GENETIC STERILIZATION AND REPRODUCTIVE BIOLOGY OF *LANTANA CAMARA*

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To my family and friends

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Completion of this project required the help of numerous individuals over the last few years. I would like to thank my parents for understanding my absence from their lives and my inability to participate in the important events that I have missed because of the commitments associated with my degree. My friends over the years have provided me with support, advice, and much needed grounding without which I would not have had the focus to complete my projects. Lastly I would like to thank my professor and advisors, who have given me the guidance and advice to learn and grow as a scientist.

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APHIS	Animal Plant Health Inspection Service
CB	Cotton blue vital stain
CP	Controlled pollination
CMS	Cytoplasmic male sterility
DNA	Deoxyribonucleic acid
DUFG	Double unreduced female gamete production
EBN	Endosperm balance number principle
FDA	Fluorescein diacetate stain
FDR	First division restitution
FFI	Female fertility index
FI	Flowering intensity
FLEEPC	Florida Exotic Plant Pest Council
FPP	Fruit per peduncle
LSD	Least significant difference
NASS	National Agriculture Statistics Service
NFREC	North Florida Research and Education Center
NTSYS	Numerical taxonomy system computer program
OP	Open pollination
PCA	Principal Component analysis
PCR	Polymerase chain reaction
PID	Percent insect damage
PFP	Percent fruiting peduncles
PQ	Plant quality
RA	Risk assessment

SAS®	Statistical Analysis Software
SDR	Second division restitution
SP	Self pollinated
SSR	Simple sequence repeat
UFBL	University of Florida breeding line
UFG	Unreduced female gamete
UMG	Unreduced male gamete
UPL	Unknown Pittsburg line
USDA	United States Department of Agriculture

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Lantana camara is an important ornamental and landscape plant. Yet, it is a Category I invasive species that that can hybridize with the Florida native species *Lantana depressa*. Sterile cultivars are needed as a preventive measure to control the invasiveness of *L. camara*. This study sought to identify the primary biological factors controlling *L. camara*'s male and female fertility, to assess *L. camara*'s hybridization potential with *L. depressa*, and to develop new sterile *L. camara* cultivars.

Male fertility was assessed based on pollen stainability. Pollen stainability varied from 1.8% to 81.1% among 32 *L. camara* cultivars. Ploidy level was found to be the most important factor determining *L. camara* pollen stainability. On average, diploids exhibited the highest pollen stainability, followed by tetraploids, pentaploids, hexaploids, and triploids. *L. camara* cultivars differed considerably in fruit production, ranging from 0.003 to 7.173 fruit per peduncle. Ploidy level, unreduced female gamete (UFG) production, and apomixis played significant roles in determining the fruit production capacity of *L. camara*. Triploids not producing UFGs and apomitic seed were most female-sterile.

Ploidy and simple sequence repeat marker analyses showed that *L. camara* could form three types of female gametes and two types of male gametes and develop seed through fertilization or apomixis, leading to six modes of reproduction and resulting in six types of progeny [$n + n$, $n + 0$ (haploidization), $2n + n$ (sexual polyploidization), $2n + 0$ (clonal seed), $4n + n$ (sexual polyploidization), and $4n + 0$ (apomictic polyploidization)]. UFG and apomictic seed production restored the female fertility of triploids in *L. camara*. The two traits were not observed in any of the five diploids but were present in three of the six tetraploids evaluated in this study.

Pollen stainability of *L. camara* was the most important factor determining the potential of *L. camara* as a male parent to hybridize with *L. depressa*. Diploid *L. camara* cultivars were the most compatible with *L. depressa*. Triploid *L. camara* with pollen stainability below 10% showed little potential to cross-pollinate *L. depressa*. When *L. camara* was pollinated with *L. depressa,* ploidy level and mode of reproduction of *L. camara* were the primary factors determining fruit production. UFG-producing triploid *L. camara* was highly crossable as a female with *L. depressa*, whereas non-UFG-producing triploid *L. camara* was the least crossable with *L. depressa.*

Interploidy crosses made between diploid and tetraploid cultivars/breeding lines not carrying the UFG production and apomixis traits resulted in four triploids that showed high levels of male and female sterility, and performed and flowered well in southern, central, and northern Florida. These triploids have shown potential to be released as new sterile cultivars.

CHAPTER 1
INTRODUCTION

Overview and Rationale

Floriculture Industry and Concerns about Introduced Plant Species

The floriculture industry is one of the most important sectors of production agriculture in the United States. According to the most recent USDA report, this industry generated a total wholesale value of $3.83 billion (www.usda.gov) [U.S. Department of Agriculture/National Agricultural Statistics Service (USDA/NASS), 2010]. Of the total wholesale value $2.3 billion were from garden/bedding plants and herbaceous perennials. Nationwide, Florida is the second largest producer of bedding plants and flower crops, generating a wholesale value of $696 million annually. More than 20% of the bedding plants and flower crops used in the United States are produced in Florida. This industry is a major segment of the State's environmental horticulture industry, which was estimated to generate an economic impact of ~$15.2 billion annually and provide 294,179 jobs (Hodges and Haydu, 2006).

One distinct feature of the floriculture industry is that it produces and uses hundreds, of plant species, to meet consumers' needs for plant materials. Both producers and consumers are constantly looking for new species and cultivars to introduce. Frequently many new introductions come from other states, countries, or continents (Anderson, 2004b). Many introductions are primarily made based on their plant, foliage and flower characteristics, i.e. attributes closely associated with ornamental values.

A major concern about introduced plant species in the floriculture industry is their potential to become weedy, even invasive. When this happens, the introduced species

may cause significant ecological, environmental, and economic damages. This may result in high costs to manage or control the introduced species, which may far outweigh the benefits the species have brought to the industry and society (Simberloff, 1996).

Plant breeding has played a critical role in providing new cultivars to fuel the growth of the floriculture industry (and other agricultural and horticultural industries) (Anderson, 2004a). It has been suggested that plant breeding should help address the invasive species issue facing industry by developing and selecting non-invasive cultivars (Anderson, 2006).

Lantana camara

Lantana camara is a member of Verbenaceae L. (1753). It originated in Central and South America and the West Indies (Sanders, 2001) but European explorers introduced and spread it to almost all the tropical colonies by 1900 (Howard, 1969). Plants of this species produce attractive flowers year-round, attract numerous species of pollinators (including at least 24 species of butterflies, Schemske, 1976; Goulson and Derwent, 2004), tolerate harsh environmental conditions (droughts, pollution, salts, etc.), and have low maintenance requirements. These attributes make lantana an ideal plant for landscape use (Arnold, 2002; Klingaman, 2000; Mungai, 1999; Starman and Lombardini, 2006; Veracion, 1983). The species has even been evaluated for chemical byproducts and biofuel production (Ghisalberti, 2000; Prasha, 2007; Sahu and Panda, 1998). Chemical byproducts produced have been useful although limited thus far. Additionally, lantana is easy to produce commercially. The species has perfect flowers borne on a spicate raceme and is self compatible and will set seed in the presence of

various pollinators (Czarnecki, 2009; Heywood 1993; Sanders, 2001). Commercial production of the species is done by vegetative stem cuttings which usually root readily.

With high ornamental values, *L. camara* is a very important floricultural crop in many parts of the world, especially in the southern United States (Beaulieu, 2008; Hammer, 2004; Howard, 1969). According to a survey conducted in 2004, 19% of responding nurseries in Florida grew lantana commercially, generating an estimated value of $40 million. This one species accounted for over one percent of Florida's total nursery industry plant sales. In Florida, lantana production provides 288 jobs (Wirth et al., 2004). *L. camara* is a very important crop in a number of other states in the southern United States as well.

Lantana breeding has been very active in increasing diversity in lantana plant growth form and flower color. It was estimated that over 650 varieties have been developed over the last 200 years. As of 1969, there were over 600 named varieties. These numbers indicate consumers' strong interest in lantana (Day et al., 2003; Howard, 1969; Hammer, 2004).

Need To Sterilize *Lantana camara*

L. camara has naturalized through most of the tropical and sub-tropical world (Ramey, 1999; Sanders, 2006) and it has been considered one of the 100 worst weeds in the world (Lowe et al., 2000). The only factor limiting the spread of this species seems to be temperature. *L. camara* does not survive extended periods of sub-zero (celcius) temperatures. In the United States, *L. camara* has been found in 14 contiguous states of the south, from North Carolina to California. It also grows in Hawaii, Puerto Rico, and the Virgin Islands (USDA NRCS 2011).

In Florida, *L. camara* is listed as a Category I invasive plant (FLEPPC, 2011). Sanders (1987a) listed 60 locations of hybridization between *L. camara* and the native *L. depressa*. All three varieties of *L. depressa,* var*. depressa*, var*. sanibelensis*, and var*. floridana,* have been shown to successfully hybridize with *L. camara* (Sanders, 1987). The vast occurrence of mutualist pollinators increases such hybridiztions (Suehs et al., 2006). Such interspecific hybridizations have resulted in genetic contamination of native species (Anderson and Ascher, 1994; Anderson, 2001).

L. camara has been shown to be able to out-compete native species in natural habitats. It does this by utilizing resources better and by hindering germination and the growth of other plants (Arora and Kohli, 1993; Duggin and Gentle, 1998). The authors also found this species to be highly opportunistic and more likely to infest disturbed sites with increased light availability, including cleared land for agricultural, commercial, and residential purposes.

These studies or observations show that *L. camara* can disturb natural areas, hybridize with native plants, and change the ecology of the environment (Hammer, 2004; Sanders, 1987a). Thus, *L. camara* has been considered one of the most invasive, persistent, and noxious weeds worldwide. It has been found to be invasive in over 30 different countries and well known with at least 96 common names (Morton, 1994). The invasiveness of *L. camara* also may threaten the floriculture industry. Because of its popularity and high demands, many nurseries are engaged in propagating and producing *L. camara,* as described above. If the invasiveness of *L. camara* is not effectively controlled and *L. camara* is subsequently banned from

propagation, production and utilization, a substantial economic impact is expected to occur to the industry (Wirth et al., 2004).

The spread of this species is also of concern due to its toxicity to animals. Wagstaff (2008) compiled a summary of over 60 reports of lantana causing illness in buffalo, cattle, sheep, and goats. Based on the type of animals already affected, it is likely that other ranging animals are also afflicted. Green fruit of *L. camara* can be toxic to young humans if consumed (Wolfson and Solomons, 1964).

A number of biological, physical, and chemical control measures have been used to control *L. camara*. (Cillers and Neser, 1991; Day et al., 2003; Graff, 1986; Graff, 1987; Ramey, 1999). However, in many situations these methods are not feasible due to high costs, labor requirements or the fact that infestation sites are inaccessible for treatments (Day et al., 2003). In many situations, biological control is the only viable long-term solution to managing *L. camara*. In Australia alone, millions of dollars and numerous projects have gone into searching for potential biological agents and introducing them to control *L. camara* (Thomas et al., 2006; Zalucki et al., 2007). Despite intensive efforts in many countries, biological control of lantana is only partially successful, and results are frequently poor or unstable (Broughton, 2000; Zalucki et al., 2007). Several native lantana species have overlapping distributions with *L. camara* (Castillo et al., 2007; Sanders, 1987a, 1987b, 2001). Consequently, the use of biological agents to control *L. camara* in Florida and the Caribbean region has been very limited.

Prolific seed and pollen production is the greatest determinant for *L. camara*'s invasiveness. Sexual sterilization of *L. camara* could eliminate its potential to spread

sexual propagules that may directly cause ecological or physical problems and prevent its cross pollination with native *Lantana spp*.

Invasive Plants

Potential Ecological and Economic Damages

Plant species have been introduced for many purposes such as ornamental use, environmental manipulation, and human or animal consumption. Introduced plant species may escape cultivation and invade natural, disturbed, and/or agricultural land. Examples of some invasive species that have altered ecosystems include climbing yams, kudzu, melaleuca, and tree of heaven (Miller, 2003; Myers, 1983; Rayamajhi et al., 2002).

Invasive plant species may cause substantial ecological, environmental, and/or economic damage. For example, invasive plants can reduce soil fertility (Wardle, 1994) and increase light competition that prevents reproduction and inhibits growth of native species (Weihe and Neely, 1997). Some invasive species may replace native flora with essentially mono-cultures of the invasive species. In Galapagos, *L. camara* out-competes a native plant that serves as the habitat of a native bird, ultimately endangering flora and fauna simultaneously (Cronk and Fuller, 1995). Plant invaders may also usurp local pollinators of showy flowering native species causing lower seed production, cause gene transfer from native species to the invading relatives allowing better adaptation to the invading hybrids further aggravating the problem (Brown and Mitchell, 2001; Kandori, 2009). Gleditsch and Carlo (2010) have shown that invasive species may provide additional food sources for local fauna. The effects on local wildlife could become a net positive for the environment or cause some species to become more prolific, thus changing the ecology of an area.

Approximately $34.5 billion is either spent or lost annually to invasive plant species (Pimentel et al., 2005). Groups responsible for control measures could invest small percentages of the total cost to stave off future problems and it would still amount to large sums to support preventive research. The economic impact associated with control and eradication of invasive ornamental species cannot be calculated directly. Since the sale of some invasive species with desirable horticultural traits is still occurring forcing nurserymen to stop their sale may be difficult. It is unknown exactly how much eliminating the sale of economically important species from a nursery will affect their bottom line and the employees needed to produce those crops (Perrings et al., 2002; Wirth et al., 2004). It has also been suggested that plant value for the horticultural industry may not only be measured by the value of sales but also the cost to control escaped species (Wirth et al., 2004).

Humans have been the primary culprits of invasive plant introductions (Mack and Erneberg, 2002; Mack and Lonsdale, 2001). Ornamental horticulturists were found to be responsible for over 230 woody plant species introduced for the landscape trade in the United States that have now naturalized (Reichard, 1994; Reichard and Hamilton, 1997). A study from Australia showed that 70% of the invasive plant species in Australia come from ornamental introductions (Virtue et al., 2004). It is expected that as human populations increase so will invasive plant species (Foxcroft et al., 2008).

Managing Invasive Plants

In the event a species takes hold and becomes of ecological or economic detriment, control measures should be taken. The best strategy for invasive species management seems to be proper screening of the materials before release to prevent the problem.

A review of measures taken to control Chinese tallow in Florida is a three step process: 1) removing mature plants, 2) revisit to remove seedlings and missed plants, 3) introduce biological controls if possible and continued surveillance. The review of this species suggests monitoring the area until the seed bank (deposited seed in the soil) is no longer a threat (Jubinsky and Anderson, 1996). This example highlights the difficulty in controlling established species and underscores the importance in prevention with sterilization and screening methods.

It seems that controlling the flow of invasive materials from the production side may be more practical than post establishment control. This is primarily because plant management regimes are rarely effective and economically feasible to control invasive species (Pimentel, 2000).

Regulatory Measures

To date the U.S. has a large number of regulations regarding the importation and use of plant materials. An extensive review of federal regulations by Freeman et al. (2009) describes that plant importation has been a continually evolving process. The initial attempt to manage invasive plants began in 1912. The first legislation was the plant quarantine act which gave the federal government the ability to regulate the import and transfer of plant material across state lines and into the country in general. The oversight of plant material was consolidated within the United States Department of Agriculture (USDA) under the Animal and Plant Health Inspection Service (APHIS). The primary function of APHIS is to regulate and control the movement and management of plant species to prevent exotic species from causing harm to the United States. Since the formation of APHIS, its role has expanded to enforce more rigorous importation standards. The problem of invasive species became so important that in 2004 the Plant

Protection Act of 2000, which generally condensed the agencies involved in invasive plant management, was amended to allow for the Secretary of Agriculture to provide grants for weed control as appropriated by Congress.

A parallel means to control these invasive species was made in 2001, when the National Invasive Species Council was formed by executive order. Similar legislation has led to recommendations in Australia for plant risk assessment (RA) agencies. Keller et al.'s (2007) recommendation suggests the costs of preventive RAs would be negligible when compared to the costs of controlling newly released invasive species. Studies of the U.S. specifically (Reichard and Hamilton, 1997) indicate models of invasion patterns with recommendation protocols to screen plants for horticultural use. These studies suggest that local or regional screening and selection panels of new materials would be best to determine appropriate and low risk plant introductions (Reichard and White, 2001).

In Florida invasive plants are a highly debated subject. To help guide issues related to invasive species, an invasive species management agency has been formed called the Florida Exotic Pest Plant Council (FLEPPC). This council designates problem species and works to make recommendations for control and management (FLEPPC, 2011).

The council has divided plants into two categories each of which determines a different level of invasiveness. Category I species are plants that have shown the ability to change the structure or ecology of an environment. Species with the ability to cross pollinate with native species are also placed in this group. Category II species have been shown to be abundant in the Florida ecosystem but have not yet met the criteria of

category I species (FLEPPC, 2009). Plants in the second category may be changed to the first if damage to the ecosystem is demonstrated.

This council has also provided several definitions that are useful when describing invasive species. These definitions include: Exotic (a species introduced to Florida), Native (a species naturally occurring in Florida), Naturalized Exotic (an exotic species that has essentially "become" native due to its self sustainability), and Invasive Exotic (a species that is expanding its presence in native plant communities). The council has listed over 140 plant species in categories I and II for the state of Florida (FLEPPC, 2009). Keller et al. (2007) suggests that boards such as the FLEPPC will cause the percentage of invasive plants introduced to decrease as better management and predictive practices are implemented to screen new plant introductions.

Biological Factors Determining Invasive Potential of Plants

Seed Production and Dispersal

The ability to produce and disperse seed is one of the most critical aspects of a species' survivability. The degree to which a plant is able to accomplish this goal is one of the main factors determining the invasive potential of a species (Dozier, 1999). Thus, seed (and fruit) production and seed germination have been the primary criteria in evaluating exotic species' invasive potential (Wilson and Mecca, 2003; Trueblood et al., 2010).

Pollen Production and Dispersal

The ability of exotic plants to produce viable pollen could cause grave problems to native congeneric species. This ability can allow exotic plants to out-cross with native relatives and other members of the species, which can contaminate the native plants' gene pool, even endanger the native species (Sanders, 1987a). Cross pollinations may

lead to the development of hybrid populations that can spread over vast distances (Moody and Les, 2007). The absence of viable male gametes would dramatically reduce the exotic plants' ability to form hybrids. Studies to determine the potential hybridization likelihood should be conducted to test the ability of pollen to cause seed set (Olsen and Ranney, 2006 and Trueblood et al., 2010). In addition, pollen-mediated gene flow from exotic species may cause native populations to change dramatically. These changes may cause natives to become increasingly prevalent or cause detrimental effects to local and native populations (Wright, 1931). Research about the effects of this type of invasive behavior needs to be conducted since limited information is available currently (Eastham and Sweet, 2002)

Vegetative Propagules

Vegetative propagation is a trait of concern for plant species that are able to produce rhizomes, stolons, or other vegetative propagules as means of plant spread. Determining the vegetative reproduction potential is critical with clonal invasive species (Bímová et al., 2003). However, in some cases (for example, invasive watermilfoil), it was not clear to what extent the spread of the invasive was due to hybridization events or the vegetative spread of the plant materials (Moody and Les, 2007). This shows the importance of assessing multiple traits that might be associated with invasive potential.

Assessing Invasive Potential of Plants

A compilation by Anderson (2006) of major invasive characteristics for screening included flowering ability, pollinator attractiveness, non-dispersed seed, unattractive fruiting structures for consumption, non-vigorous plants, lack of seed germination, and the degree of sterility of the plant affecting male and female gamete formation. However, the most common methods for assessing plant invasive potential include

pollen viability testing for male reproduction, quantifying seed production and viability through open pollination or controlled crosses for female reproduction, and characterizing the ability and amount of vegetative spread of a plant.

For different invasive species, the primary biological factors determining the species' invasive potential may be different. For example, Dozier (1999) focused on the female fertility of *Ardisia crenata*. The main aspects Dozier identified for characterization included a plant's survivability in the environment in question and its ability to disperse and produce seed. Wilson and Mecca (2003) investigated the female fertility of *Ruellia tweediana* by cataloguing the production of seed and testing the viability of that seed. A similar study evaluating invasive potential was conducted on *Nandina domestica* (Knox and Wilson, 2004). Studies such as these provide a strong basis for recommending whether a species should be considered potentially invasive. Assessment of invasive or potentially invasive species should include any tools available to quantify the relative aggressiveness of a species. These assessments will allow for better recommendations for effective management of existing populations (Roush and Radosevich, 1985).

Genetic Approaches to Reduce or Eliminate Invasive Potential of Plants

Over the last one to two decades, significant breeding efforts have been undertaken to sterilize some of the most important, yet invasive ornamental species (Ranney, 2004). These breeding efforts adopt a variety of approaches, including traditional, mutational, and transformational methods. Each method has its positive and negative attributes. Anderson (2006) reviewed the most promising avenues of plant sterilization; breeding and genetic transformation. The method used is highly species dependant. An example of this is *Hieracium aurantiacum*, an old ornamental plant that

has been found to be largely clonal (from apomixis) in its distribution across North America. Traditional breeding would be difficult with this species as the plant produces apomictic seed (Loomis and Fishman, 2009). However, genetic transformation may provide a new possibility.

The methods for sterilization will also depend on the genetic composition of the species and their ultimate values. For most ornamentals, it would only be practical to sterilize through traditional breeding manipulating existing sterility-inducing genes, incompatibility systems, ploidy levels, etc. These methods are less controversial and may have greater market acceptance (Anderson, 2006).

Traditional Breeding

Cytoplasmic male sterility (CMS) has been widely used to create sterility in plants (Duvick, 1959). Other breeding methods that have been used for plant sterility include incompatibility systems (Culley and Hardiman, 2007), sterility genes that control male or female fertility (Singh, 2003), and the production of interspecific or wide hybrids. Collectively, these sterilization mechanisms are naturally occurring and breeders can manipulate them accordingly.

Caution may need to be taken when using some of these breeding methods. The Callery pear is a good example. It was considered to be sterile due to self-incompatibility and non-invasive, but now it has become invasive because of the addition of new cultivars. These new cultivars are cross-compatible with the original Callery pear, and these cross compatibilities have allowed invasive behavior to occur (Culley and Hardiman, 2007). This case illustrates the importance of understanding the interactions of cultivars developed through traditional breeding.

As described by Anderson (2006), self-incompatible non-invasive cultivars may regain fertility due to some sort of sterility break-down, which may allow a previously safe species to now spread freely by back-crossing with the progenitor plants.

Some breeding approaches can create sterility, but they may not always be the most useful for a given breeding system. For example, generating CMS lines in a species can be done by introducing alien cytoplasm (Peohlman and Sleper, 1995). However, maintaining the CMS lines may not always be economically feasible (Duvick, 1959). A breeding dead-end may frequently be encountered when new cultivar releases have both male and female sterility.

Mutational Breeding

The premise of mutation breeding is that adding a mutagen (usually radiation or chemical) to plant material will cause irreparable harm to the DNA of the plant and will affect genes necessary for sexual reproduction or disrupt some other cytological process needed for fertility (Elkonin and Tsvetova, 2008). In general the problems associated with this process include difficulties achieving high levels of sterility, stabilizing the mutations, and obtaining adequate rates of successful mutations.

Transgenic Approaches

Transgenic approaches also show promise as a means to control invasive plants (Park et al., 2002). Potentially, genetic transformation could generate very high levels of male and/or female sterility with good stability. This potential has been realized in some model plant species either by inserting a gene that can cause sterility or by silencing a gene that is necessary for male and/or female reproduction. A good example of transgenic sterility can be found in petunia. Dotson et al. (1996) introduced a gene that caused male and female sterility. Petunia is a model system in which tissue

culture and transformation protocols have been well defined. However, many ornamental plant species do not have efficient tissue cultural protocols and/or transformation protocols, thus increasing the difficulty of applying transgenic approaches. In addition, transgenic plants are considered highly controversial, and consumer acceptance may become an issue (Anderson, 2006, Eastham and Sweet, 2002).

Ploidy Manipulation

This approach has been used very successfully in a number of fruit and vegetable crops (Kihara, 1958). It is being explored in numerous ornamental plants. A major advantage of this approach is that it is generally inexpensive to undertake.

There have been some cases in which an invasive plant species has been sterilized through ploidy manipulation and the new sterile forms have been reintroduced to the market as safe alternatives (Trueblood et al., 2010). For example, *Catalpa* section *Catalpa spp*. breeding incorporated a hybrid approach of mutation and traditional breeding to generate higher ploidy levels and the hybridization of close relatives (Olsen and Ranney, 2006). This project produced highly sterile plants while simultaneously improving the ornamental characteristics of the plants. Ploidy manipulation could be a successful and practical means of improving the acceptability of many crops, especially when it is combined with traditional breeding approaches (Anderson, 2006).

Research Objectives

L. camara is a very important ornamental and landscape crop in the United States, especially in Florida. Yet, it is an introduced naturalized exotic species that has disturbed natural and agricultural lands, hybridized with native plants, and caused

ecological damage. If the invasiveness of *L. camara* is not effectively controlled and *L. camara* is subsequently banned from propagation, production and utilization, a substantial economic impact is expected to occur. Genetic sterilization has potential as an economical, preventive measure to control the invasiveness in *L. camara*. This research seeks to 1) identify the primary biological factors controlling *L. camara*'s male and female fertility, 2) assess *L*. *camara*'s hybridization potential with *L. depressa*, and 3) develop new sterile lantana lines that can be used to replace existing invasive forms. To fulfill these objectives, numerous experiments were conducted, and they are organized into six chapters for this dissertation.

CHAPTER 2
MALE FERTILITY OF *Lantana camara*

Rationale

The primary concern of ecologists about *L. camara*'s invasiveness in Florida is the species' ability to produce viable pollen and hybridize with *L. depressa*, an extremely rare species in Florida (FLEPPC, 2011; Hammer, 2004). Little information is available regarding the pollen viability of *L. camara* cultivars currently in commercial production. Previous studies of *L. camara*'s pollen viability were mainly on naturalized populations of plants. Results from those studies indicate a wide range of pollen viability in *L. camara* (Raghavan and Arora, 1960).

The main methods used in lantana pollen viability studies have been vital dye-based staining, including aniline blue solution (Spies, 1984c; Sanders, 1987b). Other authors have examined pollen viability but did not provide methods (Raghavan and Arora, 1960; Khoshoo and Mahal, 1967). Brewbaker (1967) observed that lantana pollen grains were binuclete and might have the potential to germinate on artificial media. Several attempts have been made to germinate lantana pollen on artificial media, but so far all in vain (Khaleel, 1972; personal observations from variations of Brewbaker and Kwack, 1963). The cause(s) of such in vitro germination failures remain to be identified. Thus pollen staining has been the primary method used in lantana pollen viability assessment. The resultant pollen stainability is considered as a maximum estimate of the pollen's viability and male fertility.

Understanding the relationships between *L. camara*'s pollen stainability and ploidy level and chromosomal association was the focal point of several researchers' studies (Spies and Stirton, 1982ab; Spies, 1984a). According to Raghavan and Arora (1960)

and Khoshoo and Mahal (1967), naturalized diploid *L. camara* could have 0-90% pollen stainability, while triploids and tetraploids could have 16-47% and 16-91% pollen stainability, respectively.

As regards to chromosomal pairing, Spies and Stirton (1982b) detected that diploid and tetraploid *L. camara* plants maintained the most normal chromosomal pairings during meiosis. Their data showed that 70-100% of meiotic chromosome configurations in diploids were bivalents and 39-82% of the chromosome configurations in tetraploids were bivalents. In their observation, 80-90% of pollen mother cells in hexaploids yielded normal meiotic products at telophase II. Triploids and pentaploids examined at the same stage exhibited normal meiotic products in 0-50% and 10-20% of the pollen mother cells, respectively. Their data indicated a strong correlation between normal bivalent chromosome pairing to high levels of pollen viability in diploids and triploids and a moderate level of negative correlation between trivalent and pollen viability in tetraploids.

The current study assessed the male fertility of important commercial cultivars of *L. camara*, to examine the relationships between ploidy level and male fertility, and to determine the potential of triploid generation for reducing *L. camara*'s invasive potential.

Materials and Methods

Plant Materials

There were 26 commercial cultivars and six University of Florida breeding lines (UFBL). Two sterile accessions of *L. movevidensis* (Henderson 1969), a relative of *L. camara*, were included as a negative control (Table 2-3). *L. camara* and *L. montividensis* plants were propagated by cuttings and grown in plastic containers (15.2–cm in diameter) filled with a commercial soilless mix (Fafard® 2P mix, Florida Potting

Soil). They were irrigated through drip lines and fertilized by constant feeding with 150 ppm of a 20-20-20 water soluble fertilizer (Gainesville greenhouses, Season one) or by incorporating a controlled-release fertilizer (Osmocote®,15N-3.9P-10K, 5-6 months release at 21°C; The Scotts Company, Marysville, OH) at 6.51 kg · m^{-3} (Gulf Coast Research and Education Center, Balm, FL; Season two). All plants were grown inside the greenhouse under natural light. The day and night temperature inside the greenhouses were set at 29/21°C and was maintained below 32 and above 15°C.

