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Progress in Genetic Sterilization of *Lantana camara* through Ploidy Manipulation

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***Lantana camara* L. (Verbenaceae) is an important nursery and landscape plant in Florida and many other states in the southern United States. Plants of this species produce bright flowers, have short production cycles, require low maintenance in the landscape, attract butterflies, and can tolerate harsh environments. However, this species has been listed as a Category I Invasive Species by the Florida Exotic Pest Plant Council and has been considered invasive in Florida by the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) Invasive Plants Working Group. Sterile, noninvasive *L. camara* cultivars are much needed to protect the native lantana, *L. depressa*. Ploidy manipulation, particularly triploid production and selection, has been used to sterilize *L. camara*. Two commercial triploid cultivars (Bandana™ Landscape Yellow and Bandito™ Orange Sunrise) and one petaploid cultivar (Luscious™ Marmalade) have been found to be highly male and female sterile and have no or low potential to hybridize with *L. depressa*. The UF/IFAS ornamental plant breeding program at the Gulf Coast Research and Education Center has released four sterile, noninvasive *L. camara* cultivars, 'UF-T3', 'UF-T4', 'UF-1011-2', and 'UF-1013A-2A'. At the current time, 'UF-1011-2' and 'UF-1-13A-2A' are commercially available under the name Bloomify™ Rose and Bloomify™ Red, respectively. Main challenges in developing sterile, noninvasive lantana cultivars have been that many *L. camara* cultivars can form unreduced female gametes (UFGs) and apomictic seeds, which can lead to significant seed production even in triploid lantana. To effectively sterilize *L. camara*, it is essential to avoid using any lantana that can produce UFGs and apomictic seeds as breeding parents.**

Lantana camara, a member of the family Verbenaceae, is a popular ornamental plant widely grown in Florida and many other states in the southern United States. Consumers love this plant due to its ability to produce bright flowers year-round, require low maintenance, attract butterflies, and can tolerate harsh environments. Growers like lantana because it is easy to propagate, has a short production cycle, is disease resistant, and tolerant to low fertility (Schoellhorn, 2004). These attributes make lantana one of the favorite nursery and landscape plants in Florida. A 2003 survey of the Florida nursery industry indicated that 19.0% of the responding nurseries produced lantana, and the annual sales value of lantana in Florida was estimated to be more than $40 million (Wirth et al., 2004).

Lantana camara is native to the West Indies (Sanders, 2001). It was introduced throughout most of the tropical world by 1900 (Howard, 1969). In the United States, *L. camara* has been found in 14 contiguous southern states, from North Carolina to California. It also grows in Hawaii, Puerto Rico, and the Virgin Islands (USDA NRCS, 2018).

Plants of *L. camara* can out-compete native species in natural habitats and have the potential to invade natural and agricultural land (Arora and Kohli, 1993; Duggin and Gentle, 1998). Furthermore, Florida native *Lantana depressa* such as var. *depressa*, var. *sanibelensis*, and var. *floridana*, can hybridize with *L. camara* (Sanders 1987). Interspecific hybridizations between the two species could result in genetic contamination of the native species (Anderson and Ascher, 1994; Anderson, 2001; Hammer, 2004). The Florida Exotic Pest Plant Council (FLEPPC) (2015) listed *L. camara* as a Category I invasive species. The University of Florida/Institute of Food and Agricultural Sciences (UF/IFAS) Assessment of NonNative Plants in Florida's Natural Areas concluded that *L. camara* should not be recommended for commercial production or landscape use in South, Central, and North Florida (UF/IFAS Assessment, 2016).

A research program was initiated in 2004 at the University of Florida's Gulf Coast Research and Education Center (UF/GCREC) to select and develop sterile or infertile *L. camara* cultivars. Recent studies have shown that two commercial triploid cultivars and one commercial petaploid cultivar are highly male and female sterile and have no or low potential to hybridize with *L. depressa*. However, the majority of commercial *L. camara* cultivars are fertile (Czarnecki, 2011). Hundreds of triploid lantana lines have been generated and evaluated since 2004 (Deng et al., 2017). So far, the program has released four sterile, noninvasive *L. camara* cultivars.

