Assessment of the Female Fertility of 26 Commercial *Lantana camara* Cultivars and Six Experimental Lines

David M. Czarnecki, II and Zhanao Deng

Department of Environmental Horticulture, Institute of Food and Agricultural Sciences, Gulf Coast Research and Education Center, University of Florida, 14625 C.R. 672, Wimauma, FL 33598

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Abstract. *Lantana camara* is an important plant for the environmental horticultural industry, yet it can be invasive, cross-pollinating with native *lantana* and dispersing fruit (and seeds) to natural and agricultural lands. Identification and development of sterile cultivars is much needed to meet industry and consumer needs for noninvasive plant materials. Previously we evaluated the male fertility of 32 *L. camara* cultivars/breeding lines at five ploidy levels. This study was to assess their female fertility and understand the relationship between female fertility and ploidy level and the production of unreduced female gametes (UFGs) in *L. camara*. These cultivars/breeding lines significantly varied in percent fruiting plants (6.3% to 100.0%), percent fruiting peduncles (0.3% to 98.8%), fruit per peduncle (0.003 to 7.173), seed germination (0% to 57.1%), and female fertility index (0.003 to 2.998). Certain diploids (e.g., ‘Denholm White’) were highly female-sterile. Eleven of the 13 triploids evaluated were UFG-producing and rather fertile. The two non-UFG-producing triploids had the female fertility index of 0.005, thus most sterile. Tetraploids, especially those producing UFGs, were prolific fruit producers. These results show that ploidy level and UFG production play a significant role in determining fruit (seed) production capacity and female fertility of *L. camara*. None of the commercial triploid cultivars evaluated reached desirable levels of male and female sterility, indicating a strong need to develop new *lantana* cultivars that are male- and female-sterile. Our results suggest that production and selection of triploids can be effective to sterilize *L. camara*, but it is imperative to select diploids and tetraploids that do not produce UFGs as the breeding parents.

Lantana camara, a member of Verbenaceae L., originated in the West Indies (Sanders, 2001) and was introduced and spread by European explorers to almost all the tropical colonies by 1900 (Howard, 1969). Plants of this species produce attractive flowers, attract numerous species of pollinators [including at least 24 species of

butterflies (Goulson and Derwent, 2004; Schemske, 1976)], tolerate harsh environmental conditions (droughts, salts, etc.), and have low maintenance requirements. These attributes make *L. camara* a popular plant for landscape use (Arnold, 2002; Mugnai et al., 1999; Starman and Lombardini, 2006). *Lantana camara* is an important floricultural/nursery crop in many parts of the world, especially in the southern United States. However, *L. camara* has been a major invasive plant species, reportedly being invasive in more than 30 countries (Morton, 1994). It is especially problematic in tropical and subtropical areas around the world where the plant is only limited by cold winters (Sanders, 2006). *Lantana camara* has been cited as one of the 100 worst weeds in the world (Lowe et al., 2000). In the United States, escaped *L. camara* has been found in 14 contiguous southern states, from North Carolina to California. Its escape also has been observed in Hawaii, Puerto Rico, and the Virgin Islands (USDA NRCS, 2020). The Florida Exotic Pest Plant Council (FLEPPC) classified *L. camara* as a Category I invasive species (FLEPPC, 2020). Category I invasive plant species are those that have shown the ability to change the structure or ecology of an environment and/or to cross-pollinate native species.

The ability to produce and disperse seeds is one of the most critical aspects of a species’ survivability. The degree to which a plant is able to accomplish this goal is also one of the main factors determining the invasive potential of a species (Dozier, 1999). Seed production and seed germination have been the primary criteria in evaluating exotic species’ invasive potential (Trueblood et al., 2010; Wilson and Mecca, 2003). In *L. camara*, seed is borne inside a round, fleshy drupe (berry). Each drupe generally contains one seed and occasionally one additional seed (reviewed by Sharma et al., 2005). The fruit is initially green but turns purple then blue-black as the fruit ripens.

Lantana camara can flower and produce fruit all year round if adequate temperature, moisture, and light are available. Several previous studies examined the fruit production of naturalized *L. camara* plants or seed densities in the soil seed bank under naturalized plants. Significant intraspecific variation seems to exist. An Australian study showed that each *lantana* inflorescence could bear approximately eight fruit (Barrows, 1976), whereas in India, as many as 25 to 28 fruit were observed on individual peduncles (inflorescences) (Sharma et al., 2005). An even greater variation has been observed in the density of *lantana* seed in the soil seed bank. Reported *lantana* seed density in soil ranged from <5 to 2690 seeds per square meter (Sharma et al., 2005). However, little information is available in the literature regarding the fruit (and seed) production capacity and seed germination of commercial *lantana* cultivars that are used in the landscape.