Ploidy Analysis

Analysis was performed using fully expanded young leaves and the Partec PA I ploidy analyzer and the CyStain UV Ploidy Precise P dye (Partec, Münster, Germany). The manufacturer- recommended ploidy analysis procedure was followed with minor modifications (supplemented with 2% w/v PVP and 0.01% β-mercaptoethanol) with dye mixture kept on ice. The ploidy level of a progeny was determined by comparing to one or more commercial cultivars (reference cultivars) with known ploidy levels that were included in the same analysis.

The ploidy levels of the reference cultivars were confirmed by counting chromosomes in root tip cells. Growing root tips of 'Cream', 'Gold', 'Pink Caprice', and 'Radiation' were collected from rooted cuttings, chilled at 8°C overnight and pre-treated with 0.05% colchicine at ambient temperature for 4 hrs, then fixed in Carnoy's (3:1 methanol:acetic acid) fluid for 2 d, and stored in 70% ethanol at 4°C. Fixed root tips were hydrolyzed in 1 N HCl at 60°C for 5 to 10 min, squashed in acet0-carmine on glass slides, and observed under a 1000x magnification on Olympus BH-2 microscope.

Chromosome counting results showed that ‘Cream’ is a diploid (2*n*=2*x*=22), and ‘Gold’, ‘Pink Caprice’, and ‘Radiation’ are tetraploids (2*n*=4*x*=44).

Pollen Staining

Two experiments were conducted to assess *L. camara*’s pollen stainability: the first one in April and May 2008 (Season 1) and the second one in November 2009 (Season 2). A comparison of two vital stains was comprised of eight commercial cultivars (‘Athens Rose’, ‘Cajun Pink’, ‘Carlos’, ‘Denholm White’, ‘Lola’, ‘New Gold’, ‘Pink Caprice’, and ‘Tangerine’) and *L. depressa* var *depressa*. The vital stains used to assess the pollen stainability of *L. camara* were lactophenol cotton blue (CB) (Eng. Scientific, Inc., Clifton, NJ) and fluorescein diacetate (FDA); (Sigma-Aldrich, St. Louis, MO). The commercial cotton blue stain solution contained phenol, glycerol, lactic acid, and anailine blue and was ready for use.

Flower clusters (inflorescences) were collected when the clusters each had one or more flowers partially open. Predehiscent anthers were removed from unopened flowers of each cluster and placed in a 1.5-mL Eppendorf tube with ~100-µL of cotton blue stain. Anthers were stained overnight at 65°C in a water bath and then rinsed three times with distilled water. Care was taken not to burst anthers while rinsing them. Rinsed anthers were squashed in 50-µL of 80:20% glycerol:water on a glass slide. Pollen grains were observed under a bright field microscope (Leica MZ16F or Olympus BH-2) using a 400x magnification objective. Photos of pollen grains were taken using Olympus Q Color 5 (Olympus Corporation of America, Center Valley, PA) or a Kodak Easy Share camera (Eastman Kodak, Rochester, NY) modified to attach to the microscope. Images of pollen grains were later viewed and counted on computer.

For FDA staining, the protocol of Helsop-Harrison and Helsop-Harrison (1970) was followed with minor modifications. Fresh anthers were collected from unopened flowers and stained overnight in a FDA solution containing 10^{-6} M FDA and 0.25 M sucrose at room temperature (~22°C) in the dark. Stained anthers were transferred onto a microscope slide and covered with a coverslip. The slide was gently tapped and pressed to release pollen grains out of anthers. Pollen grains were examined using a fluorescence microscope (Olympus BX 41). Uniformly round, non-wrinkled, brightly fluorescing pollen grains were considered viable. Non-fluorescing or lightly fluorescing and wrinkled or deformed pollen grains were considered non-viable.

Experimental Designs

Lantana plants from which flowers were collected for pollen staining were arranged in the greenhouse following a randomized complete block design with four (Season 1) or three (Season 2) replicates (plants). The experimental unit was single plants propagated by cuttings and grown in plastic containers. For each experimental unit, three flower clusters, approximately 3–4 flowers, and 12 anthers were examined. One (Season 2) to two (Season 1) slides were prepared for each experimental unit; three fields on a slide were randomly selected and photographed. All pollen grains in a given field were counted (Figure 2-1).

Statistical Analysis

Pollen stainability data were analyzed using PROC GLM in SAS® for Windows 9.2 (SAS® Institute, Cary, NC) to determine the significance of differences among ploidy levels, lantana lines, seasons, and staining dyes. Data transformation of stained pollen (%) was performed using the arcsine square root function. To determine differences among lines and ploidy levels seasons were combined. When differences were

significant, mean separation analysis was performed using the Tukey's W procedure in SAS®.

Meiotic Observation

Entire immature flower clusters, 1–20 mm in size, were collected from greenhouse-grown plants, fixed in a fresh, cold solution of methanol: acetic acid (3:1, v/v) for at least 24 hours, and kept in -20°C, as described in Brandão et al. (2009). From each inflorescence 12-15 anthers were removed and placed on a glass slide. Anthers were squashed in a small drop of modified carbol fuchsin stain (Kao, 1975) anther debris was carefully removed, and a cover slip was placed on the slide. At the time when pollen mother cells, microspores or pollen grains were released in the initial squash, the cover slip was then sealed with nail polish and then firmly tapped to distribute cells across the slide. The prepared slides were observed under an Olympus BH-2 compound microscope. Photos of pollen mother cells undergoing meiosis were taken using an Olympus Oly-750 microscope camera and the computer software Image Pro 6.2. Lantana cultivars examined for meiosis include 'Athens Rose', 'Lola', 'Miss Huff', 'New Gold', 'Pink Caprice', and 'Red Bandana'. Plants were provided for meiotic work to be conducted by Amanda Herschberger in Athens Georgia at the University of Georgia.

Results and Discussion

Ploidy Analysis

Among the 26 commercial cultivars analyzed for ploidy level; 13 were triploids, six were tetraploids, three pentaploids, and one was hexaploid. Only three cultivars were diploids. This ploidy level distribution is quite different from what was reported previously from wild collected accessions (Spies, 1984b), where tetraploids were more

common than triploids. This difference may reflect the increased efforts by commercial and private lantana breeders toward producing triploid lantana cultivars.

Considering the scarcity at diploid, pentaploid and hexaploid levels, efforts were made to identify breeding lines at these ploidy levels so that the effects of ploidy level on lantana male sterility could be assessed more adequately. Toward this, two additional diploid, pentaploid, and hexaploid breeding lines were added (LAOP-9, LAOP-30, 629-1, 629-2, 620-1, and 621-4) to the 26 commercial cultivars.

Dye Comparison for Lantana Pollen Stainability Determination

CB and FDA were used in parallel to stain the pollen of nine *Lantana* lines. The two staining methods revealed similar percentages of pollen stainability or viability in these cultivars. Statistical analysis indicates that the two staining methods were not significantly different (Table 2-1) and there was no interaction between staining methods and lantana lines used. Further, the pollen stainability by CB and the viability by FDA were highly positively correlated, with an R^2 of 0.94 ($p<0.0001$) (Figure 2-2). The results indicate that when staining lantana pollen, either dye could generate similar results. The stains were more effective than aceto-carmine and aceto-orcein which were also used preliminarily. FDA staining requires the use of fluorescence microscopes, thus CB was chosen for the rest of the pollen stainability assessments.

Pollen Stainability of *L. camara* Cultivars and Breeding Lines

Significant differences were found among pollen stainability of the cultivars (Table 2-3). In Season 1, the lowest pollen stainability was 0.8% (‘New Gold’) and the highest was 88.7% (LAOP-9), i.e. a 110-fold difference. In Season 2, the lowest and highest pollen stainabilities were 1.7% (‘New Gold’ again) and 76.7% (‘Lola’), respectively, and had a 45-fold difference.

Most of the cultivars/breeding lines (17) had similar (±5%) pollen stainability between the two seasons. However, the pollen stainability of nine cultivars ('Cream', LAOP-9, LAOP-30, 'Landmark Peach Sunrise Improved', 'Lucky Red Hot Improved', 'Carlos', 'Radiation', 'Cajun Pink', and 'Spreading Sunset') fluctuated between seasons (±10 to ±20.5%). Additionally, there were six cultivars whose pollen stainability changed by plus or minus 5 to 10%. These fluctuations resulted in an overall significant difference between the two seasons and significant interactions between cultivar and season (Table 2-3).

When data were pooled by ploidy level, diploids had the highest pollen stainability, an average of 74.8%, and triploids had the lowest, an average of 9.3% (Table 2-4). Pollen stainability of tetraploids was between those of diploids and triploids, an average of 45.1%. Pentaploids had an average of 34.6% pollen stainability, slightly lower than tetraploids', but the difference was not statistically significant. Hexaploids had even lower stainability, an average of 18%, significantly different from other ploidy levels. These results showed that ploidy level is an important factor determining *L. camara*'s pollen stainability.

Diploids

All diploid cultivars and breeding lines had high pollen stainability, ranging from 54.4% to 88.7%. Pollen stainability of 'Denholm White' and 'Lola' was consistent between seasons (± 4.3% to 8.9%), while that of 'Cream', LAOP-9 and LAOP-30 changed more (± 16.8% to 20.5%). Overall, 'Lola' and its two open-pollinated progeny had similar pollen stainability.

Triploids

Based on the average pollen stainability over two seasons, triploids could be divided into three groups. The first group had pollen stainability below 10%, which includes nine cultivars, ‘Landmark Pink Dawn’, ‘Lemon Drop’, ‘Miss Huff’, ‘New Gold’, ‘New Red Lantana’, ‘Red Butler’, ‘Red Spread Lantana’, ‘Samson’, and ‘Sunset’. Among them, ‘New Gold’ and ‘Miss Huff’ had the lowest pollen stainability, 1.8% to 1.9%. Their pollen stainability changed little (± 0.1 to 4.9 in percentage) between the two seasons. The second group had pollen stainability between 10% and 20% and consisted of two cultivars, ‘Lucky Red Hot Improved’ and ‘Patriot Fire Wagon’. Another two cultivars (‘Athens Rose’ and ‘Landmark Peach Sunrise Improved’) constituted the third group, whose pollen stainability was between 20% and 30%. Overall larger seasonal variation was observed in Groups 2 and 3 (4.6 to 10.5%) than in Group 1 (0.1 to 4.9%).

Tetraploids

Three cultivars, ‘Gold’, ‘Dallas Red’, and ‘Radiation’ had similar pollen stainability (26.2% to 32.3%), but overall, a large range of variation in pollen stainability was present among lantana tetraploids. ‘Irene’ and ‘Pink Caprice’ had an average of 57.4% and 73.5% pollen stainability, respectively, statistically similar to that of the diploids. The average pollen stainability of ‘Carlos’ was 49.4%, significantly different from diploids ‘Cream’ and ‘Lola’, but not significantly different from diploid ‘Denholm White’.

Morphological and molecular marker analysis has shown that ‘Gold’ is likely an allotetraploid resulting from interspecific hybridization between *L. camara* and *L. depressa*, while the rest of the tetraploid cultivars included in this study are autotetraploids (Gong and Deng unpublished). In most plants, allotetraploids from two species with highly divergent genomes behave like diploids in chromosome paring and

assortment during meiosis, and thus they generally have higher pollen viability or fertility than autotetraploids. It is interesting that ‘Gold’ a putative allotetraploid exhibited significantly lower pollen viability than ‘Irene’, and especially ‘Pink Caprice’. This may be due to some degree of genome homology that would lead to multivalents reducing successful meiotic products.

Pentaploids and hexaploids

Two of the pentaploid cultivars had pollen stainabilities similar to tetraploids (‘Cajun Pink’ and breeding line 629-2). The average pollen stainability of ‘Spreading Sunset’ was 19.5%, similar to that of hexaploids and some triploids (Groups 2 and 3).

‘Tangerine’ was the only hexaploid cultivar, and its average pollen stainability was 20.9%, similar to that of Groups 2 and 3 triploids. Two hexaploid breeding lines had similar pollen stainability (11.9% to 21.2%).

Meiotic Abnormalities

Flower clusters containing all unopen corollas ranging from 0.5 mm to 15 mm in size were examined in an attempt to identify the best stage for meiotic analysis. Fully developed pollen was found in all flower sizes examined. This indicates that meiosis must have occurred very early in flower development but was variable in most of the sizes screened as most meiotic stages were found.

Normal meiotic products were found in diploid and tetraploid cultivars (Figure 2-3) but abnormalities were also found in those same cultivars (Figure 2-4). Based on the cytogenetic analysis by Spies (1984a), there were no individuals with completely pairing sets of chromosomes. It seems likely the abnormalities found in this study are the result of the numerous univalents, trivalents, and quadravalents described in all ploidy levels assessed (Spies 1982b; Spies 1984ab). Chromosomal formations such as these would

likely lead to lagging chromosomes, abnormal telophase, uneven tetrads, and numerous microspores. In addition other observed meiotic dysfunctions were fused microspores, which may be a result of failed telophase II or additional aborted pollen. Meiotic analyses presented here would be the result of suggested chromosomal mispairings by Spies (1982b).

Previous work has shown a large number of meiotic paring arrangements that could lead to pollen abortion and abnormalities (Spies 1984a). The current work demonstrates the results of abnormal meiosis suggested by Spies. It is likely that the cultivars assessed are an amalgam of diverse genotypes similar cytogenetically to the hybrid swarm studied by Spies.

Summary

Male fertility of *L. camara*, as revealed by pollen stainability, varied greatly among cultivars, differing by 45 to 110 fold. An important factor determining *L. camara* male sterility was the ploidy level. This was determined by grouping individuals by ploidy level. On average, diploids exhibited the highest male fertility, followed by tetraploids, pentaploids, and hexaploids. Triploids had the lowest pollen stainability or the highest sterility. Thus generating triploids could be an effective genetic approach to reduce *L. camara'*s invasive potential. Significant pollen stainability variation was also found within certain ploidy levels. This variation suggests that genetic background or pedigree may also play a significant role in determining *L. camara*'s male fertility. Some of the triploids had pollen stainability approaching 20-30%. Thus new triploids require careful examination and screening to ensure that only highly sterile breeding lines or cultivars are selected. Overall, triploids, especially those with pollen stainability below 10%, showed little variation between growing seasons. The pollen stainability of a number of

cultivars varied significantly between seasons, suggesting potential influence of environmental conditions on pollen stainability. This work provides a characterization of the maximum male fertility of commercial materials currently available in the U.S. horticultural trade Combined with our meiotic analyses which yielded similar results expected from previous research (Spies and Stirton, 1982b) indicates that meiotic failures due to triploidy and abnormal pairing greatly reduce pollen viability. .

Table 2-1. Analysis of variance table for comparing two vital stains used to determine the pollen stainability of eight *Lantana camara* cultivars and a *L. depressa* accession. Pollen stainability tests were conducted at the University of Florida Gulf Coast Research and Education Center, Balm, Florida in 2010.

Source	DF	F value	P value
Cultivar[Z]	8	137.61	<0.0001
Stain[Y]	1	0.06	0.803
Cultivar[Z]*Stain	8	0.87	0.5508

[Z]Eight *L. camara* cultivars were tested and one accession of *L. depressa*
[Y]The two vital stains tested to determine if a difference existed were Cottone Blue and Fluorescein Diacetate.

Table 2-2. Analysis of variance table for pollen stainability of 32 *Lantana camara* and two *Lantana montevidensis* cultivars. Fresh pollen was stained with cotton blue at the University of Florida, Gainesville, Florida and University of Florida Gulf Coast Research and Education Center, Balm, Florida in 2008 and 2009 respectively.

Source	DF	F value	P value
Ploidy Level[Z]	5	165.36	<0.0001
Lines[Z]	33	74.17	<0.0001

[Z]In total 32 lines of *L. camara* and two lines of *L. montevidensis* were included in this study.

Table 2-3. Ploidy levels, pollen counts, and stainability of 34 lantana lines detected with cotton blue. Season 1 experiment was conducted in 2008 in Gainesville, FL and and Season 2 experiment was conducted in 2009 in Balm, FL.

Lantana spp. cultivars/lines	Ploidy[X]	Season 1 Pollen count	Season 1 Pollen stainability	Season 2 Pollen count	Season 2 Pollen stainability	Average pollen stainability[Y]
'Cream'	2*x*	4441	87.1 ± 1.1	649	70.3 ± 0.3	78.7 ± 8.4 a
'Denholm White'	2*x*	5116	68.3 ± 1.0	1251	72.6 ± 1.0	70.4 ± 2.2 a-c
'Lola'	2*x*	3492	85.6 ± 1.8	276	76.7 ± 3.3	81.1 ± 4.4 a
LAOP-9	2*x*	4089	88.7 ± 1.1	304	69.3 ± 17.3	79.0 ± 9.7 a
LAOP-30	2*x*	5691	54.4 ± 12.3	768	74.9 ± 9.6	64.6 ± 10.3 a–d
'Athens Rose'	3*x*	5348	20.8 ± 3.1	694	20.3 ± 3.5	20.5 ± 0.3 g–j
'Landmark Peach Sunrise'[Z]	3*x*	4055	21.8 ± 2.8	402	32.3 ± 0.9	27.1 ± 5.2 f–i
'Landmark Pink Dawn'	3*x*	4621	8.9 ± 3.2	731	4.0 ± 0.6	6.4 ± 2.5 k–m
'Lemon Drop'	3*x*	4318	5.7 ± 0.9	800	1.7 ± 0.6	3.7 ± 2.0 l–m
'Lucky Red Hot'[Z]	3*x*	4812	19.4 ± 0.9	373	9.3 ± 3.0	14.4 ± 5.0 i–k
'Miss Huff'	3*x*	6414	2.0 ± 0.2	562	1.9 ± 0.8	1.9 ± 0.0 m
'New Gold'	3*x*	6074	0.8 ± 0.1	260	2.7 ± 2.0	1.8 ± 0.9 m
'New Red Lantana'	3*x*	5291	5.6 ± 0.8	514	7.0 ± 1.7	6.3 ± 0.7 k–m
'Patriot Fire Wagon'	3*x*	4512	19.3 ± 1.0	442	14.7 ± 2.5	17.0 ± 2.3 h–j
'Red Butler'	3*x*	4911	7.5 ± 1.8	236	4.0 ± 1.7	5.8 ± 1.8 k–m
'Red Spread Lantana'	3*x*	4588	6.2 ± 0.7	399	5.7 ± 0.3	5.9 ± 0.3 k–m
'Samson Lantana'	3*x*	5208	6.4 ± 0.4	483	5.2 ± 0.7	5.8 ± 0.6 k–m
'Sunset Lantana'	3*x*	6465	5.2 ± 0.7	1078	3.1 ± 1.2	4.2 ± 1.1 l–m
'Carlos'	4*x*	4102	54.5 ± 1.6	1416	44.2 ± 2.4	49.4 ± 5.2 b–e
'Dallas Red'	4*x*	4234	34.5 ± 2.3	374	29 ± 1.0	31.7 ± 2.8 e–h
'Gold'	4*x*	4727	31.0 ± 9.6	559	21.4 ± 8.2	26.2 ± 4.8 f–i
'Irene'	4*x*	3994	55.1 ± 1.2	1272	59.7 ± 3.2	57.4 ± 2.3 a–d
'Pink Caprice'	4*x*	3227	75.9 ± 2.3	966	71.1 ± 1.9	73.5 ± 2.4 a–b
'Radiation'	4*x*	3961	40.7 ± 11.2	576	23.9 ± 2.7	32.3 ± 8.4 e–h
'Cajun Pink'	5*x*	4241	41.2 ± 2.5	229	23.4 ± 2.0	32.3 ± 8.9 e–g
'Patriot Hallelujah'	5*x*	5423	41.3 ± 4.3	938	42.5 ± 3.1	41.9 ± 0.6 d–f
'Spreading Sunset'	5*x*	6609	13.6 ± 2.0	2463	25.4 ± 5.5	19.5 ± 5.9 g–j

Table 2-3 Continued.

Lantana spp. cultivars/lines	Ploidy[Y]	Season 1 Pollen count	Season 1 Pollen stainability	Season 2 Pollen count	Season 2 Pollen stainability	Average pollen stainability[Y]
629-1	5*x*	3946	33.0 ± 3.1	700	29.5 ± 3.8	31.2 ± 1.8 e–h
629-2	5*x*	3646	52.9 ± 3.0	1287	43.7 ± 5.6	48.3 ± 4.6 c–e
'Tangerine'	6*x*	3881	24.5 ± 5.2	285	17.3 ± 3.0	20.9 ± 3.6 g–j
620-10	6*x*	6668	9.9 ± 0.9	1237	14.0 ± 4.1	11.9 ± 2 j–l
621-4	6*x*	3389	24.5 ± 2.4	512	17.9 ± 2.7	21.2 ± 3.3 g–j
L. montevidensis (lavender)	3*x*	3308	1.2 ± 0.0	770	0.2 ± 0.2	0.7 ± 0.5 m
L. montevidensis (white)	3*x*	5966	2.4 ± 0.3	248	0.6 ± 0.6	1.5 ± 0.9 lm

[Z]Removed Improved from cultivar name.
[Y]Lowercase letters indicate statistical groupings with Tukey's W procedure significance at p≤0.05.
[X]Differences in ploidy level based on the average pollen stainability of all *Lantana spp.* cultivars/lines.

Table 2-4. Difference in pollen stainability among ploidy levels of *Lantana spp*. from two seasons.

Species	Ploidy level	Lines sampled	Pollen counted	Lowest stainability	Highest stainability	Average pollen stainability (%)[Z]	Standard error
L. camara	2*x* a	6	26077	64.6	81.1	64.6 a	3.1
	3*x* d	13	73591	1.8	27.1	9.3 d	2.2
	4*x* b	6	29408	26.2	73.5	45.1 b	7.5
	5*x* b	5	29482	19.5	48.3	34.6 b	4.9
	6*x* c	3	15972	11.9	21.2	18.0 c	3.1
L. montevidensis	3*x* e	2	10292	0.7	1.5	1.1 e	0.4

[Z]Lowercase letters indicate statistical groupings with Tukey's W procedure significance at p≤0.05.

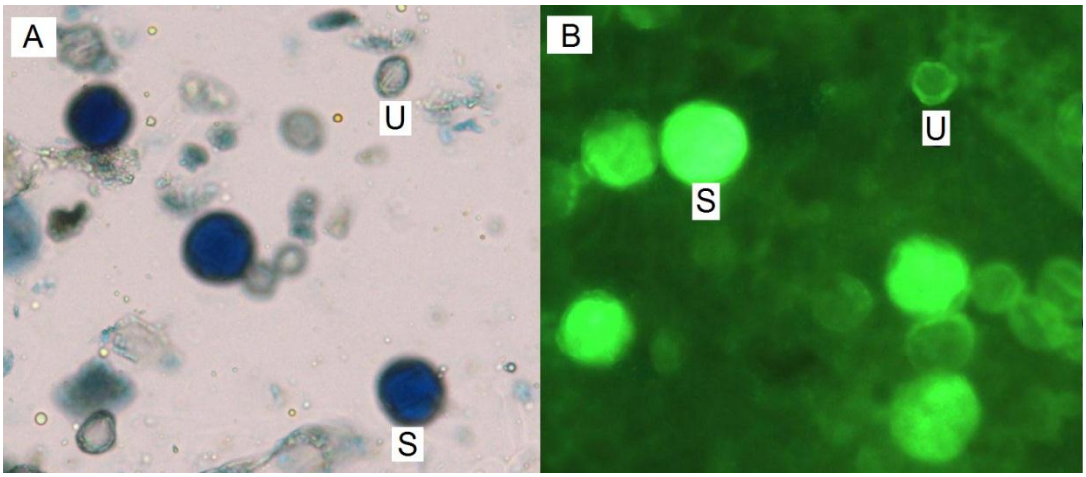

Figure 2-1. Example of stainable (S) and unstainable (U) *Lantana* pollen grains. A) Detected with cotton blue under a bright field miscroscope. B) Detected with fluorescein diacetate under a fluorescence microscope. Pollen was collected from *L. camara* 'Tangerine'.

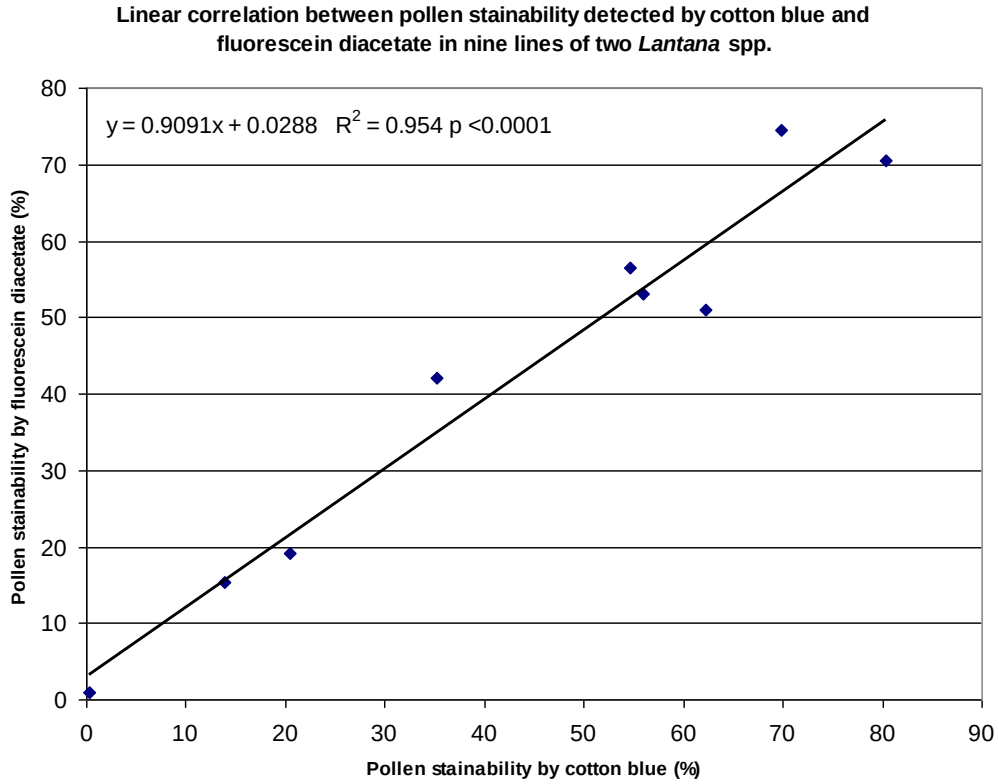

Figure 2-2. Linear correlation of pollen stainability (%) of eight *L. camara* and one *L. depressa* lines detected by two vital stains, cotton blue and fluorescein diacetate. Pollen stainability of each line revealed by the two stains were not significantly different (P=0.803).

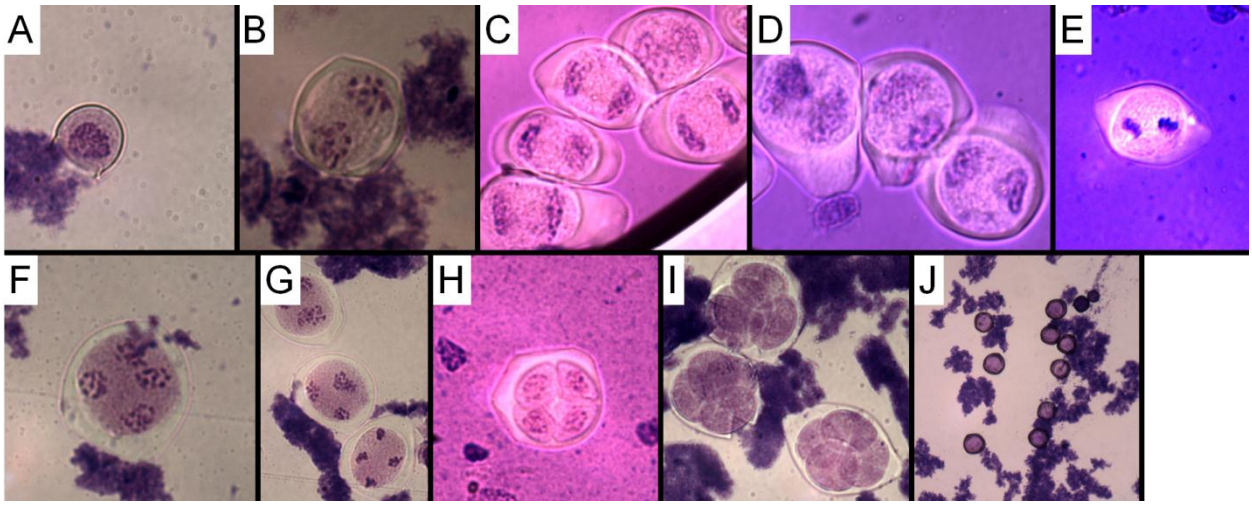

Figure 2-3. Stages of normal meiosis in *Lantana camara*. A) Prophase I ('Lola'). B) Anaphase I ('Lola'). C) Telophase I ('Bandana Red'). D) Prophase II ('Bandana Red'). E) Metaphase II ('Bandana Red') F) Late Anaphase II ('Lola'). G) Telophase II ('Lola'). H) Late telophase II ('Bandana Red'). I) Tetrad formation ('Bandana Red'). J) Pollen grains ('Lola'). Photo credit: Amanda Hershberger, Univeristy of Georgia.