Male and Female Fertility of *L. camara* Cultivars

Male and female fertility are the most important factors determining the invasive potential of *L. camara*. Commercial *L. camara* cultivars exhibit remarkable variation in both male and female fertility.

Male fertility. The male fertility of *L. camara* cultivars can be assessed based on pollen stainability or viability and hybridization potential with *L. depressa*. The main method used to assess

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lantana pollen stainability and viability has been vital dye-based staining (Czarnecki et al., 2014; Sanders, 1987c; Spies, 1984). Two vital stains, lactophenol cotton blue (CB) (Eng. Scientific, Inc., Clifton, NJ) and fluorescein diacetate (FDA) (Sigma-Aldrich, St. Louis, MO) have been used to assess the pollen stainability of *L. camara*. Czarnecki et al. (2014) evaluated the pollen stainability of eight *L. camara* cultivars using both stains and concluded that there were no significant differences in pollen stainability between the two dyes. Czarnecki et al. (2014) evaluated the ploidy level and pollen stainability of 32 *L. camara* cultivars and breeding lines and showed that ploidy level was the most important factor determining *L. camara* pollen stainability/male fertility. On average, diploids exhibited the highest pollen stainability (64.6%), followed by tetraploids (45.1%), pentaploids (34.6%), and hexaploids (18.0%). Triploids showed the lowest pollen stainability (9.3%). However, several triploid cultivars had pollen stainability approaching 20% to 30%.

Female fertility. Seed (or fruit) production and germination are the major factors determining female fertility in lantana. To take both into consideration, a female fertility index (FFI) can be calculated by multiplying fruit per peduncle (FPP) and seed germination percentage (Czarnecki et al., 2012). Previous data have shown a strong positive correlation between FFI and FPP, but little correlation between FFI and seed germination, indicating that FPP is of much greater influence on the overall female fertility of *L. camara* than seed germination (Czarnecki, 2011). Ploidy level, the formation of unreduced female gametes (UFGs), and the development of apomictic seeds have significant effects on fruit (seed) production in lantana (Czarnecki et al., 2009). Unreduced female gametes are a type of *2n* gametes; they are formed because megaspore mother cells skip meiosis. Triploids without the capacity to form UFGs and apomictic seeds are most sterile. However, triploids with the UFG-production and apomictic seed traits can produce abundant seeds (Czarnecki et al., 2009). These traits could also be transmitted to progeny.

Ploidy manipulation

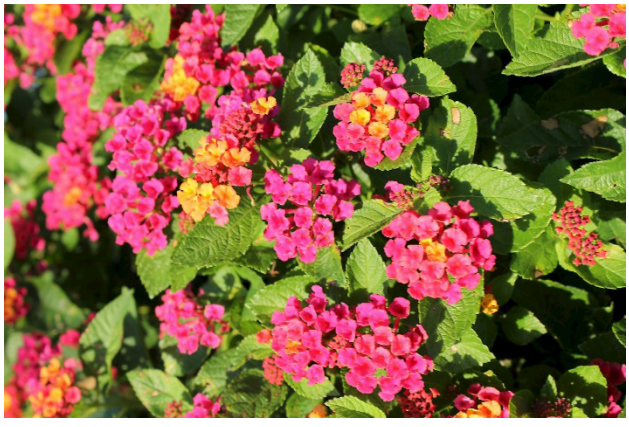
Ploidy manipulation has been widely used in fruit and vegetable crops to develop seedless cultivars. It has been also successfully used in developing noninvasive ornamental plants. The most commonly used ploidy manipulation strategy has been hybridizing tetraploids (*4×*) with diploids (*2×*), followed by screening the resulted triploids (*3×*). Triploids contain three sets of chromosomes in cells and are often highly male and female sterile (Ranney, 2004; Trueblood et al., 2010). However, as mentioned above, some lantana cultivars have the ability to form UFGs and apomictic seeds. These traits will enable triploid lantana plants to produce fruit and seeds. To obtain highly sterile triploids in lantana, diploids and tetraploids to be used as breeding parents should be screened prior to interploid hybridization. Cultivars with the ability to form UFGs and apomictic seeds should not be used as breeding parents if the breeding objective is to produce new sterile cultivars.