Several researchers have attempted to understand the relationships between ploidy level and fruit or seed production in *L. camara*. Natarajan and Ahuja (1957) suggested that ploidy level would be an influencing factor in fruit production. In their study, diploid *L. camara* plants had “no seed” to “good” seed production, whereas triploids produced no seed; 30% of tetraploid plants did not produce seeds, while the rest of tetraploid plants had “none” to “good” seed production. Two subsequent studies by Raghavan and Arora (1960) and Khoshoo and Mahal (1967) indicated that triploid plants did produce “good” amounts or at least a few seeds. Spies (1984) collected seeds from all observed ploidy levels in South Africa and found a range of seed production capacity across diploid to pentaploid plants of 0 to 2485 (per plant). These studies indicated that tetraploid and diploid plants were the highest seed producers at 856 (4x) and 565 (2x) seed per plant, respectively. The triploid plants were expected to be sterile but still produced 342 seeds per plant. In these studies, few pentaploid and hexaploid plants were available, and one pentaploid produced 638 seeds on a single plant.

Lantana camara seeds can germinate at any time of the year with sufficient conditions (Gentle and Duggin, 1997). Studies from Australia and India indicated a range of seed germination: 12% in diploids, 28% in

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D.M.C. is a former graduate student at the University of Florida. Current address: Ernst Benary of America, Inc., 195 Paulsen Road, Watsonville, CA 95076.

Z.D. is the corresponding author. E-mail: zdeng@ufl.edu.

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triploids, and 56% in tetraploids (Raghavan and Arora, 1960; Spies, 1984). An earlier study (Heit, 1946) investigating the best methods for *L. camara* seed germination determined the highest average rate of seed germination to be 53% after 40 d, with an individual accession reaching as high as 70% after 60 d. Nevertheless, in their study, only one individual was sampled for each of the three ploidy levels (diploid, triploid, and tetraploid) (Raghavan and Arora, 1960).

In a previous study, we identified five ploidy levels among 32 *L. camara* cultivars/breeding lines, determined their pollen stainability, and gained a better understanding of the relationship between ploidy level and pollen stainability or male fertility in *L. camara* (Czarnecki et al., 2014). In an earlier study (Czarnecki and Deng, 2009), we performed ploidy analysis of more than 1500 *lantana* progeny from self, open, and/or controlled pollinations and observed frequent production of unreduced female gametes (UFGs) in some *lantana* cultivars/breeding lines. The objectives of this study were to assess female fertility (fruit production and seed germination) in the 32 cultivars/breeding lines and to understand the relationship between female fertility and ploidy level and UFG production in *L. camara*.

Materials and Methods

Plant materials

Twenty-six *L. camara* cultivars and six breeding lines were used in this study. They were propagated and grown as previously described (Czarnecki et al., 2014). Propagation was done by cuttings on 17–19 Sept. 2007. When plants were $\approx$ 8 months old (after male fertility assessment was done), they were transplanted to ground beds on 29 May 2008. The beds were treated with Roundup for lawns (The Scotts Company, Marysville, OH) and Image (BASF, Research Triangle Park, NC) following the labels for weed control and covered with white-on-black plastic mulch. The field was irrigated with drip tubes, twice a week and 1 h per irrigation event. Each plant received 5 g of the commercial controlled-release fertilizer (Osmocote, 15N–9P–12K, 5- to 6-month release at 21 °C; The Scotts Company). Plants were grown in the ground beds for nearly 6 months and then harvested on 13–14 Nov. 2008.

Assessing fruit production

Commonly *L. camara* flowers take $\approx$ 3 to 5 weeks from opening to producing ripe fruit. Thus, the first fruit collection was made 6 weeks after transplanting. Fruit collection was then repeated approximately every 5 weeks until mid-November when the air temperature became too low for *lantana* plants to produce flowers and set fruit regularly. A total of four collections were made during the growing season, on 17–18 July, 25–28 Aug., 28–30 Sept., and 4–11 Nov. 2008, respectively. During each collection, 20 peduncles were randomly harvested from

each experimental unit (plant), and fruit (berries) on each peduncle, regardless of maturity, were counted to calculate the percentage of peduncles setting fruit [percent fruiting peduncles (PFP)] and the number of fruit per peduncle (FPP) (Fig. 1). In addition, every plant in the study was inspected during each collection to determine whether the plant set any fruit to calculate the percentage of plants setting fruit should the 20 peduncles collected not bear any fruit.