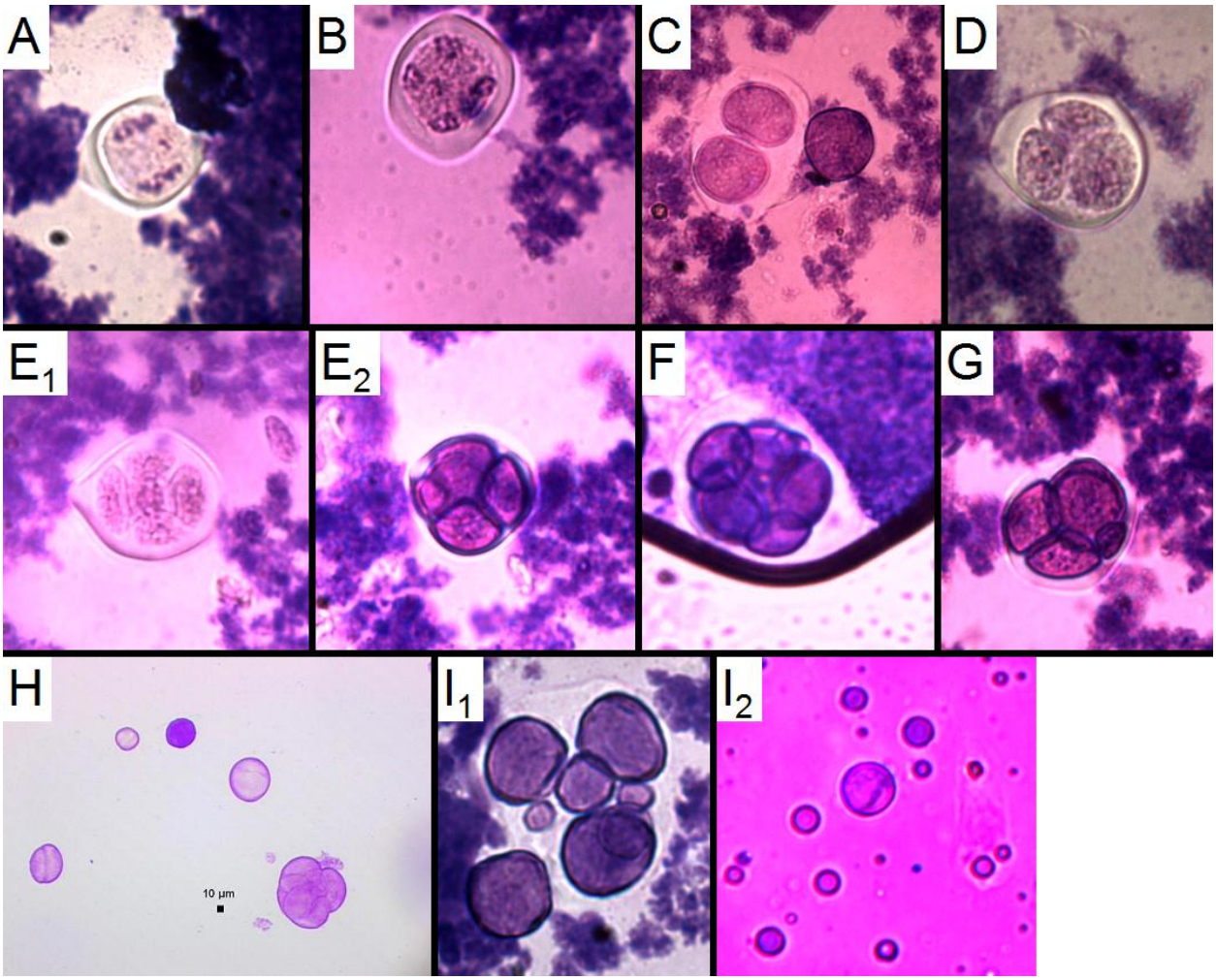

Figure 2-4. Abnormalities observed in *Lantana camara* meiosis. A) Lagging chromosome in Telophase I ('Lola'). B) Lagging chromosome in Telophase II ('Miss Huff'). C) Dyad ('Pink Caprice'). D) Triad formation ('Lola'). E) Pentad formation ($_1$ 'Miss Huff' and $_2$ 'Pink Caprice'). F) Head formation ('New Gold'). G) Uneven pollen size ('Pink Caprice'). H) Fused tetrad after tetrad sac rupture ('Miss Huff'). I) Variable sized pollen (I_1'Athens Rose' and $_2$'Miss Huff'). Photo credit: Amanda Hershberger, Univeristy of Georgia.

CHAPTER 3
FEMALE FERTILITY OF *LANTANA CAMARA*

Rationale

In plants, the most important factors determining female fertility are seed (or fruit) production and seed germination. In lantana, a seed is borne inside a round, fleshy drupe (berry). The fruit is initially green, but turns purple then blue-black as the fruit ripens. *L. camara* can produce fruit all year round if adequate temperature, moisture, and light are available (reviewed by Sharma et al., 2005). Generally each drupe contains one seed that is 0.1-0.2 cm wide. Occasionally, the fruit may contain one additional seed (reviewed by Sharma et al. 2005). Several studies have 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 about eight fruit (Barrows, 1976); whereas in India, as many as 25-28 fruits 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 seed densities ranged from less than 5 to 2,690 seed per square meter (Sharma et al., 2005). These seed have been shown to be able to germinate at any time of the year with sufficient soil moisture, light, and temperature (Gentle and Duggin, 1997). Collectively little information is available in the literature regarding fruit production capacity and seed germination of commercial lantana cultivars or in U.S production.

Several researchers have attempted to understand the relationships between ploidy levels and fruit or seed production in *L. camara*. Natarajan and Ahuja (1957) suggested that ploidy level would be an influencing factor in fruit production as diploid

plants had “no seed” to “good” seed production while no triploids in their study produced any seed. Thirty percent of tetraploid plants were found to not produce seed while the rest ranged from “none” to “good” seed production. Two later studies by Raghavan and Arora (1960) and Khoshoo and Mahal (1967) indicated that triploid plants did produce “good” amounts or at least a few seed. Spies (1984c) collected seed produced from all observed ploidy levels in South Africa and found a range in seed production across diploid to pentaploid plants of 0–2,485. These studies indicated that tetraploid and diploid plants were the highest seed producers at 856 (4*x*) and 565 (2*x*) seed per plant respectively. The triploid plants were expected to be sterile triploids but produced 342 seed per plant. Very few pentaploid and hexaploid plants were available and only one pentaploid survived the treatment (plants were cut back to the crown) to produce 638 seed on a single plant.

Studies from both Australia and India indicated a range of seed germination: 12% in diploids, 28% in triploids, and 56% in tetraploids (Raghavan and Arora, 1960; Spies, 1984c). An older study (Heit, 1946) investigating the best methods for seed germination of *L. camara* determined the highest average rate of seed germination to be 53% after 40 days with an individual accession reaching as high as 70% after 60 days. Only one individual was sampled from diploid, triploid, and tetraploid *L. camara* providing limited data (Raghavan and Arora, 1960).

These studies indicate that ploidy level likely has a significant effect on fruit production in lantana. The observed wide range of variation in fruit production within each ploidy level group also suggests that other factors such as genes and chromosomal constituents may play significant roles in determining lantana’s fruit

production capacity. The current work outlined here will focus on assessing the variation in fruit production and seed germination of U.S. commercially produced *L. camara* in controlled field trials and understanding the role of ploidy level variation in influencing *L. camara* fruit production and seed germination.

Materials and Methods

Plant Materials

This study used the same 32 commercial *L. camara* cultivars and breeding lines used for pollen stainability work described in Chapter 2. All cultivars/breeding lines were propagated by cuttings on 17–19 September 2007. When plants were ~8-month old (after male fertility assessment), they were transplanted to ground beds on 29 May 2008. The beds were treated with Roundup® (The Scotts Company, Marsville, OH) and Image® (BASF, Research Triangle Park, NC) and covered with white-on-black plastic mulch spaced at 1.8 m. The field was irrigated using drip tubes for one hour twice a week. Each plant received five grams of a commercial control-released fertilizer (Osmocote®, 15N-9P-12K, 5-6 months release at 21°C; The Scotts Company, Marsville, OH). Plants were grown in the ground beds until final plant harvest on 13–14 November 2008.

Assessing Fruit Production

Commonly *L. camara* takes about 3–5 weeks from flower opening to produce ripe fruit. Thus, the first fruit collection was made 6 weeks after transplanting. Fruit collection was then repeated about 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 August, 28–30 September, and 4–11 November 2008. During each collection, 20

flower peduncles were randomly harvested from an experimental unit (plant) and berries on each peduncle, regardless of maturity, were counted to calculate the percentage of flower peduncles setting fruit or percent fruiting peduncles (PFP) and the number of fruit per peduncle (FPP) (Fig. 3-1). In addition, every plant in the study was inspected during each collection to determine if the plant set any fruit to calculate the percentage of plants setting fruit should the 20 flower peduncles collected not bear fruit.

After each collection, ripe berries were stored in glycine bags in ambient laboratory conditions at 22.2°C for subsequent seed extraction (see below) and green/immature and visibly damaged berries were discarded. During the last 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 study.

Insect Damage

During the first fruit collection, insect larvae were found burrowing through 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 Department of Plant Industry (Clemson University, Clemson, SC). Characteristic larvae damage consisted of spiraling grooves on peduncles, blackened depressed areas, and burrowed holes through leaves, flowers, and berries (Fig. 3-1) 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

Seed was extracted on 7–9 January 2009 after fruit collection was completed in November 2008. Saved ripe berries were macerated manually using a fine metal mesh flour sieve and collected seeds were air-dried under the ambient conditions in the laboratory for ~4 weeks. For most of the *L. camara* cultivars/breeding lines, the number of seed from each experimental unit was limited, thus seed were combined by cultivar at each collection period for seed germination studies.

The bulked seed was divided into three replications. Seed was sown in plastic community trays on the surface of Fafard® 2B (Anderson, SC) potting soil on 9 February 2009, and germinated in the greenhouse, under intermittent mist. Germination rates were taken every week for 16 weeks.

Calculating Female Fertility Index

As shown later in this chapter, *L. camara* cultivars/breeding lines varied greatly in fruit production and seed germination. Some of them produced copious amounts of fruit but seed had low germination, while others set fewer fruit but their seed germinated readily. The female reproductive potential of a given *L. camara* would be 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 fruit per peduncle (FPP) and seed germination percentage. This index was expected to give a better representation of a given cultivar's female fertility.

Plant Dry Weight

To determine if differences in vigor existed after the final fruit collection, on 13–14 November 2008, the above-ground parts of each plant were harvested by cutting the stems off at the soil line and placing them into a bag (116 x 95 cm trash bags). Bags

were left open and the harvested plant materials were dried at 60°C in the drying room for 4 weeks before their weight was taken (kg).

Experimental Design

Thirty-two *L. camara* cultivars and breeding lines were arranged in the field in a randomized complete block design. The experimental unit was a single asexually propagated plant. There were four blocks and one plant per cultivar in each block.

Statistical Analysis

FPP and seed germination were analyzed using PROC GLM in SAS® for Windows 9.2 (SAS® Institute, Cary, NC) to determine the significance of differences among ploidy levels, lantana lines, and collections. Seed germination data were transformed using the arcsine square root function. Seed collection intervals were combined to better determine differences among lines. When differences were significant, mean separation analysis was performed using the LSD Procedure in SAS®.

A principal component analysis (PCA) was performed using NTSYS (NTSYSpc, version 2.2 [Rohlf, 2005]) to obtain a graphic representation of relationships among cultivars/breeding lines (Kulakow 1999). Procedurally the data were standardized and then a correlation was computed. The correlation allowed eigenvalues to be extracted (which determine the variance explained by a principal component). These values were then projected onto a two-dimensional graph.

Results and Discussion

Fruit Production in *L. camara*

Three variables, percent fruiting plants, percent fruiting peduncles (PFP), and fruit per peduncle (FPP), were used to assess the fruit production capacity of each *L. camara* cultivar/breeding line. The percentage of plants setting fruit varied from 6.3% to

100.0%; PFP ranged from 0.3% to 98.8%; and FPP ranged the highest from 0.003 to 7.173. Analysis of variance indicates that the differences among cultivars/breeding lines in FPP were highly significant (F value = 46.54, P < 0.0001).

As described in Chapter 2, the 26 cultivars and six breeding lines represented six ploidy levels, from diploid to hexaploid. As shown later in Chapters 4 and 5, 11 of the 13 triploids, three of the six tetraploids, all five pentaploids, and all three hexaploids included in this study are expected to produce unreduced female gametes (UFGs) (and apomictic seed). For the reasons to be discussed in Chapter 5 and simplicity, these polyploids are to be referred to as having the UFG producering trait. ANOVA results indicate that both the ploidy level and the UFG-producing trait played a role in fruit production.

Statistical analysis also indicated that the FPP values were significantly different among four collections (F value = 3.14, P = 0.0252). This was expected based on a preliminary study conducted in 2007 using 139 *L. camara* lines and their cyclic flowering and fruiting habit. Climatiological factors such as temperature and pollinator activity could greatly influence seed production at each collection period. Thus fruit collection was done four times over a period of five months.

Seed Germination in *L. camara*

Because of the large differences among cultivars/breeding lines in fruit production, the number of seeds available for the germination study varied considerably. No seed was available for testing the seed germination of ‘Athens Rose’ and 629-1. One seed was collected from 'Denholm White' plants and it germinated. For five cultivars/breeding lines, 4 to 119 seed were processed for sowing, but none of them germinated. Excluding these cultivars/breeding lines, the seed germination percentage

in the remaining 25 cultivars/breeding lines ranged from 9.4% to 57.1%. As expected, statistical analysis indicates that cultivars were significantly different in seed germination ($F = 4.44$, and $P=<0.0001$) (Table 3-2).

Female Fertility Index (FFI) of *L. camara*

As mentioned above, seeds of seven cultivars/breeding lines did not germinate, resulting in a FFI of 0. 'Pink Caprice' had the highest FFI, 2.998 (Table 3-3). The remaining 25 cultivars/breeding lines had a FFI between 0.003 and 0.599. In total there were 13 cultivars/lines (three diploids, three triploids, three hexaploids, and four pentaploids) whose FFI were ≤0.054.

Fruit Production, Seed Germination and Female Fertility Index of Diploid *L. camara*

Based on 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 rather low germination percentage (16.2%), resulting in a FFI of 0.149. In the second group was LAOP-30, which had a lower FPP value (0.344) but a much higher germination percentage (60.0%), resulting in a similar FFI (0.261). The third group consisted of ‘Cream’ and LAOP-9. Their seeds did not germinate well (~10.0% germination), which led to a low FFI (0.020 or 0.034), although their FPP values were not very low (0.193 and 0.435, 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 a FFI of 0.003.

These results suggest that even within diploids, *L. camara* cultivars/breeding lines can vary remarkably in fruit production (FPP from 0.003 to 0.922), seed germination (10.0% to 100%), and FFI (0.003 to 0.261) and certain diploids can be highly sterile. Understanding the genetic mechanism(s) for the high level of female sterility in these diploids, especially in 'Denholm White', could be very valuable for sterilizing *L. camara*. To determine if a gene is controlling the sterility in 'Denholm White' would require cross pollination with fertile diploids and assessment of the progeny to determine if the trait is heritable. Alternatively embryology could be described with cytological invesitigation to determine the mechanism causing sterility. Determination of the factors regulating this trait would indicate the ultimate stability of sterility in this line.

Fruit Production, Seed Germination and Female Fertility Index of Triploid *L. camara*

Of the thirteen triploid cultivars assessed for fruit production, the majority, (11) were found to have the ability to produce UFGs and apomictic seeds (refer to Chapters 4 and 5 for further discussion). Only 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 (Tables 3-1 and 3-2).

Non-UFG-producing triploids

'Athens Rose' and 'Lucky Red Hot Improved' are the only cultivars that belong to this group. A total of two berries were found on 305 peduncles of 'Athens Rose' during the entire 5-month period. None of 'Athens Rose's seed (1) germinated, resulting in a FFI of 0. The 316 peduncles surveyed on 'Lucky Red Hot Improved' plants set 30 fruit, i.e. a 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 least fertile in *L. camara* (Table 3-4). These results suggest that triploidy in combination with removal of the UFG trait could result in a high level of female sterility in *L. camara*.

UFG-producing triploids

These triploids were highly prolific in fruit production, with 100% of the plants or 16.0% to 60.7% of the flowers producing fruit, for an FPP of 0.175 to 1.379 (Table 3-3). Three triploids ('Landmark Pink Dawn', 'Lemon Drop', and 'Samson Lantana') had a 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 FPP was 0.236, higher than that of the non-UFG-producing triploids and 2.5-times higher than that of the diploids (Table 3-4).

One of the triploids in this group, 'Red Butler', had 9.3% seed germination. The remaining 10 triploids had a seed germination percentage between 18.4% and 57.1%. The average seed germination of these triploids was 29.3%, approximately 264% that of of the non-UFG-producing triploids. The average FFI of these triploids reached 0.236, 0.231 higher than that of the non-UFG-producing triploids and 0.142 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 was interesting that parents with this trait were widly used as parents and has caused UFG-production to become wide-spread in commercial triploid *L. camara* cultivars. In one experimental triploid (Czarnecki and Deng, 2009), this trait greatly increased the fruit or seed production capacity. As shown above, similar fertility-restoring effects are also present in the commercial triploid cultivars. Thus, in *L. camara*, triploidy alone may not be able to provide adequate levels

of female sterility. Rather, it would be critical and necessary to eliminate the UFG production trait in order to produce highly sterile *L. camara* cultivars.

Fruit Production, Seed Germination and Female Fertility Index 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 FPP values were 1.870, 0.573, and 1.568, respectively, with the three, collectively, averaging 1.340. Thus these tetraploids seemed to be more prolific in fruit production than *L. camara* diploids (group FPP average 0.225) (Table 3-4). The seed germination percentage of these tetraploids was 14.2, 39.1, and 11.8, respectively (Table 3-3). Their average seed germination was 21.7%, which is lower than that of the diploid *L. camara* (39.3%). 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*. Low fruit production and germination rates were unexpected in this group of plants.

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 3-3). Conversely, 'Pink Caprice' had an FFI of 2.998 higher than all 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 to 'Gold' or 'Radiation',

‘Pink Caprice’s FPP and seed germination were ~3.5- 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 3-4). 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. The production of UFGs greatly increases the overall fecundity of the species and because of this high level of female fertility and their ability to pass the UFG production trait onto progeny (Czarnecki and Deng 2009), this group of plants should be avoided for most breeding purposes.

Fruit Production, Seed Germination and Female Fertility Index of Pentaploid and Hexaploid *L. camara*

Three of the five pentaploids (‘Cajun Pink’, 629-1, and 629-2) produced small amounts of fruit and had FPP values between 0.108 and 0.426 (Table 3-3). None of the seeds extracted from their fruit germinated, resulting in a FFI value of 0. ‘Patriot Hallelujah’ produced even fewer fruit and had a very low FPP value (0.030), very close to that of non-UFG-producing triploids. None of its seeds germinated, resulting in a FFI of 0. Compared to these pentaploids, ‘Spreading Sunset’ was relatively fertile, with a FPP value of 0.906, 15.1% seed germination, and a FFI of 0.137, quite 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 3-3). 'Tangerine' was the only hexaploid cultivar that had a moderate FPP value (0.557) but a low seed germination percentage (7.1%), and a low FFI (0.040). The pentaploid and hexaploid plants were of moderate or low fertility and may not pose a significant threat in some environments.

Correlation Analysis

The strongest positive correlation was found between the FFI and FPP (r = 0.93916, *P* <0.0001) (Table 3-5). The correlation between FFI and seed germination was not significant, with r = 0.24989, P = 0.1678. This seems to indicate that FPP is of much greater influence to the overall female fertility of *L. camara* than seed germination. The highest correlation among all data collected was between FPP and the other data points. For this reason it was considered for further analysis.

Interestingly insect damage was positively correlated to FPP (0.47797, P = 0.0057) (Table 3-5). This was likely because insects feed on plants with more fruit rather than the insects causing higher seed set.

The correlation analysis also indicates significant negative correlation between plant dry weight and pollen stainability (r -0.44461, *P* = 0.0108). Most likely this correlation was largely due to the fact that 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, 1984c) but are dwarf and small (Sanders, 2001).

The results of the correlation analysis could be largely influenced by several factors such as genes and ploidy levels. Studies among individuals with similar genetic backgrounds may increase the correlary rates among the fertility characteristics.

Principal Component Analysis

The process of PCA correlates data points then extracts the most influential data to plot on a graph allowing the relation of individuals or categories to be seen in a graphical representation. This analysis will provide the opportunity for numerous additional categories of data and controls to be included in fertility assessments. In the PCA plot derived from both the FFI values mentioned above and the pollen stainability data described in Chapter 2, the 32 *L. camara* commercial cultivars/breeding lines clustered into six groups (Figure 3-2). The largest group consisted of UFG-producing triploids that had high FFIs but low pollen stainability. Diploid cultivars that had lower FFIs and high pollen stainability made up the second group. Located near the center of the graph were those with the lowest levels of male and female fertility available in *L. camara*, two non-UFG triploids and some of the pentaploids and hexaploids. The most fertile plant group is the single individual 'Pink Caprice', which had high levels of stained pollen, high FFP, and high germination rates leading to a high FFI. Two additional groups in the figure are seen among the pollen sterile high seed producing triploids and the pollen fertile low seed producing diploids.

Summary

The results of this study showed that *L. camara* cultivars differed considerably in fruit or seed production and seed germination. The difference was particularly obvious in the number of fruit produced per peduncle. Ploidy level difference and the UFG-producing trait played a very significant role in determining the fruit production capacity of *L. camara*. Triploids without the UFG-production trait were most sterile. The UFG-producing trait is wide-spread 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

showed that there are other genetic mechanisms causing female sterility in *L. camara*. Principal component analysis based on both FFI and pollen stainability provides a useful way to visualize the reproductive characteristics of different *L. camara* cultivars/breeding lines. These results indicate that low levels of fertility can be achieved through ploidy manipulation and have explained the lack of correlation between male and female fertility from previous authors.

Table 3-1. Analysis of variance table for fruit production of 32 of *L. camara* cultivars/breeding lines. Fruit was collected from July through November at five week intervals in 2008 in Balm, FL.

Source	DF	F value	P value
Cultivar/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

Table 3-2. Analysis of variance table for seed germination of 32 lines of *L. camara* cultivars/breeding lines. Seed was collected from July through November at five week intervals in 2008 in Balm, FL. Seed was sown on 9 February 2009.

Source	DF	F value	P value
Cultivars	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

Table 3-3. Average seed production and female fertility characteristics of 32 *L. camara* lines.

Cultivar	Percent plants producing fruit	Percent flower peduncles producing fruit	Flowering peduncles examined	Fruit produced by the peduncles	Fruit per peduncle (no.)[Y]	Percent flower peduncles damaged by insects	Seed germination (%)[Y]	Female fertility index[W]	Plant dry weight (kg)
'Cream' (2x)	93.8	14.7	319	62	0.193h-j	77.3	10.3 i-l	0.020	634
'Denholm White' (2x)	6.3	0.3	303	1	0.003j	42.9	100 a	0.003	50
LAOP-9 (2x)	75.0	21.3	191	107	0.435f-j	56.7	60 b	0.034	15
LAOP-30 (2x)	87.5	22.0	223	93	0.344g-j	60.8	10 k-l	0.261	26
'Lola' (2x)	100.0	43.6	307	289	0.922d-f	68.4	16.2 f-k	0.149	93
'Athens Rose' (3x)	18.8	0.6	305	2	0.006j	63.4	0 l[X]	0.000	728
'Landmark Peach Sunrise'[Z] (3x)[ω]	100.0	16.0	319	56	0.175h-j	42.1	57.1 b-c	0.100	218
'Landmark Pink Dawn' (3x)[ω]	100.0	53.1	318	392	1.232c-e	66.7	48.6 b-d	0.599	996
'Lemon Drop' (3x)[ω]	100.0	60.7	315	401	1.270c-e	47.8	9.4 h-l	0.119	506
'Lucky Red Hot'[Z](3x)	81.3	9.1	316	30	0.094h-j	71.1	11.1 k-l	0.010	167
'Miss Huff' (3x)[ω]	100.0	47.4	321	285	0.890d-f	77.5	18.4 e-k	0.164	944
'New Gold' (3x)[ω]	100.0	43.9	319	243	0.763e-g	55.4	26.8 d-j	0.205	622
'New Red Lantana' (3x)[ω]	100.0	46.0	318	264	0.832e-g	66.8	24.4 d-k	0.203	615
'Patriot Fire Wagon' (3x)[ω]	100.0	44.7	315	255	0.809e-g	62.0	45.9 b-e	0.372	517
'Red Butler' (3x)[ω]	100.0	39.0	318	184	0.579f-h	55.2	9.3 h-l	0.054	672
'Red Spread Lantana' (3x)[ω]	100.0	33.5	317	164	0.518f-j	56.8	21.6 d-k	0.112	687
'Samson Lantana' (3x)[ω]	100.0	58.6	318	436	1.379b-d	71.0	26.8 c-i	0.370	647
'Sunset Lantana' (3x)[ω]	100.0	42.2	321	288	0.895d-f	57.9	33.5 c-h	0.300	323
'Carlos' (4x)	100.0	72.3	317	592	1.870b	73.3	14.2 f-j	0.266	80
'Dallas Red' (4x)	81.3	26.0	312	179	0.573f-h	61.7	39.1 b-g	0.224	161
'Gold' (4x)[ω]	100.0	60.0	317	446	1.401b-d	64.6	9.3 h-l	0.130	663
'Iene' (4x)	93.8	68.3	298	478	1.568b-c	63.7	11.8 h-k	0.185	101
'Pink Caprice' (4x)[ω]	100.0	98.8	317	2280	7.173a	85.6	41.8 b-f	2.998	967
'Radiation' (4x)[ω]	100.0	53.4	320	510	1.594b-c	71.9	12.4 g-k	0.198	431

Table 3-3 Continued.

Cultivar	Percent plants producing fruit	Percent flower peduncles producing fruit	Flowering peduncles examined	Fruit produced by the peduncles	Fruit per peduncle (no.)	Percent flower peduncles damaged by insects	Seed germination (%)	Female fertility index[W]	Plant dry weight (kg)
629-1 (5x)[ω]	56.3	9.7	319	45	0.141h-j	63.3	0 l[X]	0.000	87
629-2 (5x)[ω]	87.5	10.1	317	34	0.108h-j	59.0	0 l	0.000	125
'Cajun Pink' (5x)[ω]	100.0	31.5	317	135	0.426f-j	54.5	0 l	0.000	383
'Patriot Hallelujah' (5x)[ω]	56.3	2.7	322	10	0.030j	52.6	0 l	0.000	328
'Spreading Sunset' (5x)[ω]	100.0	50.5	317	287	0.906d-f	50.1	15.1 f-k	0.137	404
620-10 (6x)[ω]	43.8	4.9	305	14	0.053i-j	71.3	0 l	0.000	282
621-4 (6x)[ω]	31.3	1.3	320	4	0.013j	69.6	0 l	0.000	262
'Tangerine' (6x)	100.0	41.4	314	173	0.557f-i	50.0	7.1 i-l	0.040	184

[Z]Removed Improved from the cultivar name.
[Y]Letters differentiate based on LSD procedure P ≤ 0.05.
[X]No seed available to sow. The highest seed germination rate was used from others months when seed was sown.
[W]Index from the multiplication of FPP and seed germination rate.
[ω]Indicates unreduced female gamete producer.

Table 3-4. Average fruit production, seed germination, and female fertility index of 32 *L. camara* by ploidy level and unreduced female gamete production.