Identification and Development of Sterile, Noninvasive Cultivars

Evaluation of commercial cultivars. Over the years, forty-five commercial *L. camara* cultivars have been evaluated in Florida for their male and female sterility and hybridization potential with *L. depressa*. The evaluations were conducted simultaneously at two sites, one at the UF/IFAS/GCREC, Balm and another at the UF/IFAS's Indian River Research and Education Center (IRREC) in Ft. Pierce. At each site, the 'UF-T3' cultivar (see below for more information on this cultivar) was included as a sterile control and 'Pink Caprice' as a fertile control. In these trials, two commercial triploid cultivars (Bandana™ Landscape Yellow and Bandito™ Orange Sunrise) and one petaploid cultivar (Luscious™ Marmalade) showed similar low levels of fruit production with 'UF-T3' and >99% reduction in fruit (seed) production compared to 'Pink Caprice' (Z. Deng, unpublished). The pollen stainability of 'Bandana™ Landscape Yellow', 'Bandito™ Orange Sunrise', and 'Luscious™ Marmalade' has been reduced by 80% or more compared to 'Pink Caprice' (Z. Deng, unpublished). These triploid or pentaploid cultivars did not cause fruit set or produce viable progeny when used as a male or female parent in hand-pollination with *L. depressa* (Z. Deng, unpublished).

Development of new sterile, noninvasive cultivars. The UF/IFAS ornamental plant breeding program at the GCREC has released four sterile, noninvasive *L. camara* cultivars, 'UF-T3', 'UF-T4', 'UF-1011-2', and 'UF-1013A-2A' (Czarnecki et al., 2012; Deng et al., 2017). 'UF-1011-2', and 'UF-1013A-2A' are being marketed commercially under 'Bloomify™ Rose (Fig. 1) and 'Bloomify™ Red' (Fig. 2), respectively.

'UF-T3' is a progeny of a cross between 'Dallas Red' and breeding line LAOP-9. 'UF-T4' resulted from a cross between 'Carlos' and LAOP-9. Both 'UF-T3' and 'UF-T4' are triploids and were selected in Oct. 2008. The male and female fertility and plant performance of 'UF-T3' and 'UF-T4' were evaluated in four replicated field trials in Ft. Pierce, Balm, Citra, and Quincy, FL.



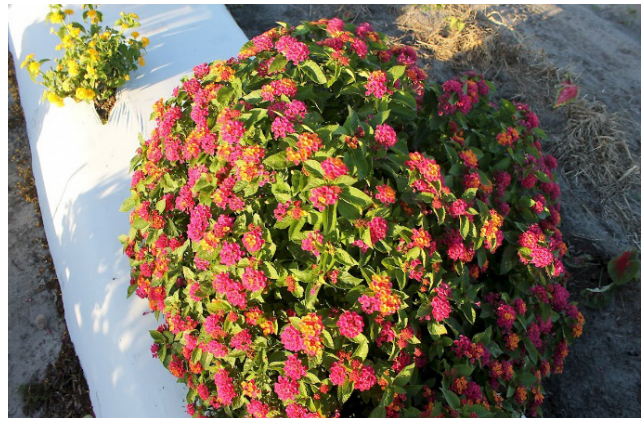


Fig. 1. Infertile, non-invasive *Lantana camara* cultivar Bloomify™ Rose.