After each collection, ripe fruit were stored in glycine bags under ambient laboratory conditions at $\approx$ 22 °C for subsequent seed extraction (described subsequently); green/immature and visibly damaged fruit were discarded. During the last fruit collection, all ripe fruit on each plant, in addition to those from the 20 randomly harvested peduncles, were collected and stored for seed extraction and germination studies.

During the first fruit collection, insect larvae were found burrowing through *lantana* flower clusters and developing fruit in the field. Some larvae were collected, reared, and sent to the Florida Department of Agriculture and Consumer Services Division of Plant Industry in Gainesville, FL for species identification. Samples were processed according to the guidelines of the Clemson Department of Plant Industry (Clemson University, Clemson, SC). Characteristic larvae damages consisted of spiraling grooves on peduncles, blackened depressed areas, and burrowed holes through leaves, flowers, and berries (Fig. 1) near the growing points of the plant. All peduncles collected thereafter were scored for insect damage and the percent insect-damaged peduncles (PID) were calculated.

Evaluating seed germination

Seeds were extracted on 7–9 Jan. 2009 after all fruit collection was completed in Nov. 2008. Saved ripe fruit were soaked in water to loosen pulp then macerated manually using a fine metal mesh flour sieve; collected seeds were air-dried under the ambient condition in the laboratory for $\approx$ 4 weeks. For most of the *L. camara* cultivars/breeding lines, the number of seeds from each experimental unit was limited, thus seeds were combined by cultivar and collection period for seed germination studies.

The bulked seeds of each cultivar/breeding line were randomly divided into three

aliquots as three replicates. Seeds were sown in plastic trays on the surface of the commercial potting mix Fafard 2B (Anderson, SC) on 9 Feb. 2009 and germinated in the greenhouse, under intermittent mist. Temperatures in the greenhouse ranged from 16 °C (night) to 30 °C (day), and no supplemental lighting was provided. Seed germination was recorded every week for 16 weeks until the end of May 2009.

Calculating female fertility index

As shown subsequently, *L. camara* cultivars/breeding lines varied greatly in fruit production and seed germination. Some of them produced copious amounts of fruit but seeds had low germination, whereas others set fewer fruit but their seeds germinated readily. The female reproductive potential of a given *L. camara* cultivar/breeding line were expected to depend on both its fruit production capacity and seed germination. To take both into consideration, a female fertility index (FFI) was calculated by multiplying FPP and seed germination (in decimal form). This index was expected to give a better representation of a given *L. camara* cultivar/breeding line's female fertility and would also serve as a data reduction method.

Plant dry weight

After the final fruit collection on 13–14 Nov. 2008, the above-ground parts of each plant were harvested by cutting the stems off at the soil line. The harvested plant materials were dried at 60 °C in a drying room for 4 weeks before plant weights (in kilograms) were taken.

Experimental design

Lantana camara cultivars and breeding lines were arranged in the field following a randomized complete block design. The experimental unit was a single asexually propagated plant. There were four blocks (or replicates) in the experiment and one plant per cultivar/breeding line in a block.

Statistical analysis

FPP and seed germination data were analyzed using the PROC GLM procedure in SAS for Windows 9.2 (SAS Institute, Cary, NC) to determine the significance of differences among cultivar/breeding line, collections, and ploidy levels. Percentage data were transformed using the arcsine square root

Fig. 1. Ripe *Lantana camara* fruit on a peduncle (A), insect pest *Crocidosema lantana* (B), and the damage caused by *Crocidosema lantana* on a *lantana* peduncle head (C).

function before analysis of variance (ANOVA). When differences were significant at $P \leq 0.05$, mean separation analysis was performed using the least significant difference procedure in SAS.

Results and Discussion

Fruit production

Three parameters, percent fruiting plants, FPP, and FPP were used to assess the fruit production capacity of *L. camara* cultivars/breeding lines. The percentage of plants setting fruit varied from 6.3% to 100.0%, i.e., a 15.8-fold difference among cultivars/breeding lines. The percentage of fruiting peduncles had a much larger range of variation, from 0.3% to 98.8%, or 329.3-fold difference. The largest variation was observed in FPP, which ranged from 0.003 to 7.173, differing by 2391 fold. ANOVA indicated that the differences among cultivars/breeding lines in FPP were very highly significant ($P < 0.0001$).