Ploidy Level	UFG producers	Average FPP[Z]	Low FPP	High FPP	Low germination	High germination	Germination at 16 weeks[Z]	Female fertility index
2*x*	No	0.47 c-d (0.38)[Y†]	0.003	0.922	10	100[Y]	24.1 (39.3) a-b[Z†]	0.094
3*x*	No	0.05 d	0.006	0.094	0[Y]	21.6	11.1 b-c	0.005
3*x*	Yes	0.85 c	0.175	1.379	9.3	57.1	29.3 a	0.236
4*x*	No	1.34 b	0.573	1.87	11.8	39.1	21.7 a-b	0.225
4*x*	Yes	3.39 a	1.401	7.173	9.3	41.8	21.2 a-c	1.109
5*x*	Yes	0.32 d	0.03	0.906	0[Y*]	15.1	3.8 c	0.027
6*x*	Yes	0.21 d	0.013	0.557	0[Y*]	7.1	2.4 c	0.013

[Z]Letters denote different statistical groupings from the LSD procedure.
[Y]Only one seed was sown.
[*]Multiple observations.
[†]Number in parenthesis includes Denholm White which was excluded from statistical analysis.

Table 3-5. Correlation of female and male fertility traits and statistical probability (p value).

	Dry weight	Plants (%) setting seed	Flowers (%) setting fruit	Insect damage	Fruit per peduncle	Germination (November)	Pollen staining[Z]
Female fertility index	0.42025 (0.0166)	0.21442 (0.2386)	0.58338 (0.0005)	0.45796 (0.0084)	0.93916 (<.0001)	0.24989 (0.1678)	0.23396 (0.1975)
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 seed (%)			0.70624 (<.0001)	0.12121 (0.5087)	0.35855 (0.0439)	-0.05966 (0.7457)	-0.17009 (0.352)
Flowers setting fruit (%)				0.31844 (0.0757)	0.78601 (<.0001)	0.06826 (0.7105)	-0.05698 (0.7568)
Insect damage					0.47797 (0.0057)	-0.2346 (0.1962)	0.13397 (0.4648)
Fruit per peduncle						0.14744 (0.4206)	0.2129 (0.242)
Germination (November)							0.17004 (0.3521)

[Z]Pollen staining data from chapter two

Figure 3-1. Effects of *L. camara* seed production. A) *L. camara* seed on peduncle. B) *Crocidosema lantana* found in seed peduncles of *L. camara*. C) Damage caused by *Crocidosema lantana*.

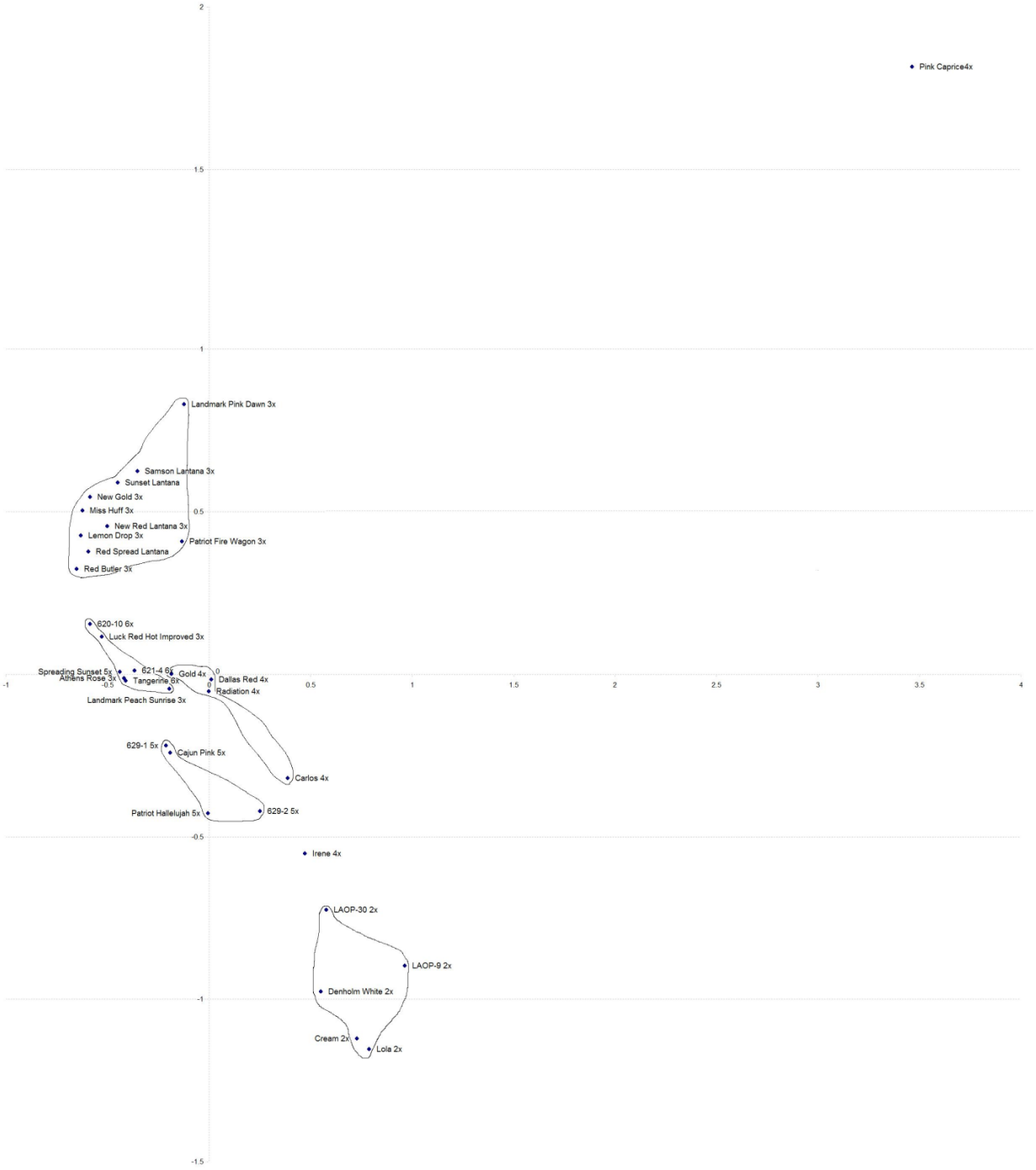

Figure 3-2. Principal component analysis of pollen stainability and female fertility index. The x-axis and y-axis represent Dimensions one and two, respectively, of the principal component analysis.

CHAPTER 4
OCCURRENCE OF UNREDUCED FEMALE GAMETES LEADS TO SEXUAL POLYPLOIDIZATION IN *LANTANA CAMARA*

Rationale

Lantana is a member of the Verbenaceae and has been widely grown as container plants, hanging basket plants, ground covers, and hedge or accent plants (Beaulieu, 2008; Howard, 1969). It is popular in the nursery industry because of easy propagation, short production cycles, interest among gardeners because it attracts butterflies, and has tolerance to drought and poor soil conditions. Nursery production of lantana is widespread, especially in the Southern United States. For example, a survey of the Florida nursery industry, which consists of more than 5,000 nurseries, indicates that 19.0% of the responding nurseries grew lantana and that the annual sales value in Florida alone was over $40 million (Wirth et al., 2004). The majority of the commercial lantana cultivars belong to *Lantana camara* L. This species can escape from cultivation through seed dispersal and invade agricultural and natural lands and can hybridize (as pollen donors) with native lantana species. Because of these behaviors, *L. camara* has been listed as an invasive species in South and Central Florida (FLEPPC, 2007). In several other countries including Australia, India, and South Africa, lantana has been considered as a noxious weed or an invasive species (Sharma et al., 2005). Polyploid manipulation, particularly triploid production, has been proposed as a genetic approach to develop sterile, non-invasive lantana cultivars (Czarnecki et al., 2008). Similar genetic approaches (polyploid production and selection) are being used to sterilize other ornamental plants for invasiveness control (Ranney, 2004).

Polyploids are common in *L. camara*. Triploids, tetraploids, pentaploids, and hexaploids have been reported in cultivated and naturalized *L. camara* (Czarnecki et al.,

2008; Natarajan and Ahjua, 1957; Spies and Stirton, 1982a). Several lines of evidence suggest that polyploidization may be associated with the species' invasive behavior (Sanders, 2001): Tetraploids are rare in the native populations of this species in tropical America, but very common in the naturalized populations in India, South Africa, and Australia, and overall, tetraploids have courser leaves, grow more vigorously, and set more seeds (Sanders, 2001), whereas diploids tend to be stunted. Tetraploids have a much wider range of distribution than diploids, and pentaploids are frequently found at high altitudes (Sinha and Sharma, 1984).

In plants, natural polyploidization can occur through somatic chromosome doubling or gametic non-reduction (Bretagnolle and Thompson, 1995). The former results from mitotic abnormalities in somatic (zygotic and meristematic) cells, while the latter results from meiotic abnormalities during gamete or gametophyte genesis and the formation of unreduced gametes (pollen and/or eggs). When an unreduced gamete unites with another unreduced gamete (bilateral) or with a normal haploid gamete (unilateral), the union leads to sexual polyploidization. To a certain extent, polyploidization via somatic chromosome doubling bears similarity to inbreeding, while polyploidization via unreduced gametes can retain heterozygosity. Thus, the two polyploidization processes can have significant differences in terms of genetic and evolutionary consequences to polyploidized species (Bretagnolle and Thompson, 1995; Hermsen, 1984).

Little information is available regarding the origin of polyploids in *L. camara*, except for a report by Khoshoo and Mahal (1967). The authors observed several tetraploids and a pentaploid in the open-pollinated (OP) progeny of a triploid and two hexaploids in

the OP progeny of a pentaploid. They inferred that these tetraploid and hexaploid progeny with chromosome numbers higher than their parents must have come from the union of unreduced female gametes (UFGs) with normally reduced pollen. The occurrence of UFGs has been reported in several plant species (Stelly and Peloquin, 1986; Ramsey and Schemske, 1998). On the contrary, unreduced pollen has been reported in numerous plants and seems to be a more common mode of sexual poloploidization in plants (Bretagnolle and Thompson, 1995). Several studies have examined the pollen size and morphology of *Lantana*, but none of them reported the occurrence of unreduced pollen in *L. camara* (Raghavan and Arora, 1960; Sanders, 1987a). In a recent pollen viability study, we examined tens of thousands of pollen grains and did not notice highly variable viable pollen grains within cultivars (D. Czarnecki and Z. Deng, unpublished).

During the course of interploidy pollination and triploid generation, we observed pentaploid progeny from a tetraploid by diploid cross, which seems to indicate the occurrence of UFGs in the lantana cultivars used. Therefore, a study was undertaken to (1) confirm the occurrence of UFGs in lantana, (2) determine its frequency and distribution in major commercial lantana cultivars, (3) test its transmissibility from generation to generation, and (4) determine if this trait would affect seed set on lantana triploids. Toward these objectives, progeny of commercial cultivars from self-pollination (SP) and open-pollination (OP) were first analyzed for ploidy levels, followed by controlled pollinations among cultivars and breeding lines and ploidy analysis of their progeny. This report presents the results from these pollinations and ploidy analyses. Based on the results, we further propose a model for the origin of the multiple levels of

polyploids in cultivated (and naturalized) *L. camara* and discuss the possible mechanisms for the formation of unreduced gametes in lantana and potential implications of this trait for lantana ploidy manipulation, particularly triploid generation and selection for sterile lantana development.

Materials and Methods

Plant Materials

Ten commercial cultivars and 6 breeding lines were used in this study (Table 4-1). Cuttings or rooted cuttings of these cultivars were generously provided by Dr. Bijan Dehgan, University of Florida Environmental Horticulture Department (Gainesville, FL) in 2004, and Robrick Nursery (Hawthorne, FL) in 2005 and 2006 respectively. Plants were grown in plastic containers filled with a commercial soilless mix, VerGro® container mix A (Verlite Co., Tampa, FL) amended with controlled release fertilizer, Osmocote® (15N-3.9P-10K, 8-9 months release at 21°C; The Scotts Company, Marysville, OH) at 7.12 kg m^{-3}.

Pollinations

Three types of pollination were completed to generate as many seeds (progeny) as possible for ploidy analysis: self, open, and controlled pollinations (SP, OP, and CP, respectively). In SP, lantana plants in 3-gallon containers were grown in a greenhouse with double doors and a thrips-proof screen. Insecticides were applied at regular intervals in the greenhouse. As lantana is autogamous (Rambuda and Johnson 2004), hand pollination is not needed for SP. Therefore, to avoid damage to flowers and maximize seed set, flowers were allowed to self-pollinate without manual emasculation or hand pollination. In OP, lantana plants in 7-gallon containers were arranged on a raised metal bench in open air. Different cultivars were interspersed to maximize cross-

pollination mediated by insect pollinators. Insecticides were not applied. Common insect pollinators observed were butterflies, moths, bees and wasps. Controlled pollinations were done on lantana plants grown in the greenhouse. Mature flowers were emasculated by pulling off the entire calyx to which the anthers were attached and pollinated immediately using a camel brush with fresh pollen from flowers that just opened. Between pollinations, the brushes were soaked in 100 percent ethanol to kill residual pollen. Controlled pollinations were done in April – June 2006, August – December 2006, February – July 2008, June – November 2008, and February 2009.

When lantana fruit (berries) turned dark purple to black and became ripe, they were collected from self, open, or control-pollinated flower heads. Seeds were extracted and cleaned within 1 to 2 weeks after harvest, with the exception of the SP seeds of 'Pink Caprice', which were cleaned and stored for over 1 ½ years before sowing.

Progeny Growing

Seeds were sown on the surface of a peat/vermiculite mix (VerGro® container mix A, Verlite, Tampa, FL) and germinated under intermittent mist in a greenhouse. Temperatures in the greenhouse ranged from 16°C (night) to 30°C (day), and no artificial lighting was used. The majority of seeds germinated within four months after sowing, but some took as long as a year to germinate. After young seedlings had developed true leaves, they were transferred to 12.7-cm plastic containers filled with VerGro® container mix. Plants were fertilized by incorporating Osmocote® (15N-3.9P-10K) at 7.12 kg · m^{-3} in the soilless mix.

Ploidy Analysis

Analysis was performed using fully expanded young leaves and the Partec PA I ploidy analyzer and the CyStain UV Ploidy Precise P dye (Partec, Germany). The manufacturer recommended ploidy analysis procedure was followed with minor modifications. The ploidy level of a progeny was determined by comparing to one or more commercial cultivars (reference cultivars) with known ploidy levels that were included in the same analysis.

The ploidy levels of the reference cultivars had been confirmed by counting chromosomes in root tip cells. Growing root tips of 'Cream', 'Gold', 'Pink Caprice', and 'Radiation' were collected from rooted cuttings, chilled at 8°C overnight and pre-treated with 0.05% colchicine at ambient temperature for 4 hrs, fixed in Carnoy's fluid for 2 d, and stored in 70% ethanol at 4°C. Fixed root tips were hydrolyzed in 1 N HCl at 60°C for 5 to 10 min, squashed in acetic carmine on glass slides, and observed under a 100x objective on Olympus BH-2 microscope. Chromosome counting results showed that 'Cream' is a diploid and 'Gold', 'Pink Caprice', and 'Radiation' are tetraploids.

Results

Ploidy Analysis

One diploid ('Lola') and two tetraploid cultivars ('Pink Caprice' and 'Gold') were allowed to self-pollinate naturally in the greenhouse (without emasculation and hand-pollination). More than 900 seed were collected and sown, and more than 500 progeny were analyzed for ploidy levels. All 103 progeny of 'Lola' were diploids, indicating no UFG formation in this cultivar. The majority (88.7% and 93.4%) of the progeny of tetraploid cultivars 'Pink Caprice' and 'Gold' were tetraploids, as expected for normal *n* (2*x*) gamete formation and fertilization, but 7.6% of 'Gold' SP progeny and 11.3% of

‘Pink Caprice’ SP progeny were hexaploids (Table 4-1). The occurrence of these hexaploids indicates that *2n* gametes (4*x*) had been formed and fertilized with *n* gametes (2*x*) from normal meiosis and sporegenesis, and the fertilized eggs had developed into embryos (6*x*). Additionally, two diploids were recovered from ‘Gold’ SP seeds, but no diploids were identified in the SP progeny of ‘Pink Caprice’, even though much more progeny (311 of ‘Pink Caprice’ vs. 91 of ‘Gold’) were examined. This is the first time that such ploidy level reduction from a tetraploid to a diploid has been observed in lantana. Understanding the origin of these diploids will require the use of cytological and molecular genetic analysis tools, which are not yet available in lantana.

The above results clearly indicate the formation of *2n* gametes in ‘Gold’ and ‘Pink Caprice’, but do not elucidate which side (maternal or paternal) produced the *2n* gametes.

Ploidy analysis of OP progeny

To confirm the above findings and to determine if *2n* gametes occur in other lantana cultivars, OP seeds (more than 2800 in total) were collected from ‘Lola’, ‘Pink Caprice’ and ‘Gold’ as well as another two diploids (‘Cream’ and ‘Denholm White’) and three more tetraploids (‘Carlos’, ‘Dallas Red’, and ‘Irene’) grown in a large screen house. If *2n* gametes were not present, it would be expected that most OP progeny of a diploid cultivar would be diploid (from self pollination or cross pollination by other diploid cultivars) and some progeny would be triploid (from cross pollination by tetraploid cultivars) (Table 4-2). Similarly, if *2n* gametes did not occur, most OP progeny of a tetraploid cultivar would be tetraploids (from self pollination or cross pollination by other tetraploid cultivars) and some progeny would be triploids (from cross pollination by diploid cultivars) (Table 4-2). On the other hand, should *2n* gametes have been formed

on the seed parent side, some OP progeny of a diploid cultivar would be expected to be tetraploids (from *2n* gametes of the diploid seed parent and the normal gametes of tetraploid pollen parents), and some OP progeny of a tetraploid cultivar would be pentaploids and hexaploids (from *2n* gametes of the tetraploid seed parent and the normal *n* gametes of diploid and tetraploid pollen parents) (Table 4-2).

A limited number of seeds (and progeny) were obtained from 'Cream' and 'Denholm White' due to their very low female fertility, especially in 'Denholm White`, where 2472 flowers were pollinated with five pollen sources and 0.0004% seed set was observed. All their OP progeny were diploids, indicating a possible lack of *2n* gamete formation in these cultivars (more supporting data below).

The majority (96.4%) of 'Lola' OP progeny were diploids and 2 out of 55 progeny were triploids (Table 4-3). In an OP environment and with both diploids and tetraploids present, these two triploids could have resulted from normal gamete formation and fertilization, or *2n* gamete formation and fertilization (Table 4-2). To determine the origin of the two triploids, we examined the leaf and flower morphology of these triploids as well as all the potential diploid and tetraploid parents present at the time when OP was conducted. The most obvious difference was in flower color: all diploids had yellow, creamy yellow or white flowers, while all tetraploids had pink, magenta or red flowers. Our lantana flower color inheritance studies indicated that crosses among yellow, creamy yellow or white flowers would result in progeny with shades of yellow to white, and crosses between yellow, creamy yellow or white flowers and pink, magenta or red flowers would result in progeny with shades of pink or red (D. Czarnecki and Z. Deng, unpublished). Flowers of the two OP triploids of 'Lola' were in shades of magenta,

indicating a probable origin from union of normal *n* (*x*) female gametes of ‘Lola’ (yellow) with *n* (2*x*) male gametes of tetraploids (pink or magenta). Thus *2n* gametes were not involved in the formation of the triploid progeny of ‘Lola’.

Four ploidy levels (3*x*, 4*x*, 5*x*, and 6*x*) were observed in 14 ‘Pink Caprice’ OP progeny (Table 4-3). The presence of 5*x* and 6*x* progeny indicates the occurrence of *2n* gametes in ‘Pink Caprice’ (Table 4-2). Further, the presence of 5*x* suggests that, more specifically, it was UFGs that led to the production of higher ploidy levels (Table 4-2). The percentage of 5*x* and 6*x* progeny was 35.7%, a much higher frequency of *2n* gamete formation than observed when ‘Pink Caprice’ was grown in the greenhouse and self-pollinated. Formation of *2n* gametes was observed again in ‘Gold’, as indicated by the presence of 11 pentaploids and 4 hexaploids in the 81 ‘Gold’ OP progeny (Table 4-3). Similarly, the frequency of *2n* gamete formation in ‘Gold’ was much higher in the OP progeny than in the SP progeny (19.8% vs. 7.7% in the SP progeny). One diploid appeared in ‘Gold’ OP progeny, and its causes remain to be understood.

In ‘Carlos’ and ‘Dallas Red’, the majority of the 77 or 50 OP progeny were tetraploids and a few were triploids. The absence of pentaploids or hexaploids indicates a lack of *2n* gamete formation in these tetraploid cultivars.

OP seeds were also collected from a commercial cultivar grown at a hotel site in Pittsburg, PA (UPL) and its progeny were either tetraploid or hexaploid, suggesting a high frequency (57.7%) of *2n* gamete formation in this cultivar (Table 4-3).

These results from ploidy analysis of OP progeny confirm the results from SP progeny analysis. They also suggest remarkable differences among lantana cultivars in *2n* gamete formation and the non-reduction is likely to occur on the maternal side.

Ploidy analysis of CP progeny

A total of over 2000 seed were sown from CP. 'Carlos' (4*x*) produced tetraploid progeny when pollinated with 'Dallas Red' (4*x*) and produced triploids (214) when pollinated with 'Denholm White' (2*x*), 'Lola' (2*x*), or a diploid breeding line LAOP-9 (progeny of 'Lola') (Table 4-4). These results support the above-mentioned observation in SP and OP progeny – 'Carlos' did not produce *2n* gametes. It seems reasonable to make a similar conclusion for 'Dallas Red', as it produced only tetraploid progeny when pollinated with 'Carlos' and produced only triploids when pollinated with 'Denholm White', 'Lola', or LAOP-9 (Table 4-4). When pollinated with three tetraploids ('Carlos', 'Dallas Red', and 'Irene'), 'Lola' produced triploids, and when pollinated with diploid LAOP-9 or selfed, 'Lola' produced diploids (Table 4-4). 'Cream' produced triploids when pollinated with 'Carlos', 'Dallas Red', or 'Irene', which indicates that 'Cream' did not form *2n* gametes and supports the observations from SP and OP progeny (Table 4-4).

Although 1137 flowers of 'Gold' were pollinated with 'Carlos' or 'Dallas Red' in 2007 and 2008, only 20 seeds were obtained and only 15 germinated for ploidy analysis. The high percentages of hexaploids (60% and 100%) appearing in these progeny indicate high percentages of *2n* gamete formation, more specifically, UFG formation, in 'Gold' (Table 4-4). When 'Gold' was pollinated onto 'Carlos', all the progeny were tetraploids (Table 4-4). This contrasts with the ploidy level distribution in progeny of 'Gold' × 'Carlos' and indicates a lack of *2n* pollen formation in 'Gold' and a lack of UFG formation in 'Carlos'. 'Pink Caprice' produced pentaploids and hexaploids when pollinated with diploid 'Denholm White' or tetraploid 'Radiation', respectively, which supports the formation of UFGs in 'Pink Caprice' (25% to 100%) (Table 4-4).

Transmission of UFG Formation

To gain understanding of the transmission of this *2n* gamete formation trait, progeny of 'Gold' and 'Pink Caprice' were analyzed for ability to form UFGs. Two 'Gold' progeny were selected because of the availability of flowers for pollination. Progeny 604-1 (P604-1, 4*x*) was from a cross-pollination between 'Carlos' (non-*2n* gamete producer) and 'Gold' (UFG producer). P604-1 produced hexaploids at high frequencies in its progeny when self- or open-pollinated (Table 4-5). This indicates that P604-1 was able to produce *2n* gametes and had possibly inherited the trait from 'Gold'. Transmission of the trait from a paternal parent to progeny also suggests that the trait may be under nuclear gene control (Figure 4-2). Progeny GDGHOP-36 was a diploid progeny of self-pollinated 'Gold'. When pollinated with 'Lola'(2*x*, non-*2n* female gamete producer), GDGHOP-36 produced triploids only (100%; Table 5), indicating *2n* gamete formation in GDGHOP-36, another line of evidence showing the transmission of *2n* gamete formation from 'Gold' to its progeny.

The transmission of *2n* gamete formation from 'Pink Caprice' to its progeny was examined using PCOP-6 (4*x*), a progeny of 'Pink Caprice' and PKGHOP-1 (2*x*), a second-generation progeny of 'Pink Caprice'. PCOP-6 produced *2n* female gametes, as shown by the occurrence of 5*x* progeny when pollinated with LAOP-9 (2*x*) (Table 4-5). PKGHOP-1 produced 4*x* progeny when pollinated with DROP-25, a 4*x* OP progeny of 'Dallas Red' (Table 4-5), indicating that PKGHOP-1 also carried the UFG formation trait from 'Pink Caprice'.

Effect of 2*n* Gamete Formation on Seed Production by Triploids

Three 3*x* breeding lines with or without *2n* gamete formation background were pollinated with one common cultivar 'Lola' (2*x*). Breeding lines 605-35 and 624-1 were

from ‘Carlos’ and ‘Dallas Red’, respectively, and both did not carry the UFG trait. Their seed set ranged from 0 to 1%. Breeding line GDOP-4 carried the UFG formation trait from ‘Gold’, and its seed set was 25%.

Discussion

In the 2*n*-gamete-producing cultivars, the frequency of 2*n* gamete formation varied widely, from 7.7% to 100.0% in ‘Gold’ and from 11.3% to 100.0% in ‘Pink Caprice’ (Table 4-1). Similar variation seems common in other plants and has been noted among individuals, flowers of an individual, and even within different anthers of a single flower. For example, the frequency of 2*n* gamete (pollen) formation varied between 4% and 37% among flowers of an individual plant of *Medicago sativa* (McCoy, 1982) and ranged from 5.6% to 61.7% among anthers of a single flower bud in *Solanum* (Veilleux et al., 1982). It has been documented that the frequency of 2*n* gamete formation in a plant may be influenced by genotype, environment, and their interactions. Temperatures, in particular, seem to have a strong impact on the production of 2*n* gametes in *Solanum* (McHale, 1983). Additional important factors noted in previous studies are variable degrees of penetrance and expressivity in the genes responsible for 2*n* gamete formation (Watanabe and Peloquin, 1989; Mok and Peloquin, 1975). In this study, except for the variable frequencies, a cultivar’s 2*n* gamete formation activity remained quite consistent under different pollination schemes (OP, SP, or CP) and under different growing conditions (shade house and greenhouse): 2*n*-gamete producers always produced 2*n* gametes, and non-2*n*-gamete producers did not.

Mechanisms of UFG Formation in *L. camara*

Two basic processes have been described as the mechanisms for *2n* gamete (pollen and egg) formation in plants: first division restitution (FDR) and second division

restitution (SDR) (Bretagnolle and Thompson, 1995). The most critical step in gamete formation is meiosis. Normal meiosis consists of two successive divisions: the first leading to the separation of paired homologous chromosomes and the second leading to the separation of sister chromatids. In FDR, the first meiotic division occurs abnormally, with homologous chromosomes not pairing during Prophase I and/or not separating to opposite poles during Anaphase I. In SDR, the second meiotic division occurs abnormally, with sister chromatids not separating to opposite poles during Anaphase II. Two experimental approaches have been used to deduce the likely mode and the relative frequency of each mode in the formation of *2n* gametes in plants: observing the cytological abnormalities in the meiotic mother cells and/or segregation analysis of morphological or molecular markers in the progeny (Bretagnolle and Thompson, 1995). Due to its fruit structure (drupe), clear identification of meiotic abnormalities in the ovules of lantana is expected to be very difficult. Morphological and molecular markers are yet to be identified or developed in lantana for segregation analysis. However, the ploidy analysis results from the present study and cytological observations from previous studies may be able to provide some indications as to the possible mechanisms underlying UFG formation in lantana. Possible explantaions could be non-functional spindle apparatus, or dysfunctional gene(s) regulating meiosis.

It has been shown that univalents and multivalents (during Prophase I) and laggards (during Anaphase I) are very common in lantana polyploids (Natarajan and Ahuja, 1957; Spies and Stirton, 1982b). It is expected, therefore, that *2n* gametes formed through SDR would be aneuploids and contain various numbers of chromosomes. The progeny of ‘Gold’ and ‘Pink Caprice’ resulting from UFGs were

either pentaploids or hexaploids (Tables 4-3 and 4-4). Similarly, the progeny of 'Red Cap' (3*x*) were either tetraploids or pentaploids, and the progeny of 'Purple Prince' (5*x*) were hexaploids (Khoshoo and Mahal, 1967). These results suggest that the UFGs leading to the production of these higher level polyploids should contain all the chromosome complements in the respective seed parents. This would be possible only through FDR, and the meiotic abnormalities occurred before or during the first division and all the unpaired (univalents) as well as paired homologous chromosomes (bivalents, trivalents and quadrivalents) did not separate during anaphase I. To confirm or refute the above inference, we have developed SSR (simple sequence repeat) markers that can be used for segregation analysis and for assessing the levels of heterozygosity in the progeny of *2n*-gamete-producing cultivars. This will help determine the stage of non-reduction.

