Breeding for sterility in invasive ornamental plants[©]

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INTRODUCTION

Invasive plants are introduced species that can thrive in areas beyond their natural range of dispersal (USDA-NISIC, 2014). They naturalize over large areas, displace native plants, and disrupt natural ecosystems (Ranney, 2004). In Florida, over 1.5 million acres (approximately 600,000 ha) of public conservation lands have been invaded by introduced plant species (Figure 1), and approximately USA$7 million was spent on management and control of invasive upland plants in 2011. In the USA, control costs and production losses due to weeds was estimated at US $30.6 billion per year (Cusack et al., 2009). For example, purple loosestrife (*Lythrum salicaria*), was introduced from Europe to USA in the early 1800s. Purple loosestrife is now found in all continental states except Florida (Blossey, 2002) and accounts for USA$50 million per year in control costs and forage losses. Mexican petunia, *Ruellia simplex* (previously also known as *R. brittoniana*, *R. coerulea*, *R. malacosperma*, and *R. tweediana*), was introduced to Florida from Mexico sometime before 1940 (Hupp et al., 2009) and has now naturalized throughout the state, plus six other southern USA states, Puerto Rico, the USA Virgin Islands and Hawaii (USDA-NRCS, 2014). It is considered as a Category I invasive species in Florida because it is altering native plant communities by displacing native species and changing community structures or ecological functions (FLEPPC, 2013). However, there is no evidence that it is hybridizing with native species (Freyre and Tripp, 2014). Sales of *R. simplex* 'Purple Showers' in Florida were ranked third for herbaceous perennials after pentas and lantana (Rick Brown, Riverview Flower Farms, pers. commun.), so a breeding program aiming to develop sterile, non-invasive cultivars was established at the University of Florida in 2007 (Freyre et al., 2012a). This species will be described in more detail in this paper.

Figure 1. Lake Jesup area invaded by *Ruellia simplex*.

CHARACTERISTICS OF INVASIVE PLANT SPECIES

The most successful non-native species, those capable of displacing natives, share several characteristics: (1) Effective reproductive and dispersal mechanisms; (2) Competitive ability superior than that of the native; (3) Few to no herbivores or pathogens;

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(4) Ability to occupy a "vacant niche"; (5) Capability of altering the site by either significantly changing resource availability or disturbance regimes or both (Gordon, 1998). *Ruellia simplex* shows many of these characteristics. Plants flower within 3 months (Wilson and Mecca, 2003), and can produce fruits from either open or self-pollination. Under low light levels, plants can produce cleistogamous flowers, which have greenish-brown, very small corollas that do not open, and form fruits from self-pollination (Khoshoo et al., 1969). Capsules contain on average 20.6 seeds per capsule. Seeds do not have a dormancy period, and have 98% to 100% germination rate under ideal conditions of 30°C day and 20°C night. Moreover, seeds are capable of germination under a wide range of temperatures and under conditions of both light and dark (Wilson et al., 2004). Explosive dehiscence of the seed capsule results in seed dispersal distances from the parent plant of 2.5 to 3 m (Witztum and Schulgasser, 1995). Seeds become mucilagenous and adhesive when wet, aiding their dispersal by animals (Ezcurra and Daniel, 2007). Seeds can even germinate under water (personal observation).

Ruellia simplex plants have the ability to grow in a wide range of environmental conditions, from wetlands to almost xeric. In Florida, the species has been reported in five different plant community types: pine flatwoods, prairies; hardwood (hammocks, tree islands, etc.); freshwater marshes; rivers, springs; and salt marsh (Hupp et al., 2009). In the meantime, native *R. caroliniensis* is found primarily in dry native woodlands (Gilman and Landrum, 1999). A study comparing growth and development of *R. caroliniensis* and *R. simplex* established that under wet conditions in laboratory experiments, *R. simplex* exhibited several traits that favor efficient use of resources and high growth rates. It was therefore concluded that under typical wetland conditions *R. simplex* might be expected to out-grow and out-compete native *R. caroliniensis*, especially if the supply of nutrients is limited (Wilson et al., 2004). In several areas where *R. simplex* has naturalized, its coverage was found to constitute 50% of the infested stratum, thus changing community structure by adding a new stratum, or increasing plant density in the stratum by 5-fold. It was also probably altering the hydrology within plant communities (Hupp et al., 2009).

BREEDING METHODS TO OBTAIN STERILITY IN ORNAMENTAL PLANTS

For several years, ornamental plant breeders have been using a number of methods to develop sterile (or nearly sterile) plants that will not be invasive by seed dispersal including the following.