As described by Czarnecki et al. (2014), the 26 cultivars and six breeding lines represented five ploidy levels, from diploidy to hexaploidy. Previously Czarnecki and Deng (2009) reported that 11 of the 13 triploids, three of the six tetraploids, all the five pentaploids, and all three hexaploids included in this study were expected to produce UFGs. These polyploids are to be referred to as having the UFG-producing trait. ANOVA results indicated that both the ploidy level and the UFG-producing trait played significant roles in determining fruit production capacity of *L. camara*. The F and P value for the ploidy level factor was 40.97 and <0.0001 , respectively (Table 1). The only two ploidy levels with UFG and non-UFG plants were triploid and tetraploid groups, and the F value for the UFG production factor were 44.46 and 17.65 for triploid and tetraploid groups, respectively, with P values of 0.0001 (Table 1).

Statistical analysis also indicated that the fruit per peduncle values were significantly different among four collections ($P = 0.0252$). This was expected based on a preliminary study in 2007 using 139 *L. camara* lines and their cyclic flowering and fruiting habit (D.M. Czarnecki and Z. Deng, unpublished data, 2007). This was also the reason for making a number of fruit collections over a period of several months.

Seed germination

Because of the large differences among cultivars/breeding lines in fruit production, the number of seeds available for germination varied considerably among cultivars/breeding lines. Seeds were not available for testing seed germination in 'Athens Rose' and breeding line 629-1. One seed was collected from 'Denholm White' plants, and it germinated. For five cultivars/breeding lines, 4 to 119 seeds were obtained and sown, but none germinated. Excluding these cultivars/breeding lines, the seed germination percentage in the remaining 24 cultivars/breeding

Table 1. Analysis of variance table for fruit production and seed germination of 32 *L. camara* cultivars/breeding lines. Seeds were sown on 9 Feb. 2009; seed germination data were collected for 16 weeks after seed sowing. Ploidy level of the cultivars/breeding lines ranged from diploidy to hexaploidy. Triploid and tetraploid cultivars/breeding lines could be divided into two groups, producing or not producing unreduced female gametes (UFGs).

Sources of variation	DF	F value	P value
Fruit production			
Cultivars/breeding lines	31	46.54	<0.0001
Ploidy level	4	40.97	<0.0001
UFG vs. non-UFG within 3x	1	44.46	<0.0001
UFG vs. non-UFG within 4x	1	17.65	<0.0001
Seed germination			
Cultivars/breeding lines	31	4.44	<0.0001
Ploidy level	4	6.3	<0.0001
UFG vs. non-UFG within 3x	1	4.86	0.0292
UFG vs. non-UFG within 4x	1	1.46	0.2316

lines ranged from 9.4% to 57.1%. As expected, statistical analysis indicated that cultivars/breeding lines were significantly different in seed germination ($P \leq 0.0001$) (Table 1).

Female fertility index

As mentioned earlier, seeds of a number of cultivars/breeding lines did not germinate, resulting in an FFI of 0 for these cultivars/breeding lines. 'Pink Caprice' had the highest FFI, 2.998 (Table 2). The remaining 24 cultivars/breeding lines had an FFI between 0.003 and 0.599. In total, there were 13 cultivars/lines whose FFI were ≤ 0.054 , including three diploids, three triploids, three hexaploids, and four pentaploid lines.

Fruit production, seed germination and FFI of diploid *L. camara*

On the basis of the FFI values, the five diploid cultivars/breeding lines could be separated into four groups. The first group consisted of 'Lola', which had a high percentage of plants setting fruit (100%) and a high FPP value (0.922) but a low germination percentage (16.2%), resulting in an FFI of 0.149. In the second group was breeding line LAOP-30, which had a lower FPP value (0.435) but a much higher germination percentage (60.0%), resulting in a similar FFI (0.261). The third group consisted of 'Cream' and breeding line LAOP-9. Their seeds did not germinate well ($\approx 10.0\%$ germination) and had a low FFI (0.020 or 0.034), although their FPP values were not low (0.193 and 0.344, respectively). The fourth group consisted of 'Denholm White'. It had the lowest FPP value (0.003) among all *L. camara* cultivars/breeding lines assessed in this study. Only one seed was collected from 303 flower peduncles surveyed. This seed germinated, and the cultivar had an FFI of 0.003. In a hand-pollination experiment involving thousands of flowers, 'Denholm White' also did not produce seed (Czarnecki, 2011).

Within diploids, *L. camara* cultivars/breeding lines could vary remarkably in fruit production (fruit per peduncle from 0.003 to 0.922), seed germination (10.0% to 100.0%), and female fertility index (0.003 to 0.261), and certain diploids could be highly female-sterile. Understanding the genetic mecha-

nism(s) of the high level of female sterility in these diploids, especially in 'Denholm White', would be valuable for sterilizing *L. camara*.

