy. Eppendorf tubes containing anthers and stain were agitated manually for 30 s. Then 25 µL of stain containing pollen was micropipetted onto a microscope slide and spread using a coverslip. After allowing another 5 min for full intercalation of stain to cytoplasm, the slides were examined using a Leica DM1000 light stereoscope at 10× magnification. Photographs of four pre-determined fields on each replicate slide were taken using a Qimaging retiga 2000R digital microscope camera for a total of 20 fields per plant. The number of

stained and unstained/abnormal pollen grains per field was then counted and totaled for each replicate slide of each line. For R10-105-Q54 there were a total of 151 pollen grains counted, and only 10% appeared normal and fully stained (as compared with wild *R. simplex* with 149 pollen grains counted and 69% staining). Male fertility of R10-105-Q54 was not tested by pollinating fertile genotypes; however, its reduced staining is comparable to 9% pollen staining in 'Mayan Purple' and 18% in 'Mayan White', which did not result in successful pollination when used as male parents (Freyre et al., 2012).

Conclusions

R10-105-Q54 has been selected out of 19 other pink flowering plants based on high-performance rankings in two field locations in Florida in 2013 as well as when grown as a potted plant. It has a more dense growth habit and is more compact than the currently existing commercial pink cultivar Chi Chi. Furthermore, it has very low fruiting when grown next to other fertile *Ruellia* plants, and fruits appear to abort before ripening. Manual pollinations confirmed that 35% to 50% fruit production is possible, but 95% of the fruits aborted before maturation, and the remaining fruit produced abnormal seeds, which did not germinate. Therefore, this plant has extremely limited if any potential to escape cultivation as a result of seed dispersal. As a result of its attractive flower color, it has considerable commercial potential in Florida and other southern states.

Availability

A patent was applied for R10-105-Q54 in 2013. This plant will be marketed under the name 'Mayan Pink'. 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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