Occurrence of UFGs and Polyploidization in *Lantana*

In the present study, diploids produced triploids and tetraploids, and tetraploids produced pentaploids and hexaploids through UFG formation and fertilization with *n* male gametes (Tables 4-3 and 4-4). In a previous study, a triploid ('Red Cap') produced tetraploid and pentaploid progeny, and a pentaploid ('Purple Prince') produced hexaploid progeny through the same process (Khoshoo and Mahal, 1967). As summarized in Figure 4-1, these results suggests that (1) all the observed polyploid levels in *L. camara* (triploids to hexaploids) can be evolved through UFG formation and fertilization with normal *n* male gametes, and (2) there exist two or more pathways for polyploidization at each of the ploidy levels in *L. camara*. For example, tetraploids could be evolved from three pathways: the union of an UFG (3*x*) of a triploid with a *n* male gamete (*x*) of a diploid, an UFG (2*x*) of a diploid with the *n* male gamete (2*x*) of a

tetraploid, or a *n* female gamete (2*x*) and a *n* male gamete (2*x*) of a tetraploid. Similarly, there are more than one pathway for the production and evolution of triploids, pentaploids, and hexaploids. It is conceivable that the existence of multiple polyploidization pathways could lead to an increased diversity (or complexity) in *L. camara*.

The significance of UFG formation in the evolution of polyploids in *L. camara* also lies in its potential to quickly generate a series of polyploids within a short period of time. A diploid with UFG forming capability could produce triploid progeny (in generation 1), which could produce tetraploid progeny (in generation 2) that could produce pentaploid and hexaploid progeny in generation 3. That is, all the polyploid levels observed in *L. camara*, from triploids to hexaploids, could emerge in three generations. Considering the short juvenile periods and ability to flower year-round and propagate vegetatively, once *L. camara*, acquired the ability to produce UFGs, it could produce the observed polyploid series in three generations and in two or three years.

With the ability to produce UFGs in the genetic background, pentaploids and hexaploids could potentially produce heptaploids and octoploids in the presence of 2*x* pollen from tetraploids. Should pentaploid or hexaploid eggs be formed in these pentaploid or hexaploid seed parents, the polar nuclei would be decaploids (10*x*) and dodecaploids (12*x*). If the UFGs and polar nuclei were fertilized by the *n* male gametes of a tetraploid parent, the ploidy level ratio between the embryo and the endosperm would be 7:12 (for the pentaploid seed parent) or 8:14 (for the hexaploid parent). It will be interesting to find out if it is possible for fertilized eggs under these genetic conditions would develop into viable embryos, seeds, or individuals at these high ploidy levels.

When this study was initiated, hexaploids were not available for controlled pollination to address this question. However, previous studies of numerous naturalized populations from India, South Africa, and Australia have not revealed any ploidy levels higher than hexaploids (Natarajan and Ahuja, 1957; Raghavan and Arora, 1960; Spies and Stirton, 1982), which may indicate negative or lethal effects associated with excessively high ploidy levels (heptaploidy or higher) in *L. camara*. So far, the most common polyploids found in commercial cultivars as well as naturalized populations of *L. camara* have been tetraploids (Czarnecki et al., 2008; Natarajan and Ahuja, 1957; Raghavan and Arora, 1960; Sanders, 1987a; Spies and Stirton, 1982b). Compared to diploids or triploids, pentaploids and hexaploids, tetraploids occupy in the widest habits and show high levels of fertility, suggesting that tetraploidy might be the most adaptable (and aggressive) ploidy level in *L. camara* (Sanders, 1987a).

Polyploids are common in other lantana species (such as *Lantana involucrata* L.) and some other genera of Verbenaceae (such as *Duranta* L., *Lippia* L., *Stachytarpheta* Vahl, and *Verbena* L.) (Sanders, 2001). A good understanding of the mechanism and pathways for polyploidization in *L. camara* may shed light on the emergence and evolution of polyploids and speciation in these highly complex species as well.

UFGs and Seed Set in Polyploids

One interesting phenomenon in *L. camara* has been the lack of correlation between meiotic irregularities during microsporegenesis and seed set in naturalized polyploids. In spite of high frequencies of meiotic irregularities in pollen mother cells, lantana polyploids produced abundant viable seeds (Natarajan and Ahuja, 1956; Raghavan and Arora, 1960). For example, the majority of tetraploids collected from different parts of India showed 2 to 3 quadrivalents, 2 to 6 trivalents, and 4 to 12

univalents in pollen mother cells, but more than half of these tetraploids produced the largest numbers of seeds, compared to diploids (Natarajan and Ahuja, 1957; Spies 1984b). Khoshoo and Mahal (1967) suggested that apoximis was responsible for the seed set in these highly male-sterile polyploids. Spies and Stirton (1982c) examined embryo sac development in *L. camara* from South Africa, but did not find definitive evidence for apomixis. Lantana producing UFGs provides an explanation for such a lack of correlation in polyploids. Without undergoing the normal meiotic first division during megasporegenesis, polyploids, even though showing high levels of meiotic abnormalities during microsporegenesis in their pollen mother cells, could still produce megaspores and egg cells that contain all the chromosome complements, which would be viable and able to be fertilized and produce viable seeds.

Effect of 2*n* Gamete Formation on Seed Production by Triploids

Sterile cultivars are needed to control the invasive potentials *L. camara* has shown in the Southern United States and other countries. Compared to diploids and tetraploids, triploid lantana plants show the lowest pollen stainability and seed set (Czarnecki et al., 2008). Because of this and the general high level of male and female sterility triploids have expressed in banana, citrus and watermelon, production of triploids has been proposed as a main genetic approach to develop new sterile lantana cultivars. Results from the present study suggest the possibility of producing triploids through crosses between diploids and tetraploids, and through crosses between diploids as well (Figure 4-1). More importantly, the results indicate a strong need to screen and select breeding parents carefully and to avoid using UFG-producing plants in crosses intended for high sterility. If triploids have inherited the ability to form UFGs from either seed or pollen parents, these triploids will be likely to produce viable UFGs

(3*x*), which can be fertilized with pollen from surrounding diploids or tetraploids and produce viable seeds, which will defy the purpose of producing such triploids. None of the diploids ('Cream', 'Denholm White', and 'Lola') in this study showed tendency to produce UFGs, but three out of the six tetraploids ('Gold', 'Pink Caprice') showed such tendency. To find more tetraploids that do not produce UFGs and can be used as breeding parents, screening of more commercial cultivars or germplasm and progeny through ploidy analysis will be required.

Table 4-1. Ploidy level, ancestry, source of plant material, and formation of unreduced female gametes in lantana cultivars and breeding lines.

Cultivars / breeding lines	Ploidy level	Ancestry	Sources of plant material	2*n* female gamete formation	2*n* female gametes (%)
'Carlos'	*4x*	Unknown	Commercial	No	-
'Cream'	*2x*	Unknown	Commercial	No	-
'Dallas Red'	*4x*	Unknown	Commercial	No	-
'Denholm White'	*2x*	Unknown	Commercial	No	-
'Gold'	*4x*	Unknown	Commercial	Yes	7.7 to 100
'Irene'	*4x*	Unknown	Commercial	No	-
'Lola'	*2x*	Unknown	Commercial	No	-
'Pink Caprice'	*4x*	Unknown	Commercial	Yes	11.3 to 100
'Radiation'	*4x*	Unknown	Commercial	Yes	50.0
UPL[Z]	*4x*	Unknown	This study	Yes	42.3
DROP-25	*4x*	OP progeny of 'Dallas Red'	This study	No	-
GDGHOP-36	*2x*	OP progeny of 'Gold'	This study	Yes	100
GDOP-4	*3x*	OP progeny of 'Gold'	This study	Yes	100
LAOP-9	*2x*	OP progeny of 'Lola'	This study	No	-
PCOP-6	*4x*	OP progeny of 'Pink Caprice'	This study	Yes	100
PKGHOP-1	*2x*	Second-generation OP progeny of 'Pink Caprice'	This study	Yes	100
P604-1	*4x*	Progeny of 'Carlos' and 'Gold'	This study	Yes	57.1 to 100

[Z]Unknown Pittsburg lantana

Table 4-2. Expected distribution of ploidy levels in the progeny of diploid and tetraploid lantana with or without unreduced female gametes and/or unreduced pollen.

Seed parents: ploidy level	Pollen parents: ploidy level	Pollination schemes	Expected ploidy levels in progeny of diploid and tetraploid parents with and/or without 2*n* gamete formation during sporogenesis													
			n gametes (female and male)			2*n* female gametes (unilateral)				2*n* male gametes (unilateral)				2*n* female x 2*n* male gametes (bilateral)		
			2*x*	3*x*	4*x*	3*x*	4*x*	5*x*	6*x*	3*x*	4*x*	5*x*	6*x*	4*x*	6*x*	8*x*
2*x*	2*x*	SP, CP [z]	yes			yes				yes				yes		
	4*x*	CP		yes			yes					yes			yes	
	2*x* and 4*x*	OP	yes	yes		yes	yes			yes		yes		yes	yes	
4*x*	2*x*	CP			yes			yes			yes					yes
	4*x*	SP, CP							Yes				yes			yes
	2*x* and 4*x*	OP		yes	yes			yes	Yes		yes		yes		yes	yes

[z] CP, OP, SP: controlled, open, and self-pollination, respectively.

Table 4-3. Distribution of ploidy levels in the progeny from self- and open-pollination (SP and OP). Pollination was performed in 2006 and 2007, and ploidy analysis done in 2007 and 2008, Wimauma, FL.

Seed parent (ploidy level)	Pollination scheme	Seeds collected & sown	Progeny available for ploidy analysis	Progeny in ploidy levels (no.)					Polyploids indicating 2*n* gamete formation	Percentage of 2*n* gametes
				2*x*	3*x*	4*x*	5*x*	6*x*		
'Lola' (2*x*)	SP	263	103	103					3*x*, 4*x*	0
'Pink Caprice' (4*x*)	SP	403	311			276		35	6*x*	11.3
'Gold' (4*x*)	SP	256	91	2		84		5	6*x*	7.7
'Carlos' (4*x*)	SP	38	15			15			6*x*	0
'Cream' (2*x*)	OP	56	9	9					4*x*	0
'Lola' (2*x*)	OP	335	55	53	2				4*x*	0
'Pink Caprice' (4*x*)	OP	403	14		2	7	4	1	5*x*, 6*x*	35.7
'Gold' (4*x*)	OP	758	81	1	2	63	11	4	5*x*, 6*x*	19.8
'Carlos' (4*x*)	OP	639	77		9	68			5*x*, 6*x*	0
'Dallas Red' (4*x*)	OP	254	50		3	47			5*x*, 6*x*	0
'Irene' (4*x*)	OP	355	52		1	51			5*x*, 6*x*	0
UPL (4*x*)	OP	65	26			11		15	6*x*	42.3

Table 4-4. Distribution of ploidy levels in the progeny of controlled pollinations grouped by seed parent. Pollinations were made in 2005, 2006 and 2007, and ploidy analysis done in 2008, Wimauma, FL.

Seed parent	Pollen parent	Flowers pollinated	Progeny available for ploidy analysis	Progeny in ploidy levels (no.)					Polyploids indicating 2*n* gamete formation	Percentage of 2*n* gametes
				2*x*	3*x*	4*x*	5*x*	6*x*		
'Carlos' (4*x*)	'Dallas Red' (4*x*)	2202	31			31			6*x*, 8*x*	0
'Carlos' (4*x*)	'Denholm White' (2*x*)	731	23		23				4*x*, 5*x*, 6*x*	0
'Carlos' (4*x*)	LAOP-9 (2*x*)	1259	56		56				4*x*, 5*x*, 6*x*	0
'Carlos' (4*x*)	'Lola' (2*x*)	3656	135		135				4*x*, 5*x*, 6*x*	0
'Dallas Red' (4*x*)	'Carlos' (4*x*)	2051	19			19			6*x*, 8*x*	0
'Dallas Red' (4*x*)	'Denholm White' (2*x*)	468	5		5				4*x*, 5*x*, 6*x*	0
'Dallas Red' (4*x*)	LAOP-9 (2*x*)	1653	28		28				4*x*, 5*x*, 6*x*	0
'Lola' (2*x*)	LAOP-9 (2*x*)	1894	100	100					3*x*, 4*x*	0
'Lola' (2*x*)	'Lola' (2*x*)	567	18	18					3*x*, 4*x*	0
'Lola' (2*x*)	'Carlos' (4*x*)	2215	16		16				4*x*, 5*x*, 6*x*	0
'Lola' (2*x*)	'Dallas Red' (4*x*)	2230	5		5				4*x*, 5*x*, 6*x*	0
'Lola' (2*x*)	'Irene' (4*x*)	564	4		4				4*x*, 5*x*, 6*x*	0
'Cream' (2*x*)	'Carlos' (4*x*)	769	7		7				4*x*, 5*x*, 6*x*	0
'Cream' (2*x*)	'Dallas Red' (4*x*)	995	4		4				4*x*, 5*x*, 6*x*	0
'Cream' (2*x*)	'Irene' (4*x*)	472	3		3				4*x*, 5*x*, 6*x*	0
'Gold' (4*x*)	'Carlos' (4*x*)	469	10			4		6	6*x*, 8*x*	60.0
'Gold' (4*x*)	'Dallas Red' (4*x*)	668	5					5	6*x*, 8*x*	100.0
'Carlos' (4*x*)	'Gold' (4*x*)	450	5			5			6*x*, 8*x*	0
'Pink Caprice' (4*x*)	'Denholm White' (2*x*)	213	2				2		5*x*, 6*x*	100.0
'Pink Caprice' (4*x*)	'Radiation' (4*x*)	273	4			3		1	6*x*, 8*x*	25.0
LAOP-9 (2*x*)	'Carlos' (4*x*)	1515	31		31				4*x*, 5*x*, 6*x*	0
LAOP-9 (2*x*)	'Dallas Red' (4*x*)	1326	44		44				4*x*, 5*x*, 6*x*	0

Table 4-5. Formation of 2*n* female gametes in the first- and/or second-generation progeny of 'Gold' and 'Pink Caprice'. Pollinations were made in 2006 to 2008, and ploidy analysis done in 2008, Wimauma, FL.

Seed parents (ploidy level)	Pollen parents (ploidy level)	Flowers pollinated	Progeny available for ploidy analysis	Progeny in ploidy levels (no.)					Polyploids indicating 2*n* gamete formation	Percentage 2*n* gametes
				2*x*	3*x*	4*x*	5*x*	6*x*		
P604-1 (4*x*)	Self-pollinated	70	2					2	6*x*	100.0
P604-1 (4*x*)	Self-pollinated		27			16		11	6*x*	59.3
P604-1 (4*x*)	Open-pollinated		7			3		4	6*x*	57.1
GDGHOP-36 (2*x*)	'Lola' (2*x*)	265	21		21				3*x*	100.0
PCOP-6 (4*x*)	LAOP-9 (2*x*)	158	2				2		5*x*	100.0
PKGHOP-1 (2*x*)	DROP-25 (4*x*)	~80	2			2			4*x*	100.0
PKGHOP-1 (2*x*)	LAOP-9 (2*x*)	~80	1		1				3*x*	100.0

Table 4-6. Seed set of three lantana triploids pollinated with 'Lola'. Hand pollination was performed in February 2009 in Wimauma, FL.

Triploid breeding lines	UFG background	Flowers pollinated	Seed set ± standard error (%)	Germination 12 weeks after sowing (%)
GDOP-4	Yes	188	25.0 ± 5.0	26
605-35	No	160	0.0 ± 0.0	0
624-1	No	222	1.0 ± 1.0	0

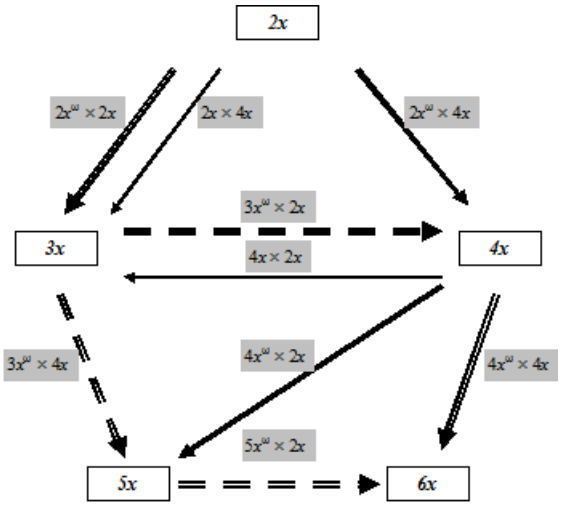

Figure 4-1. Summary of observed pathways for polyploid formation in *Lantana camara*. Solid double lines represent polyploidization pathways involving 2*n* female gamete and observed in this study; dashed double lines are polyploidization pathways involving 2*n* female gamete formation and observed by Khooshoo and Mahal (1967); and solid single lines are observed pathways not involving 2*n* female gamete formation. Symbol ω indicates parents producing 2*n* female gametes.

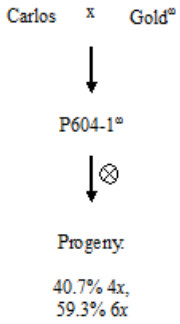

Figure 4-2. Transmission of the unreduced female gamete formation trait from 'Gold' to its progeny. 'Gold' (4*x*) was the pollen parent of breeding line P604-1, which produced tetraploid (40.7%) and hexaploid (59.3%) progeny when self-pollinated. 'Carlos' was the seed parent of P604-1. Symbol ω indicates unreduced female gamete production.

CHAPTER 5
MULTIPLE MODES OF REPRODUCTION AS REVEALED BY PLOIDY AND MICROSATELLITE MARKER ANALYSIS OF *LANTANA CAMARA*

Rationale

In evaluating female fertility of *L. camara* cultivars', many triploids were found to produce abundant fruit and seed (Chapter 3). It was suspected initially that most of the collected seeds (from OP) would have been developed from fertilized UFGs, because previous results had indicated that UFG production could result in high levels of female fertility in lantana triploids (Chapter 4). However, many of the progeny had a ploidy level identical to their maternal parents (triploids). The frequent occurrence of this type of triploid raised a question about the possibility of apomixis in *L. camara*.

A literature search revealed that attempts to understand *L. camara*'s reproductive biology could trace back to the 1960s. Raghavan and Arora (1960) noted lantana progeny that looked identical to maternal plants in morphology. Khoshoo and Mahal (1967) too described a series of reproductive anomalies in eight accessions of *L. camara*. They suspected that unreduced eggs and apomixis had led to the polyploid series found in naturalized populations. At that time, only morphological and cytological data could be analyzed to understand the relationships between parents and progeny. Nonetheless, facultative or obligate apomixis was first proposed to occur in diploid, triploid, tetraploid, and pentaploid lantana plants. Spies and Stirton (1982a) attempted to determine if apomixis was occurring in lantana. They found that the conditions for apomixis were present, but they were not able to confirm this phenomenon. Their study suggested that apospory (somatic cells forming gametes) could be occurring.

Recently the occurrence of UFGs was confirmed to exist in U.S. commercial cultivars of *L. camara* by conducting extensive ploidy analysis of progeny from OP and

controlled pollinations (CP) (Czarnecki and Deng, 2009). The significance of UFG production comes from the ability to increase the ploidy level rapidly from one generation to the next and to restore female fertility in triploid plants (Czarnecki and Deng 2009; Chapter 4).

Ploidy analysis led to the discovery of the occurrence of triploids from the offspring of triploids and the possible occurrence of apomixis. However, it was not possible to provide conclusive evidence. To facilitate understanding of the reproductive biology of *L. camara*, simple sequence repeat (SSR) markers were developed (L. Gong and Z. Deng, unpublished). This chapter focuses on analyzing the ploidy levels and SSR marker banding patterns of *L. camara'*s progeny from CP or OP. The objectives were to determine 1) the likely stage for UFGs formation, 2) the effect of UFGs on female reproduction, and 3) other possible modes of reproduction.

Materials and Methods

Plant Materials

All OP materials for this study were produced from a previous experiment (Chapter 3) to determine the fruit production of *L. camara*. The seedlings and parents from this experiment were transplanted to 15.4 cm pots on 12 May 2009 in Fafard® 2B potting mix supplemented with slow release Osmocote® (15N-3.9P-10K, 8-9 months release at 21°C; The Scotts Company, Marysville, OH) at 6.51 kg m^{-3}.

Pollinations

Pollinations were made from March 2006 through August 2009. Fresh pollen was collected and placed on the emasculated stigmas. Open pollinated seed were collected at five week intervals from field grown plants when the seed was ripe (Chapter 3).

Seed germination

Seed were collected when ripe and stored until bulked, cleaned and sown between March 2006 and February 2010. Seed from controlled crosses were sown in individual cell trays (Figure 5-1) and OP seed collected from the field were sown in community trays. Seed were sown on the surface of Fafard® 2B potting soil and germinated in the greenhouse.

Flow cytometry

Parents and progeny from 10 controlled crosses and 15 commercially available cultivars were screened for flow cytometric analysis. Also, individuals of interest from previous studies (Chapters 3 and 4) were kept for further molecular analysis. Analysis was performed using fully expanded young leaves and the Partec PA I ploidy analyzer and the CyStain UV Ploidy Precise P dye (Partec, Germany). The manufacturer recommended ploidy analysis procedure was followed with minor modifications (supplemented with 2% w/v PVP and 0.01% β-mercaptoethanol) with dye mixture kept on ice. The ploidy level of a progeny was determined by comparing to one or more commercial cultivars (reference cultivars) with known ploidy levels that were included in the same analysis. Based on flow cytometry analysis materials were divided into six groups for analysis: apomeiosis, apomixis, double unreduced female gametes (DUFG), haploids, twins (Figure 5-1), and unreduced male gametes (UMG).

DNA extraction

Fresh tender leaves were collected and dessicated in a dark box for 3-7 days with silicon gel beads. The dried leaf tissue was used for DNA extraction by first desiccating the tissue. The extraction protocol of Fulton et al. (1995) was used. Extracted DNA was dissolved in TE buffer, and DNA concentrations were determined with a ND-1000

(Thermo Scientific, Wilmington, DE, USA). DNA concentrations were adjusted to 8 ng/µL for PCR reactions.

Microsatellite analysis

Polymerase chain reactions (PCR) were done on an Eppendorf Vapo protect Mastercycler Pro 384. Each reaction (10-µL) contained 1 x PCR reaction buffer (New England Biolabs, Ipswich, MA USA), 1.5 mM of MgCl2, 2 mM dNTPs, 0.25 pmol of the forward primer with an attached M13 tail to the 5' end, 2.5 pmol of the reverse primer, 0.25 unists of Taq DNA polymerase, and 8 ηg of genomic DNA. Fluorescent labeling was done with 2.25 pmol of IRD700 and IRD800 infrared dye-labeled M13 tail primer (MWG Biotech, Highpoint, NC, USA). Primer sequences and PCR cycles used are listed in tables 5-1 and 5-2. PCR products were separated on a Li-cor 4300 DNA analyzer after denaturing at 95°C for 5 minutes. PCR products were diluted with formamide loading buffer at a 1:1 ratio and 0.8-µL of each sample was loaded into each well. Conditions for gel electrophoresis were 1500 V, 40mA at 45°C for 90 minutes after a 25 minute prerun at the same conditions. Gels were composed 6.5% Li-cor KB Plus Gel Matrix (20-mL) with, 10% APS solution (150-µL), and TEMED (15-µL). Banding results were scored as absent (0) or present (1). Each group of plants was compared to determine similarity to the parents.

Results

Ploidy Analysis of OP Progeny

Seed germination experiments in Chapter 3 resulted in 1,517 progeny from 22 *L. camara* cultivars and two breeding lines. As plants of these cultivars were grown in the open field, their progeny were expected to have resulted from open pollinations. Due to the wide differences among cultivars/lines in fruit production and seed germination

(refer to Chapter 3), the number of progeny available for ploidy analysis varied considerably, 1 to 8 for five cultivars/lines, 13 to 83 for 16 cultivars, 137 to 449 for three cultivars, and more than 1,000 for one cultivar ('Pink Caprice').

Ploidy distribution in the OP progeny of diploid *L. camara*

Two OP progeny were recovered for 'Cream'. One of the progeny was a diploid and the other a triploid (Table 5-3). In Chapter 4 and a previous report (Czarneck and Deng, 2009), 'Cream' produced diploid progeny after open pollination and triploid progeny after controlled pollination with tetraploids. The controlled pollination involved more than 2,000 'Cream' flowers. It was concluded in Chapter 4 that 'Cream' was not a UFG producer. Taking this into consideration, it is likely that the diploid would have resulted from self pollination or cross pollination with another diploid, and that the triploid would have resulted from a cross pollination with a neighboring tetraploid grown in the field.

Ten of the 13 OP progeny of 'Lola' were diploids one was triploid and one was (presumably from hexaploid pollen) tetraploid (Table 5-3). Previously 'Lola' produced diploid progeny after controlled pollination with diploids and produced triploid progeny after pollination with tetraploids, but did not produce tetraploid progeny (Chapter 4; Czarnecki and Deng, 2009). The previous analysis involved nearly 7,500 'Lola' flowers and nearly 200 progeny. Considering the diploidy of 'Lola' and other possible modes of reproduction to be discussed later in this chapter, possible cause(s) for the observed tetraploid include 1) fertilization of a 'Lola' UFG (2*x*) with a 2*x* male gamete from a neighboring tetraploid, 2) the formation of a doubled UFG by 'Lola' followed by apomictic seed development, and 3) fertilization of a normal reduced female gamete (1*x*) of 'Lola' with a reduced male gamete (3*x*) of a neighboring hexaploid.

One progeny was recovered from LAOP-9 OP seeds, and it was a diploid. Similarly the three OP progeny of LAOP-30, a progeny of 'Lola' and a sibling of LAOP-9, were diploids. Since LAOP-9 was a non-UFG producer, as demonstrated by controlled pollinations and ploidy analysis, the diploid observed here would likely be a result of self-pollination or cross-pollination with a neighboring diploid. The same seems to be applicable to the diploid OP progeny of LAOP-30.

OP progeny of triploid *L. camara*

Only one progeny was recovered from 'Lucky Red Hot Improved' OP seeds, and it was found to be aneuploid (3-4*x*) (Table 5-3). No progeny were recovered from the seeds of 'Athens Rose'. Except for these two triploid cultivars, appreciable numbers (14 to 154) of OP progeny were recovered for each of the remaining 11 triploid cultivars. Four ploidy levels (3*x*, 4*x*, 5*x*, and 6*x*) were observed in the 14 OP progeny of 'Landmark Peach Sunrise Improved'. The same series of polyploids were present in the OP progeny of 'Sunset', though at a somewhat different ratio. Three ploidy levels (3*x*, 4*x* and 5x) were found in the OP progeny of 'New Red Lantana', 'Red Butler' and 'Red Spread Lantana'. Most of the OP progeny of 'Landmark Pink Dawn', 'Lemon Drop', 'Miss Huff', and 'Samson' were 3*x*, 4*x*, or 5*x*, and occasionally some aneuploid progeny (3-4*x*, 4-5*x*, or 5-6*x*). Progeny of 'Patriot Fire Wagon' were very diverse in ploidy level, from 2*x* to 6*x*, plus aneuploids.

For most triploid cultivars (9 out of 11), 20.5% to 38.7% of their OP progeny were triploids, 55.4% to 76.9% tetraploids, and 1.3% to 10.8% pentaploids. In two triploid cultivars, 54.2% to 57.1% of OP progeny were triploids, 21.4% to 37.5% were tetraploids, and 8.3% to14.3% were pentaploids. Hexaploids appeared at 1.2-7.1% in three triploid cultivars.

In summary, the majority of the OP progeny of the female-fertile triploid cultivars were euploids, having the same ploidy level as their parents or having higher ploidy levels. Aneuploids were rare or did not occur in the OP progeny. When aneuploids were present, some of them had higher ploidy levels as well.

Unreduced (2*n*) pollen grains are rare in *L. camara*, as shown in a previous publication (Czarnecki and Deng, 2009). Thus, the occurrence of OP progeny with ploidy levels higher than their respective maternal parents' (4*x*, 5*x*, and occasionally 6*x*) suggests that these triploid parents had produced UFGs. As *n* pollen grains of 1*x*, 2*x*, or 3*x* became available from 2*x*, 4*x*, or 6*x* lantana plants grown in close proximity, these 3x UFGs would form 4*x*, 5*x*, and 6*x* embryos (and OP progeny) after fertilization. Through the same process, these 3*x* UFGs could produce some of the aneuploid progeny (4-5*x* or 5-6*x*) if aneuploid pollen grains were produced by the polyploid paternal plants and participated in the fertilization. Conversely, the occurrence of the numerous 3*x* individuals in the progeny of 11 triploid cultivars suggests that these triploid parents not only had formed UFGs, but also their UFGs had developed into embryos (and progeny) without double fertilization, i.e. apomixis. A similar phenomenon had been suspected by Spies and Stirton (1982c). As shown later in this chapter, apomixis did occur and was the source of the triploids in the OP progeny of the triploid cultivars.