Fruit production, seed germination, and FFI of triploid *L. camara*

Of the 13 triploid cultivars assessed for fruit production, the majority (11) was able to produce UFGs (and apomictic seeds) (Czarnecki and Deng, 2009; Czarnecki, 2011), and two triploid cultivars did not have this trait. The two groups of triploids had some differences in seed germination, but their most significant differences were in fruit production, and consequently in FFI (Table 3).

Non-UFG-producing triploids. 'Athens Rose' and 'Lucky Red Hot Improved' belong to this group. Two berries were collected from 305 peduncles of 'Athens Rose' throughout the 5-month period. 'Athens Rose's seed (1) didn't germinate, resulting in an FFI of 0. The 316 peduncles surveyed on 'Lucky Red Hot Improved' plants set 30 fruit, i.e., an FPP value of 0.094. The seeds of this triploid had 11.1% germination. As a group, non-UFG-producing triploids had the lowest FPP value (0.05) and the lowest FFI (0.005) and thus were the most sterile in *L. camara* (Table 3). These results suggest that triploidy in combination with the removal of the UFG trait could result in high levels of female sterility in *L. camara*. As a matter of fact, this strategy has been used in the development of new sterile *L. camara* cultivars (Czarnecki et al., 2012; Deng et al., 2017).

UFG-producing triploids. These triploids were highly prolific in fruit production, with 100% plants and 16.0% to 60.7% flower peduncles producing fruit and 0.175 to 1.379 fruit per peduncle (Table 3). Three triploids ('Landmark Pink Dawn', 'Lemon Drop', and 'Samson Lantana') had an FPP value of 1.232 to 1.379 and produced more fruit per peduncle than 'Lola', the most prolific diploid (0.922). As a group, their average FFI was 0.236, ≈ 47 -fold higher than that of the non-UFG-producing triploids and 2.5-fold higher than that of the diploids (Table 3).

Two of the triploids in this group, 'Red Butler' and 'Lemon Drop', had 9.3% or 9.4% seed germination. The remaining nine

Table 2. Fruit production capacity, seed germination, female fertility index, and plant biomass of 32 *L. camara* cultivars/lines grown in ground beds in Florida (2008). Plants were grown in full sun in Balm, FL, and fruit was collected from July through Nov. 2008 at 5-week intervals.

Cultivars/breeding lines (ploidy level)	Plants producing fruit (%)	Peduncles producing fruit (%)	Peduncles examined	Fruit produced by peduncles	Fruit per peduncle (no.)	Peduncles showing insect damage (%)	Seed germination (%)	Female fertility index ^z	Plant dry wt (g)
Cream (2x)	93.8	14.7	319	62	0.193 h-j ^y	77.3	10.3 i-j ^y	0.020	634
Denholm White (2x)	6.3	0.3	303	1	0.003 j	42.9	100.0 a	0.003	50
LAOP-9 (2x)	75.0	21.3	191	93	0.344 g-j	56.7	10.0 k-l	0.034	15
LAOP-30 (2x)	87.5	22.0	223	107	0.435 f-j	60.8	60.0 b	0.261	26
Lola (2x)	100.0	43.6	307	289	0.922 d-f	68.4	16.2 f-k	0.149	93
Athens Rose (3x)	18.8	0.6	305	2	0.006 j	63.4	0 l ^x	0.000	728
Landmark Peach Sunrise ^w (3x) ^y	100.0	16.0	319	56	0.175 h-j	42.1	57.1 b-c	0.100	218
Landmark Pink Dawn (3x) ^y	100.0	53.1	318	392	1.232 c-e	66.7	48.6 b-d	0.599	996
Lemon Drop (3x) ^y	100.0	60.7	315	401	1.270 c-e	47.8	9.4 h-l	0.119	506
Lucky Red Hot ^z (3x)	81.3	9.1	316	30	0.094 h-j	71.1	11.1 k-l	0.010	167
Miss Huff (3x) ^y	100.0	47.4	321	285	0.890 d-f	77.5	18.4 e-k	0.164	944
New Gold (3x) ^y	100.0	43.9	319	243	0.763 e-g	55.4	26.8 d-j	0.205	622
New Red Lantana (3x) ^y	100.0	46.0	318	264	0.832 e-g	66.8	24.4 d-k	0.203	615
Patriot Fire Wagon (3x) ^y	100.0	44.7	315	255	0.809 e-g	62.0	45.9 b-e	0.372	517
Red Butler (3x) ^y	100.0	39.0	318	184	0.579 f-h	55.2	9.3 h-l	0.054	672
Red Spread Lantana (3x) ^y	100.0	33.5	317	164	0.518 f-j	56.8	21.6 d-k	0.112	687
Samson Lantana (3x) ^y	100.0	58.6	318	436	1.379 b-d	71.0	26.8 c-i	0.370	647
Sunset Lantana (3x) ^y	100.0	42.2	321	288	0.895 d-f	57.9	33.5 c-h	0.300	323
Carlos (4x)	100.0	72.3	317	592	1.870 b	73.3	14.2 f-j	0.266	80
Dallas Red (4x)	81.3	26.0	312	179	0.573 f-h	61.7	39.1 b-g	0.224	161
Gold (4x) ^y	100.0	60.0	317	446	1.401 b-d	64.6	9.3 h-l	0.130	663
Irene (4x)	93.8	68.3	298	478	1.568 b-c	63.7	11.8 h-k	0.185	101
Pink Caprice (4x) ^y	100.0	98.8	317	2280	7.173 a	85.6	41.8 b-f	2.998	967
Radiation (4x) ^y	100.0	53.4	320	510	1.594 b-c	71.9	12.4 g-k	0.198	431
629-1 (5x) ^y	56.3	9.7	319	45	0.141 h-j	63.3	0 l ^x	0.000	87
629-2 (5x) ^y	87.5	10.1	317	34	0.108 h-j	59.0	0 l	0.000	125
Cajun Pink (5x) ^y	100.0	31.5	317	135	0.426 f-j	54.5	0 l	0.000	383
Patriot Hallelujah (5x) ^y	56.3	2.7	322	10	0.030 j	52.6	0 l	0.000	328
Spreading Sunset (5x) ^y	100.0	50.5	317	287	0.906 d-f	50.1	15.1 f-k	0.137	404
620-10 (6x) ^y	43.8	4.9	305	14	0.053 i-j	71.3	0 l	0.000	282
621-4 (6x) ^y	31.3	1.3	320	4	0.013 j	69.6	0 l	0.000	262
Tangerine (6x)	100.0	41.4	314	173	0.557 f-i	50.0	7.1 i-l	0.040	184