Selecting and breeding for double flowers

Many plant species have forms exhibiting double flowers, which have more than the normal number of petals in the corolla. The reproductive organs (stamens and carpels) are modified into additional petals, thus conferring sterility or near sterility. Many garden plants have been selected for having double flowers, for example roses, carnations, camellias, and double columbines, petunias, and impatiens. Recently, a molecular model that accounts for the formation of double flowers was described (Lohmann et al., 2001; Lenhard et al., 2001).

Induced mutagenesis

Induced mutations have successfully assisted in developing improved and new cultivars among both seed- and vegetatively-propagated crops (Jain, 2006). Mutations resulting from treatment with X-ray or gamma irradiation or chemicals such as ethylmethanosulfonate (EMS) can result in sterility. However, mutations are random, resulting in the need to screen large numbers of individuals. Irradiation treatments have been successful in inducing male and/or female sterility in several ornamental crops that are clonally propagated for commercial production, including *Chrysanthemum*, *Cineraria*, and *Verbena* (Broertjes and Dejong, 1984; Huang and Hong, 1995; Saito et al., 2005).

Wide hybridization

This involves interspecific or intergeneric crosses between distantly related individuals. Chromosome dissimilarities between the parental genomes can result in meiotic

failure during gamete formation, leading to sterility. Some examples include interspecific crosses between *R. caroliniensis × R. simplex* (Freyre and Tripp, 2014), and ×*Chitalpa*, an intergeneric cross between *Chilopsis linearis × Catalpa bignonioides* (see also ×*Chitalpa tashkentensis*) (Rusanov, 1964). In some cases, breeders may need to use ovule or embryo culture in vitro to obtain hybrid plantlets that would not otherwise survive (Bridgen, 1994).

Polyploidization and development of triploids

Ploidy manipulation is an important tool in plant breeding, exemplified by the development of seedless triploid sugar beet and water melon (Stebbins, 1956). The development of triploid plants (with three sets of chromosomes) involves first the induction of tetraploids (with 4 sets of chromosomes) from original diploid plants (with two sets of chromosomes) by use of the chemicals colchicine or oryzalin, followed by cross pollination between tetraploids and diploids. Triploids typically grow and function normally, but they have an inherent reproductive barrier in that the three sets of chromosomes cannot be divided equally during meiosis (Ranney, 2004). In ornamental plants, triploids have been bred in rose-of-sharon and spurflower (Brits and Li, 2008) and this approach has also been utilized to breed triploid sterile selections of invasive tutsan (Olsen et al., 2006) and lantana (Czarnecki and Deng, 2008).

BREEDING STERILE MEXICAN PETUNIA

Polyploidization experiments were performed at the University of Florida in Gainesville in 2008 using oryzalin on the apical meristem of seedlings of *R. simplex*. Ploidy levels were determined on mature plants using flow cytometry as described by Czarnecki and Deng (2009). Treatments of three applications of 25 or 50 μM oryzalin every 12 h were most successful in inducing polyploidy. Hybridizations were performed with plants of different ploidy levels, such as $4x × 2x$ and $2x × 4x$, aiming to obtain sterile triploid plants. 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 *Ruellia* hybrids and five controls were selected for field trials and propagated vegetatively.

In 2011, plants were trialed in three simultaneous field experiments conducted at the North Florida Research and Education Center in Quincy, Florida, at the Plant Science Research and Education Unit in Citra, Florida; and the Indian River Research and Education Center in Ft. Pierce, Florida (northwestern, north central, and southeastern Florida, respectively). The experimental design 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 and 'Snow White' ($4x$) as white-flowered controls. Each plant was evaluated every 4 weeks, from May to October (24 weeks), for landscape performance, flowering and fruiting (Freyre at al., 2012a).

Three $4x$ plants with different flower colors were outstanding and better than their respective controls at all locations. 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 (Freyre at al., 2012b), and were commercialized in 2013 (Figure 2).

Figure 2. 'Mayan Pink', 'Mayan White', and 'Mayan Purple'.

Fruits were collected at the three field locations in 2011 from open pollination of pink-flowered R10-105. Seed was germinated obtaining 148 progeny, which were then trialed in the field in Citra in 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 in Citra and in Fort Pierce, and for potted plant trials in Gainesville.

The plant R10-105-Q54 was selected as the best performing pink-flowered plant that also had low fruit count. In Citra it was observed that R10-105-Q54 produced some fruits from open pollination 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 the 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 UF/IFAS Invasive Plants Working Group. This line will be commercialized under the name 'Mayan Pink' (Freyre and Wilson, 2014).

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