The suspected pathway for embryogenesis (Figure 5-3) also suggests that when UFGs were formed during megasporogenesis, they could follow two paths, sexual or asexual, to produce progeny. When UFGs took the sexual pathway, they produced 2*n* + *n* progeny, and when UFGs took the asexual pathway, they produced 2*n* + 0 progeny.

Interestingly, when the frequencies of the two types of progeny were averaged across the 11 triploid cultivars, the ratio was close to 2:1 [65.8% (2*n* + *n*): 33.1% 2*n* + 0]. This ratio may indicate that on average, the UFGs might be twice as likely to produce 2*n* + *n* progeny through fertilization as to produce 2*n* + 0 progeny through apomixis.

One progeny of 'Patriot Fire Wagon's was a diploid. Diploid individuals were also observed in the progeny of tetraploid cultivars 'Gold', 'Pink Caprice', and 'Radiation' (Chapter 4; Czarnecki and Deng, 2009). The occurrence of these diploids in the progeny of tetraploids indicates that some reduced female gametes might also have the ability to develop into embryos (and progeny) through apomixis, leading to low frequencies of haploidization in *L. camara*.

OP progeny of tetraploid *L. camara*

The ploidy levels of the OP progeny of 'Carlos', 'Dallas Red', 'Gold', 'Irene', 'Pink Caprice', and 'Radiation' were described in chapter 4 and a previous publication (Czarnecki and Deng, 2009). Analysis of the ploidy level distribution in the OP progeny of these cultivars suggests that 'Gold', 'Pink Caprice' and 'Radiation' produced UFGs, while 'Carlos', 'Dallas Red', and 'Irene' did not.

'Carlos', 'Dallas Red', and 'Irene' produced triploids when pollinated with diploids and produced tetraploids when pollinated with tetraploids (Chapter 4). A similar ploidy level distribution was observed in the OP progeny (35 to 56 progeny) of these cultivars: low frequencies of triploids (5.4% to 11.1%), high frequencies of tetraploids (77.1% to 91.1%), and low frequencies of aneuploids. This distribution was similar to the one shown in Chapter 4, except for the occurrence of low frequencies of aneuploids. An aneuploid (5-6*x*) appeared in the OP progeny from 'Carlos'. This was likely the result of a viable pollen grain from a pentaploid or hexaploid plant. The field planting map

indicated that several pentaploids and hexaploids were within 1.8 to 3.6 meters of ‘Carlos’ plants.

Four ploidy levels were observed among the ‘Gold’ OP progeny, including a low frequency (1.7%) of *n* + 0 progeny, a low frequency (3.4%) of 2*n* + *n* progeny (5*x* and 6*x*), and a high frequency (94.9%) of 4*x* progeny (Table 5-3). This ploidy level distribution again indicates that UFGs had been formed and apomixis has occurred in ‘Gold’. Considering these new possibilities, the 4*x* progeny may be the result of normal fertilization of n female and male gametes (*n* + *n* progeny) or apomictic seed development from UFGs without fertilization, 2*n* + 0 progeny.

A similar ploidy level distribution was found among ‘Radiation’ OP progeny, confirming the ability of this cultivar to produce UFGs as shown in Chapter 4 and suggests that apomixis led to 2*n* or *n* progeny in addition to *n* + *n* progeny. Compared to ‘Gold’, the exception among ‘Radiation’ OP progeny was the occurrence of a low frequency (11.9%) of triploids, which would have been the result of fertilization of *n* female and *n* male gametes.

A large population of ‘Pink Caprice’ OP progeny (449) was analyzed for ploidy levels (Table 5-3). One triploid and one aneuploid with a lower ploidy level (3-4*x*) were observed among the progeny. Haploids were not observed among this group of OP progeny, but one haploid did occur previously (Czarnecki and Deng, 2009), suggesting apomixis occurred in ‘Pink Caprice’. Hexaploids (2*n* + *n*) occurred at a frequency of 14.3%. The majority of the OP progeny was 4*x* (84.2%). The higher rates of 2*n* + *n* plants in ‘Pink Caprice’ may be the result of higher pollen fertility 220 to 280% higher than ‘Gold’ and ‘Radiation’, the other UFG tetraploids (Chapter 2). The observed

tetraploid plants are from *n* + *n* fertilization of reduced female gametes with reduced male gametes and/or 2*n* + 0 progeny from UFGs undergoing apomixis.

OP progeny of pentaploid and hexaploid *L. camara*

There was a wide range of ploidy variation among the 49 OP progeny of 'Spreading Sunset' (5*x*) (Table 5-3). The ploidy level of euploids ranged from 4*x* to 7*x* and aneuploids had 3-4*x*, 4-5*x*, or 7-8*x*. This was the first time that 7*x* (2) and 7-8*x* progeny (3) were observed in this study. This was also the first time that they were observed in *L. camara* as numerous reports have noted only 2*x* to 6*x* ploidy levels (Khoshoo and Mahal, 1967; Natarajan and Ahuja 1957; Ojha and Dayalo, 1992; Raghavan and Arora 1960; Spies, 1984ab; Spies and Stirton 1982a, b, c; Sanders 1987ab). The occurrence of hexaploids and heptaploids in the OP progeny demonstrates that 'Spreading Sunset' had the ability to produce UFGs. The 5*x* gametes had had been fertilized with 1*x* or 2*x* reduced male gametes from neighboring diploids or tetraploids and developed into 6*x* or 7*x* embryos and progeny. This answers a question raised in Chapter 4 if 7*x* and 8*x* plants could be formed and may suggest the possibility to continually increase ploidy levels beyond what has been observed.

Of the eight progeny from 'Tangerine' (6*x*) OP seeds were four triploids, one tetraploid, one hexaploid, and one aneuploid (3-4*x*) (Table 5-3). The occurrence of a high frequency (50%) of triploids suggests that some of 'Tangerine's reduced female gametes (3x) might produce apomictic seeds and progeny. However, the observed ploidy level distribution could not discern whether or not 'Tangerine' produced UFGs.

Ploidy Analysis of CP Progeny

The ploidy analyses above and in Chapter 4 suggest several possible modes of reproduction in *L. camara* and the need to use molecular markers to elucidate some of

the proposed hypotheses. Toward this objective, three diploids, one triploid and three tetraploids were selected as seed parents, and three diploids and two tetraploids as pollen parents to make seven controlled crosses (Table 5-4). Three of the seven seed parents were known to produce UFGs (GDGHOP-36, GDOP-4, and PKGHOP-1) and three not to produce UFGs ('Carlos', 'Dallas Red', and DROP-25). It was unknown whether 'Landmark White' would produce UFGs. Seeds from each cross were germinated individually. Progeny were first analyzed for ploidy levels and then analyzed with molecular markers (see below).

The cross between 'Landmark White' (2*x*) and 'Denholm White' (2*x*) produced 77 progeny. All progeny were diploids, and many demonstrated flower colors similar to 'Landmark White' and 'Denholm White' (mostly creamy yellow and white shades). These results suggest that most likely the progeny were hybrids and 'Landmark White' and 'Denholm White' did not produce unreduced female or male gametes. Four of the progeny emerged from two seeds (Fig. 5-1).

Of the 63 progeny from the cross between GDGHOP-36 (2*x*) and 'Denholm White' (2*x*), 11 were diploids, 51 triploids, and one tetraploid (Table 5-4). In an earlier cross with diploid 'Lola', 100% progeny of GDGHOP-36 were triploids (Czarnecki and Deng, 2009). Thus, the high frequency (80.1%) of triploids was expected in the current cross, but not the diploids and the tetraploid. Potentially the diploids could have resulted from fertilization of reduced female and reduced male gametes or from UFGs developing into embryos through apomixis. Several possibilities might lead to the occurrence of a tetraploid in a diploid x diploid cross: fertilization of an UFG (2*x*) and a *n* unreduced male gamete (UMG) (2*x*) (2*n* + 2*n* progeny), the formation of an UFG followed by

chromosome doubling (doubled UFG) and apomixis (4*n* + 0 progeny) or fusion of UFGs in one embryo sac (4*n* + 0 embryo) followed by apomixis (4*n* + 0 progeny). Spies and Stirton (1982a) noted that diploid *L. camara* will produce two sexual embryo sacs in one ovule at a frequency of 26.7% which could explain the twin seedlings.

The cross between PKGHOP-1 (2*x*) and PCOP-6 (4*x*) produced 10 progeny; half of them were diploids and the other half tetraploids. The tetraploids were expected, as PKGHOP-1 had been shown to produce UFGs (Chapter 4). A possible origin of the diploids could be PKGHOP-1's UFGs undergoing apoximis (2*n* progeny).

The majority (24 out of 25) of the progeny of GDOP-4 (3*x*) and Lola (2*x*) were tetraploids, indicating that GDOP-4 produced UFGs that fertilized with reduced male gametes of 'Lola' and developed into embryos (2*n* + *n* progeny). This was anticipated based on the results from a previous controlled cross, in which GDOP-4 also produced a high frequency of UFGs (Czarneck and Deng, 2009). However, one progeny was found, unexpectedly, to be a pentaploid. The occurrence of a progeny of this ploidy level seems to suggest that an UFG (3*x*) formed by GDOP-4 had fertilized with an UMG from 'Lola' (2*n* + 2*n* progeny).

Two crosses were between a tetraploid and a diploid: 'Carlos' x 'Lola' and DROP-25 x LAOP-9. A large number of progeny (135 or 92) were analyzed for each cross, and all of their progeny were triploids. The results support the conclusion in Chapter 4 that 'Carlos' and DROP-25 were not UFG producers. Twin seedlings were observed in the progeny of these crosses: six pairs in 'Carlos' x 'Lola', and 10 pairs in DROP-25 x LAOP-9.

Nineteen progeny were obtained for the cross between ‘Dallas Red’ and ‘Carlos’, and they were all tetraploids, in agreement with the earlier conclusion (Chapter 4) that ‘Dallas Red’ and ‘Carlos’ produced reduced female and male gametes. One pair of twin seedlings was also observed in this cross.

Summary of Ploidy Analysis Results

Ploidy analyses of OP and CP progeny above and in Chapter 4 describe six embryo and fertilization combinations for reproduction in *L. camara* (Figure 5-2), including 1) reduced female and male gametes fertilized and developed into $n + n$ progeny, 2) reduced female gametes underwent apomixis ($n + 0$ progeny, or haploidization), 3) UFGs fertilized with reduced male gametes producing $2n + n$ progeny, 4) UFGs took the apomixis pathway and directly developed into embryos and $2n + 0$ progeny, 5) doubled UFGs fertilized with reduced male gametes, resulting in $4n + n$ progeny, and 6) doubled UFGs developed into $4n$ progeny through apomixis ($4n + 0$ progeny) (Figure 5-3). In contrast, the primary reproductive pathway during microsporogenesis was the formation of reduced male gametes. UMGs were possibly observed, but they occurred at very low frequencies.

SSR Marker Analysis

Four SSR markers, M1, M51, M76, and M87, were used to analyze 195 progeny from 22 females crossed with five males (Table 5-4). They amplified 23 alleles in total. Twenty two of the 23 alleles were found to be polymorphic with one allele present in all lines used (M87-5). All allele banding patterns to be discussed can be seen in table 5-4.

Putative 2*n* + *n* progeny

As described above, 2*n* + *n* progeny were observed in the progeny of three diploid breeding lines, nine triploid cultivars, three tetraploid cultivars, one petaploid cultivar, and one hexaploid cultivar. Ploidy analyses had provided evidence for the occurrence of UFGs and 2*n* + *n* progeny. A key question about lantana UFG formation is when is the stage of nonreduction? It was unknown whether it was first division restitution (FDR) or second division restitution (SDR). The high frequencies of 4*x* and 5*x* (euploids) individuals in the progeny of triploid cultivars, lack of aneuploids, and restoration of 3*x* and 5*x* fertility indicated a FDR mechanism. A mechanism (such as diplospory) to restore the fertility prior to a failure in prophase of meiosis I or apospory replacing the aborted gamete would both potentially lead to gametes resulting in euploid progeny.

The progeny of two crosses were analyzed using SSR markers to provide further evidence to explain how female gametes are formed. The selected seed parents for the two crosses were GDGHOP-36 (2*x*) and GDOP-4 (3*x*), known to produce UFGs and 2*n* + *n* progeny, and the pollen parents were 'Denholm White' and 'Lola', which were known to be non-UFG producers. UFG-producing tetraploids, pentaploids or hexaploids were not used in the crosses in order to avoid complex marker banding patterns.

GDGHOP-36 and 'Denholm White' and their 2*n* + *n* progeny: Three markers (M1, M51, and M76) detected 11 polymorphic alleles between GDGHOP-36 and 'Denholm White'. Alleles M1-4, M1-7, M1-9, M51-4, M51-5, M76-2, and M76-3 were present in GDGHOP-36, while alleles M1-1, M51-1, M51-2, and M76-4 were present in 'Denholm White'. Out of the 50 progeny (3*x*) analyzed, 45 produced clear gel images for reliable marker scoring. All 45 progeny carried all the alleles from the maternal

parent GDGHOP-36. The two alleles amplified by M51 (M51-1 and M51-2) in 'Denholm White' segregated among the progeny, with M51-1 present in 31 progeny and M51-2 present in another 16 progeny. These results showed that all progeny had the complete sets of alleles (or chromosomes) of their maternal parent and some of the marker alleles from their paternal parent. The paternal parents's alleles were segregating among the progeny (Table 5-4).

GDOP-4 (3*x*) and 'Lola' (2*x*) and their progeny: Controlled crosses produced 21 progeny (2*n* + *n*, 4*x*). Four SSR markers detected seven alleles present only in the maternal parent (M1-1, M1-7, M51-1, M51-4, M51-5, M87-1, and M87-3) and three alleles present only in the paternal parent (M1-4, M76-1, and M87-2). There were five alleles shared by both parents (M1-9, M51-2, M76-2, M76-3, and M87-5). Complete marker data were obtained for 18 of the progeny. All progeny carried all the marker alleles unique to the maternal parent (7). Two of the paternal parent's unique alleles, M1-4 and M87-2, were present in all the progeny, indicating that these alleles were homozygous in 'Lola' and not segregating in the progeny. One of the paternal parent's alleles, M76-1, was segregating, present in 16 progeny and absent in 5 progeny.

In summary, the results from these marker analyses confirmed that all the progeny analyzed were of the 2*n* + *n* type with invariant female alleles present. The gametes that are formed from the female are essentially clones of the maternal plant that is then fertilized by a male gamete.

Putative 2*n* + 0 progeny from controlled pollinations

PKGHOP-1 (2*x*) and PCOP-6 (4*x*) and their 2*n* progeny: Four SSR markers revealed five alleles (M1-3, M1-5, M51-1, M76-3, and M87-4) unique to the maternal parent, four alleles (M1-4, M1-9, M51-3, and M87-1) unique to the paternal parent, and

another five alleles (M51-2, M51-5, M76-1, M76-2, and M87-5) common to both parents. The five putative 2*n* + 0 (2*x*) progeny identified above (refer to ploidy analysis of CP progeny) all carried the five alleles unique to the maternal parent. None of the unique alleles from the five paternal parents were present in the progeny. These results, thus, confirm that all the suspected progeny were indeed 2*n* + 0 progeny resulting from UFGs developing into seeds through apomixis.

GDGHOP-36 and 'Denholm White' and their 2*n* + 0 progeny: As described above, the four SSR markers amplified seven alleles present only in the maternal parent, four alleles present only in the paternal parent, and four alleles present in both parents. All the 11 progeny suspected to be 2*n* + 0 progeny contained all the alleles present in the maternal parent but none of the alleles unique to the paternal parent. Again, the results were as expected for 2*n* + 0 progeny resulting from UFGs and apomixis.

Putative 2*n* + 0 progeny from open-pollinated triploids: As described in the above ploidy analysis section, 11 triploid cultivars were suspected to have produced 2*n* + 0 progeny (3*x*) through UFG formation and apomixis during open pollination. Three progeny were randomly selected out of the suspected individuals and subjected to SSR marker analysis. As the progeny were from OP with their paternal parents unknown, their SSR marker profiles were compared only to that of their respective maternal parents.

Marker M1 amplified a total of nine alleles among the triploid parents, and these parents each carried 2 to 4 alleles. All nine alleles were polymorphic. M51 revealed five alleles among the parents, and each parent contained 2 to 5 alleles. M76 detected 2 to

4 alleles, all the marker alleles were polymorphic. M87 amplified five alleles, four of which were polymorphic and one monomorphic. In total 20 polymorphic alleles were available for screening.

'Landmark Pink Dawn' and its OP progeny: M1, M51, M76, and M87 each produced three alleles in 'Landmark Pink Dawn': M1-1, M1-4, M1-9, M51-1, M51-2, M51-5, M76-1, M76-2, M76-4, M87-1, M87-3, and M87-5. Two of the three OP progeny had a marker profile identical to 'Landmark Pink Dawn's', and one progeny was missing the allele M76-4.

'Lemon Drop' and its OP progeny: M1, M51, M76, and M87 amplified three (M1-4, M1-7, and M1-9), two (M51-3 and M51-5), three (M76-1, M76-3, and M76-4), and two (M87-3 and M87-5) alleles in 'Lemon Drop', respectively. All three OP progeny shared the same marker profile with 'Lemon Drop'.

'Miss Huff' and its OP progeny: 'Miss Huff' carried four alleles of M1 (M1-2, M1-4, M1-5, and M1-9), three alleles of M51 (M51-2, M51-3, and M51-5), two alleles of M76 (M76-1 and M76-4), and four alleles of M87 (M87-1, M87-2, M87-3, and M87-5). All three OP progeny had a marker profile identical to that of 'Miss Huff'.

'New Gold' and its OP progeny: M1, M51, M76, and M87 detected 10 alleles (M1-1, M1-7, M1-9, M51-4, M51-5, M76-2, M76-3, M87-1, M87-3, and M87-5) in 'New Gold'. OP progeny shared the same marker profile as 'New Gold'.

'New Red Lantana' and its OP progeny: All OP progeny had an identical marker profile at all the 10 alleles amplified by the four SSR markers: M1-4, M1-8, M51-2, M51-3, M51-5, M76-2, M76-4, M87-2, M87-4, and M87-5.

‘Patriot Fire Wagon’ and its OP progeny: M1, M51, M76, and M87 revealed 11 alleles in ‘Patriot Fire Wagon’: M1-4, M1-9, M51-2, M51-3, M51-5, M76-1, M76-2, M76-3, M76-4, M87-1, and M87-5. The marker profile of the three OP progeny was identical among them and the same as ‘Patriot Fire Wagon’s.

‘Red Butler’ and its OP progeny: Markers M1, M51, M76, and M87 amplified three (M1-4, M1-5 and M1-9), two (M51-2 and M51-3), three (M76-1, M76-2, and M76-3), and four (M87-2, M87-3, M87-4, and M87-5) alleles in ‘Red Butler’ were identical to the OP progeny.

‘Sunset’ and their OP progeny: M1, M51, M76, and M87 each revealed a total of 12 alleles (M1-4, M1-7, M1-8, M51-2, M51-3, M76-2, M76-3, M76-4, M87-1, M87-2, M87-4, and M87-5). All three OP progeny carried these alleles from ‘Sunset’. No additional alleles were observed in the progeny.

Putative 2*n* + 0 progeny of open-pollinated pentaploid and hexaploid

‘Spreading Sunset’ (5x) carried three alleles at M1, four alleles at M51, four alleles at M76, and three alleles at M87. All three OP progeny of ‘Spreading Sunset’ possessed the same marker profile as the maternal parent, as expected for offspring originating from UFGs and apomixis.

‘Tangerine’ had the same alleles as ‘Spreading Sunset’ at M1 and M87 but one fewer allele than ‘Spreading Sunset’ at M51 and M76, providing a total of 11 alleles for the four SSR markers. Only one OP progeny was available for marker analysis, and it had the same marker profile as its maternal parent.

Putative 2*n* + 0 and *n* + *n* progeny of diploids and tetraploids

The confirmed occurrence of UFGs and apomixis in triploids raises a question: what gametes form the diploid progeny of diploid cultivars and the tetraploid progeny of

tetraploid cultivars? Potentially the diploid progeny of a diploid cultivar could be from fusion of reduced female and male gametes (*n* + *n* progeny) or from UFGs and apoximis (asexual reproduction) (2*n* + 0 progeny). A similar scenario applies to the tetraploid progeny of a tetraploid cultivar. They could be 2*n* + 0 or *n* + *n* progeny.

Cross between GDGHOP-36 (2*x*) and 'Denholm White' (2*x*) and its diploid progeny: M1, M51, M76, and M87 revealed eight alleles that were present in GHGHOP-36 but absent in 'Denholm White', four alleles that were absent in GHGHOP-36 but present in 'Denholm White', and five alleles present in both parents. All 12 diploid progeny had the same marker profile and it was identical to the maternal parent's, GDGHOP-36, thus, they were 2*n* + 0, not *n* + *n*, progeny.

Cross between LAOP-9 (2*x*) and 'Lola' (2*x*) and its diploid progeny: The four SSR markers identified four alleles (M1-1, M5101, M76-4, and M87-1) that were present in LAOP-9 but absent in 'Lola'. These alleles segregated among the six diploid progeny analyzed in a ratio of 2:3, 5:1, 4:2, or 3:3 (present: absent), respectively. There were two additional alleles (M76-2 and M76-3) that were present in both parents. They segregated in the progeny in a ratio of 3:3 or 4:2 (present: absent). Each of the diploid progeny possessed a distinct marker profile. These results suggest that the progeny resulted from sexual reproduction or fertilization of reduced female and male gametes. Thus, these progeny were of *n* + *n* type, in contrast to the diploids from UFG-producing GDGHOP-36 (2*n* + 0).

Cross between 'Dallas Red' (4*x*) and 'Carlos' (4*x*) and its tetraploid progeny: Allele M76-2 was present in 'Dallas Red' but absent in 'Carlos', while M51-3 and M76-1 were absent in 'Dallas Red' but present in 'Carlos'. Two tetraploid progeny were

available for SSR marker analysis. M76-2 was not observed in progeny, but both M51-3 and M76-1 were present, indicating that the progeny had resulted from fertilization of reduced female and male gametes, rather than UFGs and apomixis. Thus, these progeny belong to the *n* + *n* type.

'Pink Caprice' (4*x*) and its tetraploid OP progeny: Four SSR markers amplified 17 alleles in 'Pink Caprice', including five by M1 (M1-3, M1-4, M1-5, M1-6, and M1-9), four by M51 (M51-1, M51-2, M51-3, and M51-5), four by M76 (M76-1, M76-2, M76-3, and M76-4), and four by M87 (M87-1, M87-3, M87-4, and M87-5). The five tetraploid OP progeny randomly selected had a marker profile identical to 'Pink Caprice's. Thus they were 2*n* + 0 progeny resulting from UFGs and apomixis.

'Radiation' (4*x*) and its tetraploid OP progeny: M1, M51, M76, and M87 detected two (M1-4 and M1-9), three (M51-2, M51-3, and M51-4), three (M76-1, M76-2, and M76-3), and two (M87-1 and M87-5) alleles in 'Radiation', respectively. None of the three tetraploid OP progeny carried M87-1. One descendant had one allele (M87-3) not present in 'Radiation' and two progeny had three alleles (M1-1, M51-1, and M87-3) not present in 'Radiation'. The disappearance of one 'Radiation's allele and presence of alleles not existing in 'Radiation' indicate that these tetraploid progeny were of n + n type resulting from fertilization of reduced female and male gametes.

Putative 4*n* + 0, 2*n* + 2*n*, and 4*n* + *n* progeny

Tetraploids were recovered from the progeny of two diploid by diploid crosses, one (917-56) from the cross GDGHOP-36 x 'Denholm White', and another one from the cross between Myst-107 and 'Lola'. Initially they were suspected to have resulted from the fertilization of UFGs and UMGs. As described above, GDGHOP-36 and 'Denholm White' differed at nine alleles: five alleles present in the former diploid and four alleles

present in the latter. The tetraploid descendant contained all five alleles from its maternal parent GDGHOP-36 but none of the four alleles from its paternal parent 'Denholm White' (Table 5-4). Thus, it was a 4*n* + 0 (4*x*) progeny. This is likely the result of the fusion of two 2*n* embryos or an additional mitosis-like chromosome doubling process.The tetraploid from Myst-107 and 'Lola' seemed to originate from a 4*n* female gamete fused with an *n* male gamete. Myst-107 carried five alleles (M1-3, M51-3, M76-1, M87-1, and M87-3) that were absent in 'Lola', while 'Lola' had one allele (M87-2) that was absent in Myst-107. The tetraploid progeny contained all the alleles from both parents. Thus the progeny belongs to a 2*n* + 2*n* type resulting from the fusion of an UFG and an UMG (Table 5-4). To our knowledge this is the first observance of an unreduced pollen grain successfully fertilizing an embryo.

One pentaploid was also identified among the progeny of Myst-107 and 'Lola'. This pentaploid carried the same banding pattern as the tetraploid from the fusion of a UFG and UMG described above, but also was a ploidy level that can only be explained by the formation of a 4*n* gamete from Myst-107 that was fertilized by a *n* gamete from 'Lola'.

Collectively the formation of these three progeny from two controlled crosses indicated two reproductive modes that formed these individuals. The first is the formation of a double UFG (DUFG). The tetraploid progeny was not likely to be the result of an UMG due to the almost complete lack of pollen fertility of GDGHOP-36 (1.3% stainable pollen appendix A). In addition the formation of a pentaploid from Myst-107 could not be explained by self fertilization alone. The pentaploid progeny would have required the formation of a DUFG and pollination (Myst-107 also has largely

nonviable pollen: 1.4% appendix A) which from the banding pattern indicates ‘Lola’ pollen did successfully fertilize this gamete.

The other individual of interest was a tetraploid formed from the formation of a UFG and a UMG. Based on the ploidy level of the plant (4*x*) it was the result of a successful cross pollination or a DUFG. The banding pattern of the plant had alleles unique to ‘Lola’ indicating a successful cross pollination of a UFG and a UMG to form the tetraploid plant. As previously discussed UMG pollen appears to be extremely rare in *L. camara* and is likely an infrequent random occurrence.

Putative *n* + 0 progeny

From 2006 to 2010, 11 haploids were identified in the OP progeny of three tetraploid cultivars (‘Pink Caprice’, ‘Gold’, and ‘Radiation’) and one hexaploid cultivar (‘Tangerine’). Four haploids (2*x*) were from ‘Pink Caprice’ OP progeny, four haploids (2*x*) from ‘Gold’ OP progeny, two (2*x*) from ‘Radiation’ progeny, and one (3*x*) from ‘Tangerine’ progeny. The continued occurrence of these individuals provided enough evidence to suspect haploidization and conduct marker analysis.

M1, M51, M76, and M87 amplified a total of 16 alleles in ‘Pink Caprice’. Five of the alleles (M51-2, M76-1, M76-2, M76-3, and M87-5) were present in all ‘Pink Caprice’s haploids. The remaining 11 alleles (M1-3, M1-4, M1-5, M1-6, M1-9, M51-1, M51-3, M51-5, M87-1, M87-3, and M87-4) segregated among the four haploids, PCH1, PCH2, Myst-107, and PKGHOP-1. Each haploid possessed a distinct marker profile, containing 5 to 6 of the 11 segregating alleles in different combinations and the five non-segregating alleles. Thus, these haploids lost 5 to 6 alleles of their maternal parent ‘Pink Caprice’.

‘Radiation’ contained 11 alleles for the four SSR markers, two alleles for M1, three alleles for M51, three alleles for M76, and three alleles for M87. Seven alleles were not segregating, but the remaining four alleles (M51-3, M76-2, M76-4, and M87-1) segregated in the two ‘Radiation’ haploids (RAD H1 and RAD H2). The haploids each carried two of the segregating alleles plus seven non-segregating alleles, and they differed from each other at two alleles.

M51, M76, and M87 amplified 10 alleles in ‘Gold’, six of which were segregating in the four ‘Gold’s haploids, GDH1, GDGHOP-10, GDGHOP-36, and GDOP-31. These haploids each carried 2 to 3 of ‘Gold’s segregating alleles and all non-segregating alleles.

Three markers, M51, M76, and M87, detected 10 alleles in ‘Tangerine’. Its haploid carried eight alleles but missed two alleles (M51-3 and M87-1) of ‘Tangerine’.

These results confirmed that the haploids were indeed developed from ‘Pink Caprice’, ‘Gold’, ‘Radiation’, and ‘Tangerine’s reduced female gametes. Given that the process of apomixis has been shown to occur with 2*n* gametes (discussed above), it seems likely that at some frequency apomixis may be triggered with *n* gametes in the ovule causing haploidization to occur. This confirms previous speculation from Chapter 4 and demonstrates that at a low frequency haploidization will occur. Potentially these haploids may have had served a purpose in evolution and could be manipulated for breeding.