^xFemale fertility index resulted from multiplication of fruit per peduncle and seed germination (in decimal form).

^yDifferent letters indicate significant mean differences within the column based on the least significant difference procedure at $P \leq 0.05$.

^zNo seed available for germination experiments. The highest seed germination rate was used from other experiments where seeds were available and sown.

^wThe word "Improved" was removed from the cultivar name.

^vIndicates unreduced female gamete producers.

Table 3. Average fruit production, seed germination, and female fertility index of 32 *L. camara* cultivars/breeding lines by ploidy level and unreduced female gamete (UFG) production.

Ploidy level	UFG producers	Cultivars/breeding lines (no.)	Avg fruit per peduncle ^z	Lowest FPP	Highest FPP	Lowest germination	Highest germination	Avg germination ^z	Female fertility index
2x	No	5	0.47 ^c c-d (0.38) ^x	0.003	0.922	10.0	100.0 ^y	24.1 a-b (39.3) ^x	0.094
3x	No	2	0.05 d	0.006	0.094	0 ^w	21.6	11.1 b-c	0.005
3x	Yes	11	0.85 c	0.175	1.379	9.3	57.1	29.3 a	0.236
4x	No	3	1.34 b	0.573	1.87	11.8	39.1	21.7 a-b	0.225
4x	Yes	3	3.39 a	1.401	7.173	9.3	41.8	21.2 a-c	1.109
5x	Yes	5	0.32 d	0.03	0.906	0 ^{y,w}	15.1	3.8 c	0.027
6x	Yes	2	0.21 d	0.013	0.557	0 ^{y,w}	7.1	2.4 c	0.013

^zDifferent letters within a column denote significant differences among the mean values by the least significant difference procedure at $P \leq 0.05$.

^yOnly one seed was available and sown.

^xValues in the parentheses included the data of 'Denholm White', which was excluded from the statistical analysis.

^wNo seed germination also observed in other experiments we have conducted.

triploids had a seed germination percentage between 18.4% and 57.1%. The average seed germination of these triploids was 29.3%, $\approx 264\%$ of that of the non-UFG-producing triploids. The average FFI of these triploids reached 0.236, higher than that of the non-UFG-producing triploids and higher than that of the diploids.