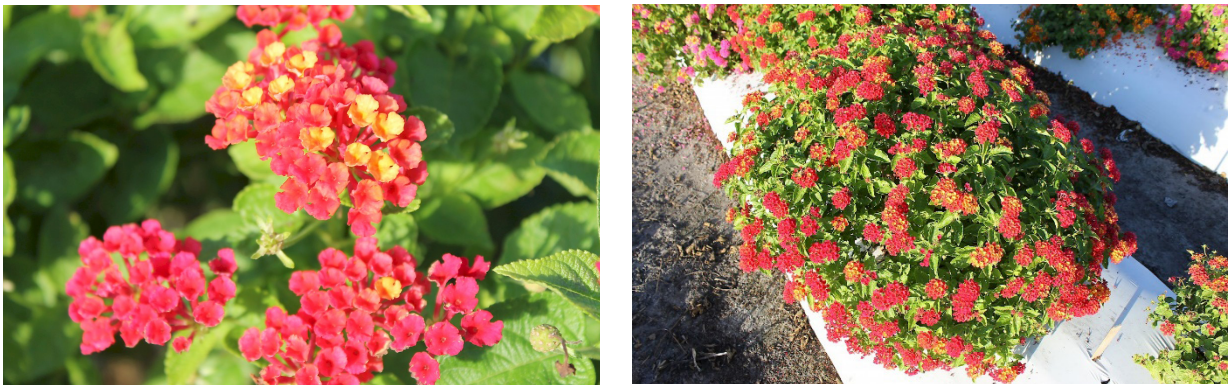


Fig. 2. Infertile, non-invasive *Lantana camara* cultivar Bloomify™ Red.

(Czarnecki et al., 2012). The average pollen stainability of 'UF-T3' and 'UF-T4' was 5.3% and 3.2%, respectively. The average fruit per peduncle for 'UF-T3' and 'UF-T4' was 0.019 and 0.023, respectively. In these trials, the fertile, invasive cultivar Pink Caprice had an average of 65.6% pollen stainability and produced 10.451 fruit per peduncle. Thus, 'UF-T3' and 'UF-T4' exhibited significant reduction in both male and female fertility compared to 'Pink Caprice'. Hand pollination experiments performed at the GCREC showed that 'UF-T3' and 'UF-T4' had little or no potential to hybridize with *L. depressa*.

'Bloomify™ Rose' and 'Bloomify™ Red' are the latest cultivars released from the GCREC research program. 'Bloomify™ Rose' was the progeny of a cross between breeding line CAOP-73 ($2n = 4\times$) and 'Landmark Flame Improved' ($2n = 2\times$). 'Bloomify™ Red' resulted from a cross between breeding line DROP-25 ($2n = 4\times$) and 'Landmark Flame Improved'. Ploidy analysis showed that 'Bloomify™ Rose' and 'Bloomify™ Red' are likely triploids. The average pollen stainability of 'Bloomify™ Rose' and 'Bloomify™ Red' was 9.7% and 3.0%, respectively. Compared to 'Pink Caprice', the pollen stainability of 'Bloomify™ Rose' and 'Bloomify™ Red' was reduced by at least 85%. Fruit production in 'Bloomify™ Rose' and 'Bloomify™ Red' represented > 99% reduction from the fruit production of 'Pink Caprice'. The low level of fruit production in 'Bloomify™ Rose' and 'Bloomify™ Red' was similar to that of 'UF-T3'. The hybridization potential of 'Bloomify™ Rose' and 'Bloomify™ Red' with *L. depressa* was extremely low. 'Bloomify™ Red' did not set any fruit (seeds) after it was pollinated with *L. depressa*, nor did it effect any fruit (seed) set on *L. depressa*. 'Bloomify™ Rose' effected 0.2 % fruit (seed) set on *L. depressa*, but none of these few seeds germinated. 'Bloomify™ Rose' did not set fruit (seeds) after it was pollinated by *L. depressa*. Overall, 'Bloomify™ Rose' and 'Bloomify™ Red' are as highly sterile as 'UF-T3'. In garden and container trials, 'Bloomify™ Rose' and 'Bloomify™ Red' had much better plant performance than 'UF-T3'. Plants of these two new cultivars have very good plant branching and growth habit and produce many bright flower colors.

Future Study

The UF/IFAS ornamental plant breeding program at the GCREC has made significant progress in development and identification of sterile, noninvasive lantana cultivars. There is still a huge demand for new noninvasive lantana cultivars by the nursery and landscape industry. The main challenges in developing sterile, noninvasive lantana cultivars have been that many *L. camara* cultivars can form UFGs and apomictic seeds. To develop new noninvasive lantana cultivars, it is necessary to identify more breeding parents that do not produce UFGs and apomictic seeds.

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