Twin progeny

‘Carlos’ (4*x*) x ‘Lola’ (2*x*): The six pairs of twins (3*x*) identified in the progeny of this cross were different among and within pairs at one to five alleles. Within the six pairs one differed at M87-1, two twins differed at all M76 alleles while at two different

alleles in primer M 51 (M51-3 and M51-5). Another twin only differed at M51-3 and M51-5. The other differed at M51-5, M76-2 and M76-3. The last twin differed only at the M76 primer (M76-1, M76-2, and M76-3). Within this group of twins differences were more common within the M76 locus as 13 of the 18 differences within the group were found with this primer. In total five of the alleles were missing from the maternal parent indicating different female gametes were fertilized to produce twins.

LAOP-9 (2*x*) x 'Lola' (2*x*): Two pairs of twin seedlings (2*x*) were identified from the progeny of this cross. The first pair of twins differed at three alleles (M1-1, M76-2, and M76-3), and the second pair differed at four alleles (M1-1, M76-2, M76-3, and M87-1), indicating that each pair of twins were developed from different zygotes within a seed. Alleles M1-1, M76-4, and M87-1 were present in the maternal parent LAOP-9 but absent in the paternal parent 'Lola'. M76-4 segregated among pairs of twins. M87-1 segregated within one pair and M1-1 segregated within both pairs of twins. These results suggest that the female gametes involved in the production of each pair of twin seedlings were different as well.

'Landmark White' x 'Denholm White': Two pairs of twin seedlings were analyzed. The seedlings within the first pair were different at three alleles, M51-1, M51-3, and M76-3, and the seedlings within the second pair were different at alleles M51-1, M51-2, and M87-1. M51-3 and M76-3 were present in 'Landmark White' but absent in 'Denholm White'. These two alleles were segregating within the first pair of twins, but present in both seedlings of the second pair. Nevertheless, M51-2, an allele present in both parents, segregated in the second pair of twins. Additionally, M87-1, an allele present in 'Denholm White' segregated in this pair of seedlings.

DROP-25 (4*x*) x LAOP-9 (2*x*): A total of 10 pairs of twin seedlings (2*x*) were analyzed using SSR markers. They could be divided into two groups, based on their marker profile. The first group consisted of eight pairs of seedlings from eight seeds. These individuals differed among and within pairs at 1 to 4 alleles (an average of 2.1). Thus, each of the twins originated from separate zygotes.

The second group consisted of two pairs of twins (3*x*). Their marker profiles were identical. Each progeny contained one (M76-1) of the three alleles that were present in DROP-25 and two (M51-1 and M87-2) of the three alleles that were present in LAOP-9. Thus these twins did carry some alleles from each parent, that is, these twins were from zygotes. Alternatively, the identical marker profile may suggest that each pair of twins might have originated from a single gamete.

GDOP-4 (3*x*) x 'Lola' (2*x*): Two pairs of twins were collected from the progeny of this cross, and they were tetraploids known to result from fertilization of UFGs and reduced male gametes (refer to above). The four progeny were identical in SSR marker profile, carrying all eight alleles (M1-1, M1-7, M51-1, M51-4, M51-5, M76-4, M84-1, and M84-3) that were present in GDOP-4 (and absent in 'Lola) and the three alleles (M1-4, M76-2, and M87-2) that were present in 'Lola' (and absent in GDOP-4). M1-4 and M87-2 seemed to be homozygous in 'Lola', thus they were expected to appear in every progeny of 'Lola'. However, M76-2 seemed to be heterozygous in 'Lola', as it segregated at 16:5 in all the progeny from this cross. The allele present from 'Lola' was of the more common band and may be from two pollen grains carrying the same allele. The identical marker banding pattern within each of the two pairs of twins does raise a

question whether or not the twins originated from single zygotes similar to the previous cross discussed.

GDGHOP-36 (2*x*) x 'Lola' (2*x*): Two pairs of twins were identified in the progeny of this cross, and they were triploids known to originate from UFGs and reduced male gametes (see above). These twins shared the same marker profile: containing all five alleles (M1-7, M51-4, M51-5, M87-1, and M87-3) of GDGHOP-36 and two alleles (M51-2 and M87-2) of 'Lola'. These data confirmed that the twins were from sexual reproduction, but they were not able to discern whether or not each pair of twins originated from the same or separate zygotes.

Discussion

Multiple Modes of Reproduction in *L. camara*

The above ploidy and microsatellite marker analyses have shown that *L. camara* can form three types of female gametes (reduced female gametes or RFGs, UFGs, and DUFGs) and two types of male gametes (reduced male gametes or RMGs and UMGs), and can develop seed through fertilization or apomixis. Taking into consideration these various types of gametes and apomixis, potentially there could be nine possible modes of reproduction. Seven of these (Modes 1 -7) were observed in this current study (Figure 5-3). So far, the observed frequencies of DUFG and UMG seem to be quite low. The observed primary modes of reproduction have been Modes 1, 3, 4, and 5, which would result in $n + n$, $n + 0$, $2n + n$, and $2n + 0$ progeny (Figure 5-3). Differences in reproductive modes have been noted among *L. camara* cultivars/breeding lines. For example, three cultivars ('Carlos', 'Dallas Red', and 'Irene') seem to have reproduced primarily through Mode 1 ($n + n$), while three other cultivars ('Gold', 'Pink Caprice', and 'Radiation') have reproduced through Modes 1, 3, 4, and 5.

Formation of UFGs and DUFGs

UFG formation is one of the two primary factors that cause such diverse types of reproduction in *L. camara*. Two basic processes have been described as the mechanisms for formation of unreduced gamete in plants: first division restitution (FDR) and second division restitution (SDR) (Bretagnolle and Thompson, 1995). In FDR, the first meiotic division occurs abnormally, with homologous chromosomes not pairing during Prophase I and/or not separating to opposite poles during Anaphase I. In SDR, the second meiotic division occurs abnormally, with sister chromatids not separating to opposite poles during Anaphase II. Univalents and multivalents (during Prophase I) and laggards (during Anaphase I) are common in lantana polyploids (Natarajan and Ahuja, 1957; Spies and Stirton, 1982b). Thus *2n* gametes formed by SDR would be aneuploids and contain various numbers of chromosomes. However, the progeny of 'Gold', and 'Pink Caprice' resulting from UFGs were euploids (Chapter 4, and Tables 5-4 and 5-5). Microsatellite marker data showed that these euploids carried all the alleles of their respective seed parents. This would be possible only through FDR, more specifically apomeiosis.

The occurrence of DUFGs was rare, but how they were formed is very intriguing. Determining the mechanism by which DUFGs are formed may also be explained by the mechanism that forms UFGs or potentially a different chromosome doubling mechanism may be involved. Conceivably, several possible processes could lead to the formation of this type of female gametes, including another chromosome doubling event after UFG formation, or merging of two embryo sacs. Regardless of the specific processes, DUFGs could result in rapid increase of ploidy levels in the progeny (Figure 5-3, Modes 6 and 7). This has similarly been seen in *Fragaria* (Bringhurst and Gill 1970).

Apomixis

This type of asexual reproduction was suspected to occur in *L. camara* because of the appearance of apparently matriclinical progeny (Khoshoo and Mahal 1967; Raghavan and Arora 1960). Based on extensive ploidy and molecular marker analysis, this study confirmed that apomixis occurred frequently, especially in some cultivars/breeding lines. Apomixis can be either aposporous or diplosporous. Aposporous apomixis arises from somatic cells within the ovule, commonly from the nucellar or integumentary cells, while diplosporous apomixis initiates from megaspore mother cells, like the normal megasporogenesis. Three types of apomictic progeny have been observed in *L. camara*: those identical to their maternal parents in ploidy level and microsatellite marker banding pattern (2*n* + 0 progeny), those identical to their maternal parents in microsatellite marker banding pattern, but with an increased ploidy level (4*n* + 0 progeny, Figure 5-3, Mode 7), and those with reduced ploidy levels (haploids) and containing a portion of the microsatellite marker alleles of the maternal parents (*n* + 0 progeny) (Figure 5-3, Mode 3). The first two types of progeny could be either from somatic cells or doubled somatic cells undergoing aposporous apomixis or from UFGs or DUFGs going through diplosporous apomixis. However, the third type of apomictic progeny (*n* + 0) could be produced only through the formation of reduced female gametes and diplosporous apomixis.

Twin seedlings

Twin seedlings seem to be common in some cultivars/breeding lines. Previously, Spies and Stirton (1982c) observed the occurrence of two sexual embryos in numerous embryo sacs in *L. camara*. SSR marker analysis in this study showed that progeny in many pairs of twins had different marker banding patterns. This suggests that twins in

these pairs have resulted from separate fertilization events in a single embryo sac rather than from the splitting of single embryos. Evolutionarily this formation of two viable embryos within an embryo sac may seve two purposes. The first is the potential for two progeny to come from a single and the second is that two embryos may increase the successful rates of fertilization.

Implications for Lantana Breeding

The fact that *L. camara* can reproduce through multiple modes can greatly affect the manner in which lantana breeding is to be conducted. In order to achieve high levels of sterility, parental materials must have progeny screened prior to use to know their primary gamete types and modes of reproduction. Such screening can be timely especially when seed germination is considered. In addition, a flow cytometer is needed to analyze the ploidy levels of progeny.

In *L. camara* breeding, open-pollinated (OP) seeds are often used to develop new germplasm. The use of this type of seed may save time, but this practice may introduce UFG production and apomictic traits into new germplasm. These traits would reduce our ablity to control the sexual reproduction of the species. Thus, careful planning to preclude UFG-producers is necessary before OP seed are collected and used for germplasm development.

The available diploid cultivars identified in this study (and previous studies) have creamy white to yellow flowers. Transferring other flower colors (red, pink, etc.) and other desirable ornamental traits into diploids has been an important objective in many lantana breeding programs. For this reason, it was thought that the obtained haploids could be very valuable. However, these haploid lines carried the UFG trait and it was very difficult to transfer desirable traits from UFG-producing haploids to diploid

germplasm. When used as seed parents, these haploids (haploids PKGHOP-1, GDGHOP-36, and MYST-107; Table 5-5) produced mostly 2*n* + 0 or 2*n* + *n* (occasionally 4*n* + 0) progeny, which are of little value for improving diploid germplasm. These haploids potentially could be used as pollen sources, but presently all haploids evaluated had very low pollen viability (Appendix A).

Evolutionary and Ecological Significance

L. camara is a woody to semi-woody perennial plant (Floyd 1998) with the ability to self- and cross-pollinate (Mathur and Ram 1986) and produce seed that can be dispersed by birds (National Weeds Strategy Executive Committee, 2000) or other natural means such as gravity. Based on the analysis of its life history characteristics, *L. camara* is expected to have moderate to high levels of genetic diversity (Hamrick and Godt 1996). High levels of genetic diversity could have contributed to *L. camara*'s success in colonizing and invading habitats. Results from this study have shown that *L. camara* has substantial reproductive versatility which may assist in genome evolution (Soltis and Soltis 1999). This versatility may be another important factor contributing to the success of this species in colonizing and invading new habitats.

Population dynamics of *L. camara* can be very complicated in nature as the individuals of a population may take different reproduction pathways (*n* + *n*, 2*n* + *n*, 2*n* + 0, *n* + 0, etc.). Figure 5-4 presents a summary of likely interactions among individuals of a population and potential impacts on population dynamics. In this model normal *n* + *n* reproduction is shown by the red solid arrows. To simplify the model, it is assumed that only diploids, tetraploids, and hexaploids produce viable reduced male and female gametes. It is expected that in open pollination, these diploids, tetraploids, and hexaploids would produce some 3*x* and 5*x* progeny. Normally, these 3*x* and 5*x*

progeny would be highly sterile and not be able to cross-pollinate with individuals of other ploidy levels. This would raise a question how does a species overcome such genetic isolation? *L. camara* is known to produce UFGs, which have been shown to restore female fertility (Czarnecki and Deng, 2009). This trait leads to the production of 2*n* + *n* progeny in the population, represented by solid blue triangular pointed lines (Figure 5-4). *L. camara* may best be characterized as a successful polyploid metapopulation as described by Rausch and Morgan (2005). Specifically, this trait would enable 3*x* and 5*x* plants to participate in seed production and population formation. Additional fitness to colonizing plants may come from the formation of apomeiotic gametes. This would prevent the formation of lethal allele combinations during establishment (Noyes, 2006). Maintaining previously productive genotypes may provide a conservative, yet successful colonization approach providing an explanation for the success of the species. Subsequent to colonization, how would this species begin to adapt? There may be two answers to this question. The first explanation may lie in genome or allele additions. Similar to Noyes (2005), *L. camara* is able to participate in 2*n* + 0 and 2*n* + *n* seed production (Figure 5-4 modes 2 and 4). The 2*n* + *n* seed could incorporate relative genomes and locally adapted alleles safely. Evidence of this is seen in a cytological investigation of naturalized *L. camara* accessions. This work led to the conclusion that *L. camara* was comprised of at least 3 genomes (Spies and Stirton, 1982a). It could be expected that based on life history and the reproductive systems of *L. camara*, genetic diversity would likely be maintained and further increased with local cross pollination. It is possible that *L. camara* may represent a compilospecies that assimilates local genes so successfully that it eventually invades

and overtakes the native or local species (Harlan and de Wet, 1963). Another similar model is that of species homogenization causing the loss of relatives in the area and possibly endemic species (Lockwood and McKinney, 2001). Both of these lead to the adaptation of one species at the expense of another.

The second adaptive mechanism found in *L. camara* is an extensive plasticity in ploidy level. As previously discussed normal ploidal blocks (odd ploidy levels) may not exist on the female side of this species and is not always correlated to ploidy level (Czarnecki and Deng, 2009; Khoshoo and Mahal, 1967; Raghavan and Arora, 1960; Spies, 1984b). Polyploidization has been thoroughly discussed in previous sections, but little has been discussed about haploidization. A model similar to Harlan and de Wet's 1970 diploid-tetraploid-diploid-tetraploid cycling may be possible although more complex (Figure 5-4 modes 2, 3, 4, and 5). Albeit at different rates of production of $n + 0$, $2n + 0$, and $2n + n$ similar reproductive means were also shown by Bicknell and Koltunow (2004) in *Hieracium*. Spontaneous haploidization was shown to occur when reduced gametes go though apomixis. This process may force foreign alleles to recombine with invading genomes. Although fertility levels may vary among ploidy levels (Ramsey and Schemske 2002) any lack of fertility is removed by the presence of the UFG trait thus allowing this process to continue. A previous study has shown that plants became larger at higher altitudes (Matthew 1971) possibly indicating adaptation from gene recombinations or an increase in ploidy level. A similar phenomenon may be occurring with polyploidization, where increased ploidy level may encourage a rapid means for adaptation. The adaptation may have come from changing ploidy levels as Sanders (2001) suggested diploids are stunted, dwarfed, or generally less vigorous

while polyploids are generally larger. The ability to increase ploidy levels from 2*x* to 5*x* in one generation (Figure 5-4 mode 3) suggests this to be highly plausible adaptive trait. It is our suspusion that both mechanisms are working together. The additional genomes would allow increased heterosis that could lead to improved fitness.

Collectively this work provides an explanation of the reproductive capabilities leading to *L. camara*'s invasion, colonization, and eventual establishment. The formation of UFGs leading to clonal apomixis that allows initial establishement, then UFGs provide a foundation for successful gametes to fuse with other local genotypes and then after colonization normal gametes and reproduction can occur to adapt more quickly to ecotype. For the purposes of varietal development UFG-production appears to be a trait that can be selected against (Czarnecki and Deng 2009) thus removing a large component of the invasive potential of *L. camara*.

Table 5-1. Polymerase chain reaction cycles to detect differences in *L. camara* parents and progeny produced from different modes of reproduction *Lantana* reproduction.

	Hold	Cycle X 10			Cycle X 30			Hold	Hold
1345	95°C 2 min.	95°C 45 sec.	55°C 45 sec.	72°C 1 min.	95°C 45 sec.	45°C 45 sec.	72°C 1 min.	72°C 5 min.	8°C infinity
1350	95°C 2 min.	95°C 45 sec.	68°C 45 sec.	72°C 1 min.	95°C 45 sec.	50°C 45 sec.	72°C 30 sec.	72°C 5 min.	8°C infinity

Table 5-2. Primer sequences for SSR markers used to detect differences between parents and progeny of *L. camara* produced from different modes of reproduction of *Lantana spp*

Primer code	Repeat motif	Primer	Primer Pairs	PCR Cycle
M1	$(AG)_{12}$	Forward[Z]	TGAGAACAGCTCAGTTGACCA	1350
		Reverse	CAACATGAATTAAAGGACTAAACTGC	
M51	$(GA)_9$	Forward	TGGAATGGAAAGCAAGCAG	1345
		Reverse	TCCAGGGAAAAATCATCACC	
M76	$(TC)_{10}$	Forward	CCCGCATTTTAATTCAAGAC	1345
		Reverse	GGAGGGTGTATGTCCATGAG	
M87	$(TC)_{10}$	Forward	TCACCTATTTTGCGTCTCTGTG	1350
		Reverse	GGGGTGGAAAAAGGTTGTCT	

[Z]M13 tail code CCCAGTCACGACGTTG was added to the forward primer for fluorescence labeling.

Table 5-3. Distribution of the ploidy level of progeny from field open pollination experiments of *L. camara* commercial cultivars and breeding lines. Lines are followed with ploidy level in parenthesis. *L. camara* lines and progeny were used from seed collection in Chapter 3.

		Percentage of progeny in different ploidy levels									
L. camara cultivars/lines	OP progeny analyzed (no.)	2*x*	3*x*	4*x*	5*x*	6*x*	7*x*	3-4*x*	4-5*x*	5-6*x*	7-8*x*
'Cream' (2*x*)	2	50.0	50.0	-	-	-	-	-	-	-	-
LAOP-9 (2*x*)	1	100	-	-	-	-	-	-	-	-	-
LAOP-30 (2*x*)	3	100	-	-	-	-	-	-	-	-	-
'Lola' (2*x*)	13	84.6	7.7	7.7	-	-	-	-	-	-	-
'Landmark Peach Sunrise'[Z] (3*x*)	14	-	57.1	21.4	14.3	7.1	-	-	-	-	-
'Landmark Pink Dawn' (3*x*)	137	-	35.8	56.9	6.6	-	-	0.7	-	-	-
'Lemon Drop' (3*x*)	81	-	24.7	65.4	3.7	-	-	6.2	-	-	-
'Lucky Red Hot' (3*x*)	1	-	-	-	-	-	-	100	-	-	-
'Miss Huff' (3*x*)	78	-	23.1	65.4	9	-	-	-	2.6	-	-
'New Gold' (3*x*)	39	-	20.5	76.9	-	-	-	-	-	2.6	-
'New Red Lantana' (3*x*)	40	-	22.5	75	2.5	-	-	-	-	-	-
'Patriot Fire Wagon' (3*x*)	83	1.2	24.1	67.5	3.6	1.2	-	2.4	-	-	-
'Red Butler' (3*x*)	24	-	54.2	37.5	8.3	-	-	-	-	-	-
'Red Spread Lantana' (3*x*)	31	-	38.7	58.1	3.2	-	-	-	-	-	-
'Samson' (3*x*)	154	-	31.2	66.2	1.3	-	-	1.3	-	-	-
'Sunset' (3*x*)	65	-	32.3	55.4	10.8	1.5	-	-	-	-	-
'Carlos' (4*x*)	56	-	5.4	91.1	-	-	-	1.8	-	1.8	-
'Dallas Red' (4*x*)	36	-	11.1	83.3	-	-	-	5.6	-	-	-
'Gold' (4*x*)	59	1.7	-	94.9	1.7	1.7	-	-	-	-	-
'Irene' (4*x*)	35	-	5.7	77.1	-	-	-	17.1	-	-	-
'Pink Caprice' (4*x*)	449	-	0.2	84.2	-	14.3	-	0.2	0.2	0.9	-
'Radiation' (4*x*)	59	3.4	11.9	67.8	3.4	13.6	-	-	-	-	-
'Spreading Sunset' (5*x*)	49	-	-	4.1	51	2	4.1	8.2	20.4	4.1	6.1
'Tangerine' (6*x*)	8	-	50.0	12.5	-	12.5	-	25	-	-	-

[Z]Removed Improved from cultivar name.

Table 5-4. SSR marker analysis and ploidy level of parents and progeny (in quotations) from controlled and open pollinations of *L. camara* cultivars/breeding lines to determine reproductive modes. The five modes of reproduction found were unreduced female gamete production (UFG) double UFG, apomixis, haploidization, and twinning. When possible individuals with the same alleles are grouped and the number of individuals is indicated.

SSR markers				M1									M51					M76				M87				
Allele				1	2	3	4	5	6	7	8	9	1	2	3	4	5	1	2	3	4	1	2	3	4	5
Mode	Group	Ploidy	No.																							
	GDOP-4 (F)[Z]	3*x*	-	1	0	0	0	0	0	1	0	1	1	1	0	1	1	0	0	1	1	1	0	1	0	1
	'Lola' (M)	2*x*	-	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1
	"Progeny type 1"[Y]	4*x*	16	1	0	0	1	0	0	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1
	"Progeny type 2"	4*x*	5	1	0	0	1	0	0	1	0	1	1	1	0	1	1	0	0	1	1	1	1	1	0	1
2*n* + *n*	GDGHOP-36 (F)[Z]	2*x*	-	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	'Denholm White' (M)	2*x*	-	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1	0	1
	"Progeny type 1"[Y]	3*x*	31	1	0	0	1	0	0	1	0	1	0	1	0	1	1	0	1	1	1	1	0	1	0	1
	"Progeny type 2"	3*x*	16	1	0	0	1	0	0	1	0	1	1	0	0	1	1	0	1	1	1	1	0	1	0	1
	PKGHOP-1 (F)[Z]	2*x*	-	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0	1	1
	PCOP-6 (M)	4*x*	-	0	0	0	1	0	0	0	0	1	0	1	1	0	1	0	1	1	0	1	0	0	0	1
	"Progeny type 1"[Y]	2*x*	5	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	1	1	0	0	0	0	1	1
	GDGHOP-36 (F)[Z]	2*x*	-	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	'Denholm White' (M)	2*x*	-	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1	0	1
	"Progeny type 1"[Y]	2*x*	12	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	Myst-107 (F)[Z]	2*x*	1	0	0	1	1	0	0	0	0	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1
	'Lola' (M)	2*x*	-	0	0	0	1	0	0	0	0	1	0	1	0	0	0	1	1	1	0	1	0	1	0	1
	"Progeny type 1"[Y]	2*x*	-	0	0	1	1	0	0	0	0	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1
2*n* + 0	'Sunset' (OP)[Z]	3*x*	-	0	0	0	1	0	0	1	1	0	0	1	1	0	0	0	1	1	1	1	1	0	1	1
	"Progeny type 1"[Y]	3*x*	3	0	0	0	1	0	0	1	1	0	0	1	1	0	0	0	1	1	1	1	1	0	1	1
	'Red Butler' (OP)	3*x*	-	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	1	0	0	1	1	1	1
	"Progeny type 1"[Y]	3*x*	3	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	1	0	0	1	1	1	1
	'Red Spread' (OP)	3*x*	-	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	1	0	0	1	1	1	1
	"Progeny type 1"[Y]	3*x*	3	0	0	0	1	1	0	0	0	1	0	1	1	0	0	1	1	1	0	0	1	1	1	1
	'Pink Caprice' (OP)	4*x*	-	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	0	1	1	1
	"Progeny type 1"[Y]	4*x*	5	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	0	1	1	1
	'Samson' (OP)	3*x*	-	.	.	.	.	.	.	.	.	.	0	0	1	0	1	1	1	1	0	1	0	1	1	1
	"Progeny type 1"[Y]	3*x*	3	0	0	1	1	0	1	0	0	1	0	0	1	0	1	1	1	1	0	1	0	1	1	1

Table 5-4 Continued.

SSR markers				M1									M51					M76				M87				
Allele				1	2	3	4	5	6	7	8	9	1	2	3	4	5	1	2	3	4	1	2	3	4	5
Mode	Group	Ploidy	No.																							
	'New Gold' (OP)	3*x*	-	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	"Progeny type 1"[Y]	3*x*	3	1	0	0	0	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	'Landmark Peach Sunrise'[X] (OP)	3*x*	-	1	0	0	1	0	0	0	0	1	1	1	1	1	0	1	0	0	1	1	0	1	0	1
	"Progeny type 1"[Y]	3*x*	3	1	0	0	1	0	0	0	0	1	1	1	1	0	0	0	0	0	1	1	0	1	0	1
	'Miss Huff' (OP)	3*x*	-	0	1	0	1	1	0	0	0	1	0	1	1	0	1	1	0	0	1	1	1	1	0	1
	"Progeny type 1"[Y]	3*x*	3	0	1	0	1	1	0	0	0	1	0	1	1	0	1	1	0	0	1	1	1	1	0	1
	'Landmark Pink Dawn' (OP)	3*x*	-	1	0	0	1	0	0	0	0	1	.	.	.	.	.	1	1	0	1	1	0	1	1	1
	"Progeny type 1"[Y]	3*x*	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	1	0	1
	"Progeny type 2"[Y]	3*x*	2	0	0	0	1	0	0	0	0	1	1	1	0	0	1	1	1	0	1	1	0	1	0	1
2*n* + 0 (cont)	'New Red Lantana' (OP)	3*x*	-	0	0	0	1	0	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	1	1
	"Progeny type 1"[Y]	3*x*	3	0	0	0	1	0	0	0	1	0	0	1	1	0	1	0	1	0	1	0	1	0	1	1
	'Spreading Sunset' (OP)	5*x*	-	0	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1
	"Progeny type 1"[Y]	5*x*	3	0	0	0	1	0	0	1	0	1	0	1	1	1	1	1	1	1	0	1	0	1	0	1
	'Lemon Drop' (OP)	3*x*	-	0	0	0	1	0	0	1	0	1	0	0	1	0	1	1	0	1	1	0	0	1	0	1
	"Progeny type 1"[Y]	3*x*	3	0	0	0	1	0	0	1	0	1	0	0	1	0	1	1	0	1	1	0	0	1	0	1
	'Patriot Fire Wagon' (OP)	3*x*	-	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	0	1	1	1	0	0	0	1
	"Progeny type 1"[Y]	3*x*	3	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	0	1	1	1	0	0	0	1
	'Tangerine' (OP)	6*x*	-	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0	1	0	1	0	1
	"Progeny type 1"[Y]	6*x*	1	0	0	0	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0	1	0	1	0	1
	'Pink Caprice' (OP)	4*x*	-	0	0	1	1	1	1	0	0	1	1	1	1	0	1	1	1	1	0	1	0	1	1	1
	"Progeny type 1"[Y] (PCH1)	2*x*	1	0	0	0	0	1	1	0	0	1	0	1	0	0	1	1	1	1	0	0	0	1	1	1
	"Progeny type 2" (PCH2)	2*x*	1	0	0	1	1	0	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1
	"Progeny type 3" (Myst-107)	2*x*	1	0	0	1	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1	0	1	0	1
n + 0	"Progeny type 4" (PKGHOP-1)	2*x*	1	0	0	1	0	1	0	0	0	0	1	1	0	0	1	1	1	1	0	0	0	0	1	1
	'Gold' (OP)	4*x*	1	.	.	.	.	.	.	.	.	.	1	1	0	1	1	0	1	1	1	1	0	1	0	1
	"Progeny type 1"[Y] (GDH1)	2*x*	1	0	0	0	1	0	0	1	0	1	1	0	0	0	1	.	.	.	.	1	0	1	0	1
	"Progeny type 2" (GDGHOP-36)	2*x*	1	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	"Progeny type 3" (GDGHOP-10)	2*x*	1	1	0	0	0	0	0	1	0	1	1	0	0	0	1	0	1	0	0	1	0	1	0	1
	"Progeny type 4" (GDOP-31)	2*x*	1	1	0	0	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	0	0	1	0	1

Table 5-4 Continued.