The UFG production trait was initially observed only in a number of *L. camara* tetraploids (Czarnecki and Deng, 2009). It is interesting that this trait is also widespread in commercial triploid *L. camara* cultivars. In one experimental triploid (Czarnecki and Deng, 2009), this trait greatly increased the fruit or seed production capacity in *L. camara*. As shown earlier, similar fertility-restoring effects are also present in many commercial triploid cultivars. Thus in *L. camara*, triploidy alone is not able to provide adequate levels of female sterility. Rather, it would be critical and necessary to eliminate the UFG production trait to produce highly sterile *L. camara* cultivars.

Fruit production, seed germination, and FFI of tetraploid *L. camara*

Non-UFG-producing tetraploids. As reported previously (Czarnecki and Deng, 2009), 'Carlos', 'Dallas Red', and 'Irene' do not have the UFG-producing trait. Their fruit per peduncle values were 1.870, 0.573, and 1.568, respectively, averaged to 1.340. Thus, these tetraploids seemed to be more prolific in fruit production than *L. camara* diploids (group FPP average 0.225) (Table 3). The seed germination percentage of these tetraploids was 14.2%, 39.1%, and 11.8%, respectively (Table 3). Their average seed germination was 21.7%, which is lower than that of the diploid *L. camara* (39.3%). Their FFI value was similar, between 0.185 ('Irene') and 0.266 ('Carlos'). As a group, their average FFI was 0.225, close to the average FFI of UFG-producing triploids but 239% higher than the average FFI of diploid *L. camara*.

UFG-producing tetraploids. Three commercial cultivars belong to this group. 'Gold' and 'Radiation' had high FPP values (1.401 and 1.594) but relatively low seed germination (9.3% and 12.4%), and consequently a moderate FFI (0.130 and 0.198), similar to the FFI of the non-UFG-producing tetraploids and many of UFG-producing triploids

(Table 3). In contrast, 'Pink Caprice' had ≈ 15 - to 23-fold higher FFI (2.998) than the other UFG-producing triploids, the highest among all *L. camara* examined. This high FFI was due to its extremely high FPP (7.173) and high seed germination (41.8%). Compared with 'Gold' or 'Radiation', 'Pink Caprice's FPP and seed germination were ≈ 3.5 -fold more and ≈ 2.4 -fold higher, respectively.

As a group, the average FPP of UFG-producing tetraploids was 3.39, indicating that they were the most prolific fruit producers in *L. camara* (Table 3). The average seed germination of UFG-producing tetraploids was 21.2%, which is similar to that of non-UFG-producing tetraploids and UFG-producing triploids. The average FFI of UFG-producing tetraploids was 1.109, which is at least four times the average FFI of non-UFG-producing tetraploids and UFG-producing triploids and the highest in all groups of *L. camara* examined. Because of this high level of female fertility and their ability to transmit the UFG production trait to progeny (Czarnecki and Deng, 2009), this group of plants should be avoided for breeding efforts toward producing sterile lantana cultivars.

Fruit production, seed germination, and FFI of pentaploid and hexaploid *L. camara*

Three of the five pentaploids ('Cajun Pink', breeding line 629-1, and 629-2) produced small amounts of fruit and had the FPP value between 0.108 and 0.426 (Table 2). None of the seeds extracted from their fruit germinated, resulting in an FFI value of 0. 'Patriot Hallelujah' produced even fewer fruit and had a very low FPP value (0.030), close to that of non-UFG-producing triploids. None of its seeds germinated, resulting in an FFI of 0. Compared with these pentaploids, 'Spreading Sunset' was relatively fertile, with an FPP value of 0.906, 15.1% seed germination, and an FFI of 0.137, similar to the respective values of many of the diploids.

The two hexaploid breeding lines (620-10 and 621-4) produced few fruit (0.013 or 0.053 FPP), and none of the few seeds extracted from the fruit germinated, thus their FFI was 0 (Table 2). 'Tangerine' was the only hexaploid cultivar, which had a moderate FPP value (0.557) but a low seed germination percentage (7.1%) and a low FFI (0.040).

Correlation analysis

Strongest positive correlation was found between the FFI and FPP ($R = 0.93916$, $P < 0.0001$) (Table 4). The correlation between FFI and seed germination was not significant ($R = 0.24989$, $P = 0.1678$). This indicates that FPP is of much greater influence to the overall female fertility of *L. camara* than seed germination.

Interestingly, insect damage was positively correlated to FPP ($R = 0.47797$, $P = 0.0057$) (Table 4). The cause(s) of this positive correlation remains to be found. We suspect that this might be due to insects being more likely to feed on plants with more fruit rather than the insects caused higher seed sets.

Correlation analysis also indicates a significant negative relationship between plant dry weight and pollen stainability ($R = -0.44461$, $P = 0.0108$). Most likely this correlation was largely because diploids had the smallest plant dry weight values and the highest pollen stainability. This correlation was expected as diploid *L. camara* plants often have high pollen fertility (Spies, 1984) but are dwarf and small (Sanders, 2001).

Further discussion

As has been shown, *L. camara* cultivars differed considerably in fruit (or seed) production and seed germination, the primary determining factors of lantana female fertility. The difference was particularly obvious in the number of fruit produced per peduncle. Ploidy level and the UFG-producing trait played a significant role in determining the fruit production capacity of *L. camara*. Triploids without the UFG-production trait produced the least amount of fruit per peduncle, thus most sterile. The UFG-producing trait is widespread in many *L. camara* cultivars. It is critical and necessary to eliminate this trait to achieve high levels of female sterility in *L. camara*. Results also have showed that there are other genetic mechanisms causing female sterility in some *L. camara* cultivars (such as 'Denholm White'), although the mechanisms remain to be revealed.

Ploidy manipulation, particularly development and selection of triploids, has been used to produce sterile cultivars in various fruit and vegetable crops. This approach is being employed to sterilize ornamental plants that are highly valuable yet invasive

Table 4. Correlation coefficients (R) among female and male fertility-related traits and statistical probability (P values) in *L. camara* cultivars/breeding lines.

	Plant dry wt	Plants setting fruit	Peduncles setting fruit	Insect damage on peduncles	Fruit per peduncle	Seed germination	Pollen stainability ^a
Female fertility index	0.42025 (0.0166)	0.21442 (0.2386)	0.58338 (0.0005)	0.45796 (0.0084)	0.93916 (<0.0001)	0.24989 (0.1678)	0.23396 (0.1975)
Plant dry weight		0.28524 (0.1136)	0.41635 (0.0178)	0.35861 (0.0439)	0.4138 (0.0186)	-0.01172 (0.9492)	-0.44461 (0.0108)
Plants setting fruit			0.70624 (<0.0001)	0.12121 (0.5087)	0.35855 (0.0439)	-0.05966 (0.7457)	-0.17009 (0.3520)
Peduncles setting fruit				0.31844 (0.0757)	0.78601 (<0.0001)	-0.06826 (0.7105)	-0.05698 (0.7568)
Insect damage on peduncles					0.47797 (0.0057)	-0.2346 (0.1962)	0.13397 (0.4648)
Fruit per peduncle						0.14744 (0.4206)	0.2129 (0.242)
Seed germination							0.17004 (0.3521)

^aPollen stainability data from Czarniecki et al., 2014.

The correlation coefficient (R) between fruit per peduncle and female fertility index was bolded as the R value is above 0.9 and its P value was below 0.0001.

(Anderson, 2006; Bechtloff et al., 2019; Deng et al., 2017; Phillips et al., 2015; Ranney, 2004; Vining et al., 2012). A major advantage of this approach is that it is generally much less costly and much less controversial to undertake than transgenics-based approaches. Results from this study clearly indicate that production and selection of triploids will be an effective approach to sterilizing *L. camara*. Nevertheless, it is imperative to integrate this approach with selecting proper diploid and tetraploid breeding parents that do not carry the UFG-producing trait. Should the breeding parents carry the UFG-producing trait and produce unreduced female gametes, the resulting triploids will likely produce significant amounts of fruit (and seeds) and have considerable female fertility and invasive potential.

In our previous study (Czarniecki et al., 2014), eight triploid cultivars out of 26 commercial cultivars evaluated for pollen stainability were highly male-sterile, with <10% pollen stainability. However, all these eight cultivars produced considerable amounts of fruit (0.518 to 1.379 per peduncle) and were female-fertile (FFI 0.119 to 0.599) in this study. Two of the 26 cultivars evaluated in this study, 'Athens Rose' and 'Patriot Hallelujah', had low female fertility (0.006 or 0.030 FPP; FFI 0); however, they were male-fertile (pollen stainability 20.5% or 41.9%). Thus, none of the commercial triploid cultivars evaluated were simultaneously male and female sterile. These results indicate a strong need to develop new lantana cultivars that will be both male- and female-sterile. The results also suggest significant challenges toward this goal in lantana breeding. More research is needed to identify sources of germplasm that do not carry the UFG-producing trait, understand the inheritance of this undesirable trait, and develop molecular and genomic tools that could allow selection against this trait. Additional research is also needed on the following questions: 1) Would the germinated seedlings actually survive and reproduce in landscapes or the wild? and 2) Would non-germinating seeds in this study have germinated in subsequent years and were simply dormant? In future studies, vital staining with tetrazolium may be used to assess lantana seed viability before seed germination experiments.

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