SSR markers				M1									M51					M76				M87				
Allele				1	2	3	4	5	6	7	8	9	1	2	3	4	5	1	2	3	4	1	2	3	4	5
Mode	Group	Ploidy	No.																							
	'Tangerine' (OP)	6*x*	-	0	0	0	1	0	0	1	0	1	0	1	1	1	1	0	1	1	1	1	0	1	0	1
	"Progeny type 1"[Y] (TANG H1)	3*x*	1	.	.	.	.	.	.	.	.	.	0	1	0	1	1	0	1	1	1	0	0	1	0	1
n + 0 (cont)	'Radiation' (OP)	4*x*	-	0	0	0	1	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	1	0	0	1
	"Progeny type 1"[Y] (RAD H1)	2*x*	1	0	0	0	1	0	0	0	0	1	0	1	1	0	1	0	1	1	0	0	1	0	0	1
	"Progeny type 1"[Y] (RAD H2)	2*x*	1	0	0	0	1	0	0	0	0	1	0	1	0	0	1	0	0	1	1	1	1	0	0	1
	GDGHOP-36 (F)	2*x*	-	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	'Denholm White' (M)	2*x*	-	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	1	1	0	1	0	1
4*n* + 0 and 4*n* + *n*	"Progeny type 1"[Y] (917-56)	4*x*	1	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	Myst-107 (F)	2*x*	-	0	0	1	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1	0	1	0	1
	'Lola' (M)	2*x*	-	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1
	"Progeny type 1"[Y] (815-7)	5*x*	1	0	0	1	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	.	.	.	.	.
	Myst-107 (F)	2*x*	-	0	0	1	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1	0	1	0	1
2*n* + 2*n*	'Lola' (M)	2*x*	-	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1
	"Progeny type 1"[Y] (813-9)	4*x*	1	0	0	1	1	0	0	0	0	1	0	1	1	0	0	1	1	1	0	1	1	1	0	1
	LAOP-9 (F)	2*x*		1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	1	1	1	1	1	0	0	1
	'Lola' (M)	2*x*		0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1
	"9-1"[W]	2*x*	1	**0**	0	0	1	0	0	0	0	1	1	1	0	0	0	0	**1**	**0**	1	1	1	0	0	1
	"9-2"	2*x*	1	**1**	0	0	1	0	0	0	0	1	1	1	0	0	0	0	**0**	**1**	1	1	1	0	0	1
	"13-1"	2*x*	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	0	**0**	1	0	0	1	0	0	1
	"13-2"	2*x*	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	**1**	1	0	0	1	0	0	1
	"15-1"	2*x*	1	**0**	0	0	1	0	0	0	0	1	1	1	0	0	0	0	**0**	1	1	**1**	1	0	0	1
2*n* + *n* and *n* +*n*	"15-2"	2*x*	1	**1**	0	0	1	0	0	0	0	1	1	1	0	0	0	0	**1**	0	1	**0**	1	0	0	1
	GDOP-4 (F)	3*x*	-	1	0	0	0	0	0	1	0	1	1	1	0	1	1	0	0	1	1	1	0	1	0	1
	'Lola' (M)	2*x*	-	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1
	Twins (2 pairs)[W]	4*x*	4	1	0	0	1	0	0	1	0	1	1	1	0	1	1	0	1	1	1	1	1	1	0	1
	'Landmark White' (F)	2*x*		1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	1	1	0	1
	'Denholm White' (M)	2*x*		1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1	0	1	0	1
	"911-74-1"[W]	2*x*	1	.	.	.	.	.	.	.	.	.	**1**	1	**0**	0	0	0	0	**0**	1	1	1	0	0	1
	"911-74-2"	2*x*	1	1	0	0	1	0	0	0	0	1	**0**	1	**1**	0	0	0	0	**1**	1	1	1	0	0	1

Table 5-4 Continued.

SSR markers				M1									M51					M76				M87				
Allele				1	2	3	4	5	6	7	8	9	1	2	3	4	5	1	2	3	4	1	2	3	4	5
Mode	Group	Ploidy	No.																							
	“911-75-1”	2*x*	1	1	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	1	1	1	0	1	0	1
	“911-75-2”	2*x*	1	1	0	0	1	0	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	1	0	1
	DROP-25 (F)	4*x*	-	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	0	0	1	1	0	0	0	1
	LAOP-9 (M)	2*x*	-	1	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	1
	Twins (2 pairs)[W]	3*x*	4	0	0	0	1	0	0	0	0	1	1	1	0	0	0	1	0	0	1	1	1	0	0	1
	“31-1”[W]	3*x*	1	0	0	0	1	0	0	0	0	1	1	1	0	0	**1**	1	0	**1**	**0**	1	1	0	0	1
	“31-2”	3*x*	1	1	0	0	1	0	0	0	0	1	1	1	0	0	**0**	1	0	**0**	**1**	1	1	0	0	1
	“32-1”	3*x*	1	0	0	0	1	0	0	0	0	1	1	1	0	0	1	1	0	0	1	1	**1**	0	0	1
	“32-2”	3*x*	1	0	0	0	1	0	0	0	0	1	.	.	.	.	.	1	0	0	1	1	**0**	0	0	1
	“33-1”	3x	1	**1**	0	0	1	0	0	0	0	1	1	1	0	0	1	1	0	**1**	1	1	1	0	0	1
	“33-2”	3x	1	**0**	0	0	1	0	0	0	0	1	1	1	0	0	1	1	0	**0**	1	1	1	0	0	1
	“34-1”	3x	1	**1**	0	0	1	0	0	0	0	**1**	1	1	0	0	0	1	0	0	1	1	0	0	0	1
	“34-2”	3x	1	**0**	0	0	1	0	0	0	0	**0**	1	1	0	0	0	1	0	0	1	1	0	0	0	1
	“35-1”	3x	1	0	0	0	1	0	0	0	0	0	1	1	0	0	**1**	1	0	0	1	1	0	0	0	1
2*n* + *n* and *n* +*n* (cont)	“35-2”	3x	1	0	0	0	1	0	0	0	0	0	1	1	0	0	**0**	1	0	0	1	1	0	0	0	1
	“36-1”	3x	1	1	0	0	**1**	0	0	0	0	0	1	1	0	0	0	**1**	0	**0**	1	1	**0**	0	0	1
	“36-2”	3x	1	1	0	0	**0**	0	0	0	0	0	1	1	0	0	0	**0**	0	**1**	1	1	**1**	0	0	1
	“37-1”	3x	1	1	0	0	0	0	0	0	0	0	1	1	0	0	**0**	1	0	0	1	1	**1**	0	0	1
	“37-2”	3x	1	1	0	0	0	0	0	0	0	0	1	1	0	0	**1**	1	0	0	1	1	**0**	0	0	1
	“38-1”	3x	1	**1**	0	0	1	0	0	0	0	0	1	1	0	0	**1**	1	0	0	1	1	0	0	0	1
	“38-2”	3x	1	**0**	0	0	1	0	0	0	0	0	1	1	0	0	**0**	1	0	0	1	1	0	0	0	1
	‘Carlos’ (F)	4x	-	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	0	0	1	1	0	0	0	1
	‘Lola’ (M)	2x	-	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1
	“19-1”[W]	3x	1	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	1	0	0	**1**	1	0	0	1
	“19-2”	3x	1	0	0	0	1	0	0	0	0	1	0	1	1	0	1	1	1	0	0	**0**	1	0	0	1
	“20-1”	3x	1	0	0	0	1	0	0	0	0	1	0	1	**0**	0	0	**1**	**0**	**1**	**0**	0	0	1	0	1
	“20-2”	3x	1	0	0	0	1	0	0	0	0	1	0	1	**1**	0	0	**0**	**1**	**0**	**1**	0	0	1	0	1
	“21-1”	3x	1	0	0	0	1	0	0	0	0	1	0	1	0	0	**1**	**0**	**0**	**1**	**1**	0	0	1	0	1
	“21-2”	3x	1	0	0	0	1	0	0	0	0	1	0	1	0	0	**0**	**1**	**1**	**0**	**0**	0	0	1	0	1

Table 5-4 Continued.

SSR markers				M1									M51					M76				M87				
Allele				1	2	3	4	5	6	7	8	9	1	2	3	4	5	1	2	3	4	1	2	3	4	5
Mode	Group	Ploidy	No.																							
$2n + n$ and $n + n$ (cont)	"22-1"	3x	1	0	0	0	1	0	0	0	0	1	0	1	**0**	0	**1**	1	0	0	1	.	.	.	.	.
	"22-2"	3x	1	0	0	0	1	0	0	0	0	1	0	1	**1**	0	**0**	1	0	0	1	0	0	1	0	1
	"23-1"	3x	1	0	0	0	1	0	0	0	0	1	0	1	0	0	**0**	1	**0**	**1**	0	1	0	1	0	1
	"23-2"	3x	1	0	0	0	1	0	0	0	0	1	0	1	0	0	**1**	1	**1**	**0**	0	1	0	1	0	1
	"26-1"	3x	1	0	0	0	1	0	0	0	0	1	.	.	.	.	.	**1**	**0**	**1**	1	1	0	1	0	1
	"26-2"	3x	1	0	0	0	1	0	0	0	0	1	0	1	0	0	0	**0**	**1**	**0**	1	1	0	1	0	1
	GDGHOP-36 (F)	2x	-	0	0	0	1	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1	0	1	0	1
	'Lola' (M)	2x	-	0	0	0	1	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	1	0	0	1
	"Twins (2 pairs)"[W]	3x	4	0	0	0	1	0	0	1	0	1	0	1	0	1	1	0	1	1	0	1	1	1	0	1

[Z]Begins new group of female and male controlled or open pollinations.
[Y]Begins the progeny type for the female and male combination directly above.
[X]Removed Improved in cultivar name.
[W]Begins twin progeny. Twin numbers indicate the order in which they were discovered among crosses then as either the first of second twin within the pair. Bolded text indicates differences between twins.

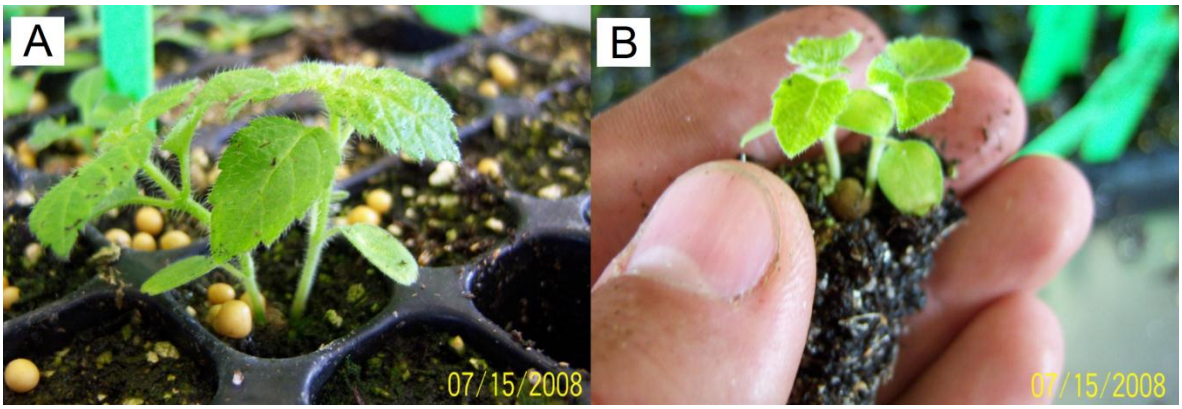

Figure 5-1. Identification of twins from germination. A) Twin germination from a single tray cell. B) Two plants germinating from a single seed.

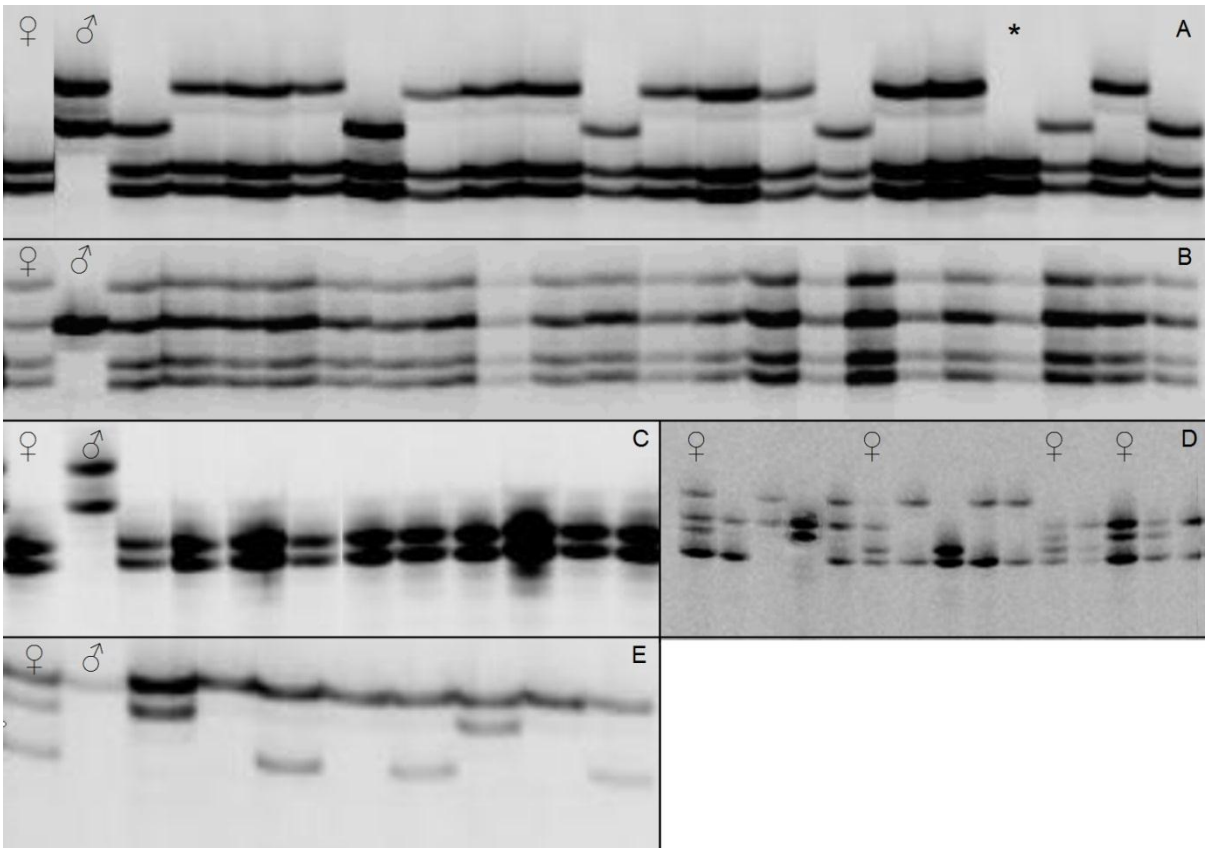

Figure 5-2. Microsatellite analysis of *Lantana camara* lines to confirm modes of reproduction. Female and male lanes are represented with ♀ and ♂ symbols respectively. A) Unreduced female gametes. The lane with an * indicates a double unreduced female gamete from GDGHOP-36 and 'Denholm White'. B) Apomeiosis from GDOP-4 x 'Lola'. C) Apomixis from GDGHOP-36 x 'Denholm White'. D) Haploidization from cultivars as females in order 'Pink Caprice', 'Gold', 'Radiation', and 'Tangerine' cultivars followed by haploidized progeny. E) Twin individuals from 'Carlos' x 'Lola'.

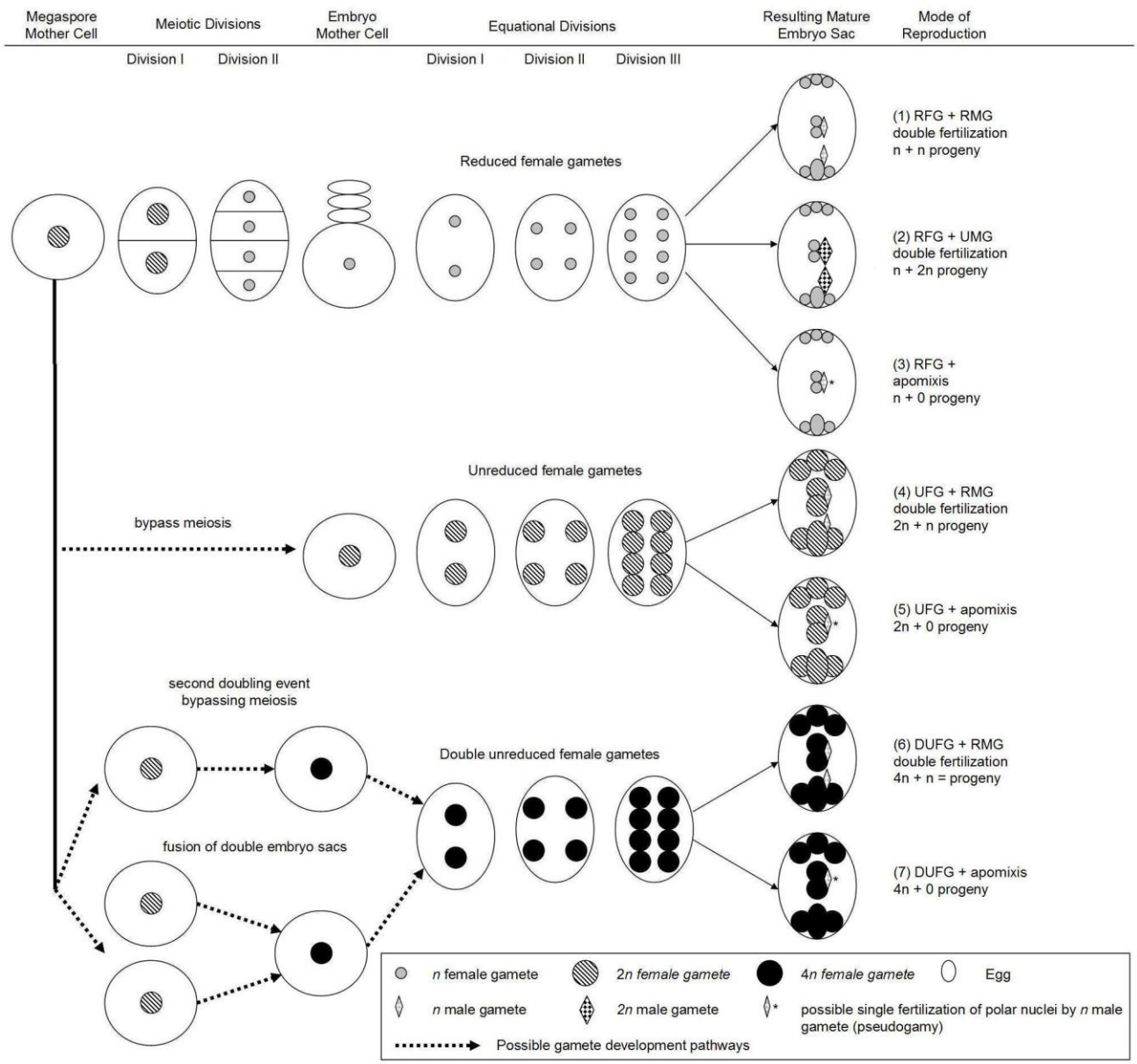

Figure 5-3. Multiple modes of reproduction demonstrated by *L. camara*.

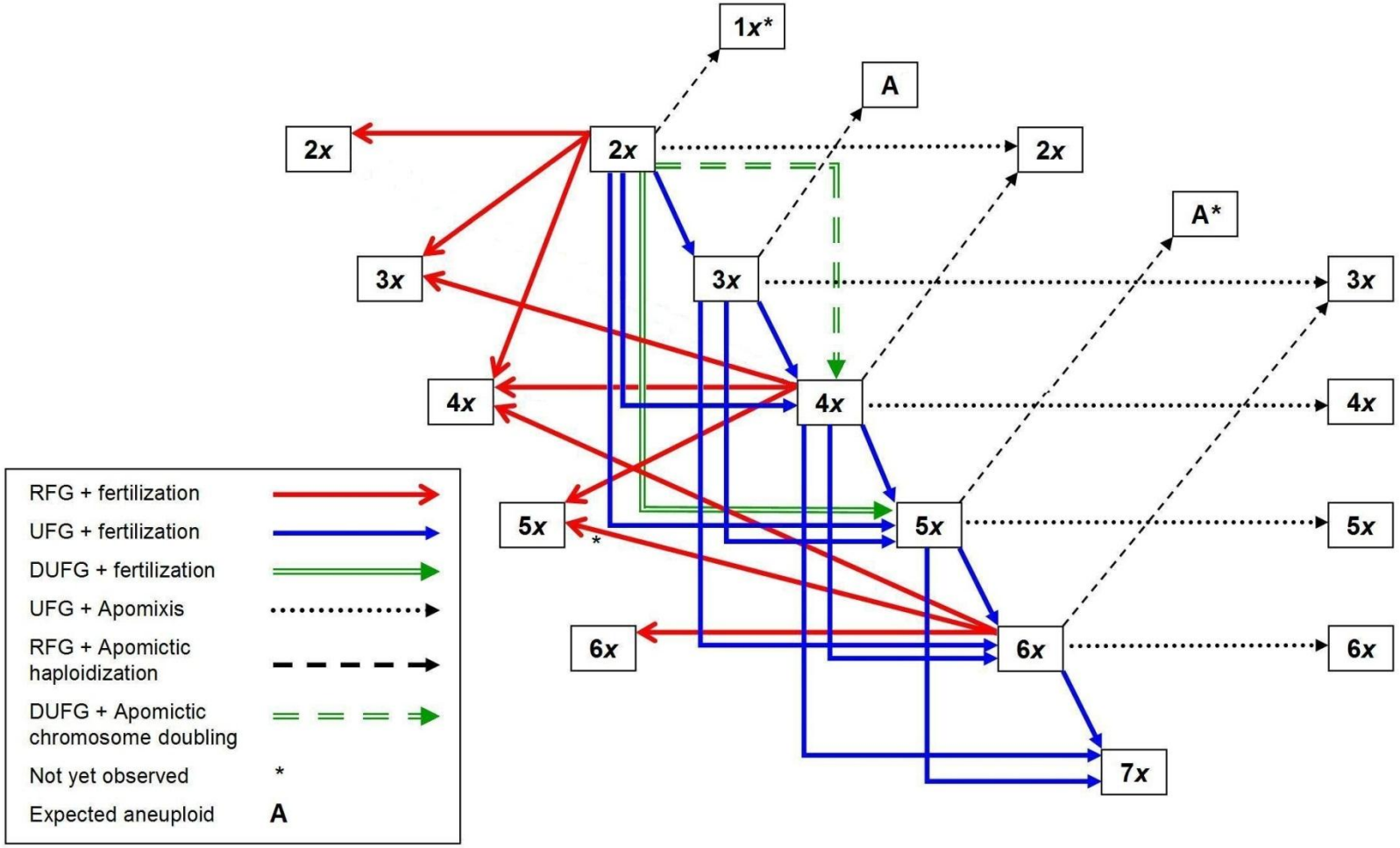

Figure 5-4. Population interactions of *L. camara* from controlled and open pollination observations. Red lines indicate normally reduced gamete fusion events. Blue and green lines indicate polyploidization events with and without gamete fusion. Black dotted and dashed lines represent paths of clonal and haploidized seed formation. To reduce complexity only 2*x*, 4*x*, and 6*x* ploidy levels were assumed to contribute normally reduced female gametes (RFG) and reduced male gametes. All ploidy levels are assumed to contribute unreduced female gametes (UFG). Only observed double UFG (DUFG) events were included as this is expected to be uncommon. All polyploidization events greater than 7*x* were not included but may be possible.

CHAPTER 6
HYBRIDIZATION POTENTIAL BETWEEN CULTIVATED *LANTANA CAMARA* AND *LANTANA DEPRESSA*

Rationale

Lantana depressa is a small native shrub endemic to Florida, growing in pine rockland of Miami-Dade County in southern Florida. Plants of this species are small, usually <0.5 m tall, with branches radiating, prostrate, reddish or purplish, more or less hispid. Leaves of *L. depressa* are induplicate or longitudinally incurved ovate to elliptic, 1-3.5 cm long; the angle between the midrib and the basal margin of lamina is 50° or less. The flowers of *L. depressa* open yellow and turn tawny orange with age, and they produce fleshy, shiny black fruits (drupes) (Sanders, 2001). All *L. depressa* accessions surveyed by Sanders (1987) were diploids ($2n = 2x = 22$) with normal bivalent pairing. In a subsequent publication, Sanders (2006) mentioned autotetraploidy in *L. depressa*.

Sanders (1987) recognized the above described *L. depressa* as *L. depressa* var. *depressa* and two additional varieties that occupy ecologically and geographically distinct areas. *L. depressa* var. *floridana* occurs on stabilized dunes of the Atlantic coast barrier islands and relictual dunes of central Florida; and var. *sanibelensis* occupies calcareous dunes along the Gulf of Mexico and wet limestone prairies in southwestern Florida (Sanders, 1987). These two varieties are nearly identical in vestiture, laminar shape, laminar curvature, laminar lustrousness, inflorescence bract shape and persistence, and fruit color to *L. depressa* var. *depressa*, but they are large, bushy shrubs. Recently taxonomic authorities working on Florida taxa revised their classification and asserted that *L. depressa* var. *floridana* and var. *sanibelensis* were synonymous for *L. camara*, while *L. depressa* var *depressa* remained a separate taxon (Wunderland and Hansen, 2004).

Lantana camara originated from South America through Mexico, and the West Indies (Sanders, 2001; Sanders, 2006). It was introduced as early as 1687 to Europe as a landscape plant. Substantial selection and hybridization occurred in Europe, resulting in various combinations of plant growth habits, flower colors, etc. By the late nineteenth century, there were over 600 named cultivars (Howard, 1969). European colonists further introduced *L. camara* cultivars throughout both the Old and New World Tropics.

L. camara has naturalized in Florida, (Sanders, 1987a) and is documented in nearly every Florida county and many habitats. Naturalized plants are vigorous, large, and weedy. They can grow in thickets reaching 4 m in height and produce dense prickles on branches. Their leaves are cordate to ovate elliptic, flat or slightly undulate, not induplicate or longitudinally incurved. Flowers are multi-colored red, pink, orange, and yellow.

Cytological studies revealed that Florida-naturalized *L. camara* comprised only tetraploids and occurred almost exclusively in disturbed habits (Sanders, 1987). However, *L. camara* probably originated as a diploid, because some diploid cultivars are still available (Sanders, 2006). Post-origin allopolyploidy has contributed to *L. camara*'s aggressive growth and success in the wild (Sanders, 2006). Numerous natural hybrids between *L. depressa* and naturalized *L. camara* have been observed sympatrically or in close proximity to one or both species (Sanders, 1987). Hybrids were triploid and morphologically intermediate between *L. depressa* and *L. camara*. These observations indicate that *L. depressa* and naturalized *L. camara* are hybridizing, which puts *L. depressa* in great danger. Over the last 40-50 years, the native habits for *L. depressa*

have been altered and/or destroyed substantially due to oceanside development, urban expansion, and agriculture. As the natural habitats are disturbed, naturalized *L. camara* invades and hybridizes with the native species and produce triploids. These triploids seem to have combined the vigor of *L. camara* and the adaptations to local habits of *L. depressa*, and they can persist and spread (Sanders, 1987a). Sanders (1987a) confirmed by hand pollination in the greenhouse that naturalized *L. camara* tetraploids and *L. depressa* were fully crossable and interspecific pollinations resulted in 31% to 40% fruit set.

As a popular nursery and landscape plant, *L. camara* receives a considerable amount of attention from corporate or independent breeders. Over the last several decades, numerous new cultivars have been developed in *L. camara*. Many of these cultivars have much reduced pollen viability and/or female fertility. As shown in Chapter 2, pollen stainability of current *L. camara* cultivars ranged from 1.8% (‘New Gold’) to 81.1% (‘Lola’). A great range of variation in fruit production per peduncle [a 1,200 fold difference, from 0.003 (‘Denholm White’) to 7.173 (‘Pink Caprice’)] has been observed among these cultivars (Chapter 3). As shown in Chapters 4 and 5, some cultivars can adopt multiple modes of reproduction, either sexual or asexual, to produce seeds. These factors can have significant effects on the hybridization potentials of *L. camara* cultivars with *L. depressa.* Little information on such aspects is available. Thus, the objectives of this study are to 1) Assess the hybridization potential of *L. camara* as a male parent with *L. depressa*, 2) Assess the hybridization potential of *L. camara* as a female parent with *L. depressa*, and 3) analyze the ploidy level of the resultant interspecific hybrids.

Materials and Methods

Plant Materials

Ten *L. camara* cultivars and 15 breeding lines were used, including two diploids, eight triploids, 10 tetraploids, two pentaploids, and three hexaploids. They represented a range of ploidy levels and pollen stainability (from 1.5 to 79.1%) (Table 6-1). The *L. depressa* accession used was provided by Pro Native Consulting (Miami, FL). Flow cytometry analysis found *the L. depressa* material provided to be tetraploid. All plants were grown in a commercial soilless potting mix (Fafard® 2B, Anderson, SC) supplemented with a controlled-release fertilizer (Osmocote®,15N-3.9P-10K, 8-9 months release at 21°C; The Scotts Company, Marysville, OH) at the rate of 6.51 kg m-3.

Hand Pollination

Plants used for pollination were grown in the greenhouse and evenly spaced on metal benches with a spacing of 30.5 cm. Pollinations were conducted over two seasons. The first was from 29 September to 11 November 2009 and the second from 6 April 2010 to 12 May 2010. Flowers were emasculated prior to anther dehiscence and pollinated immediately after emasculation with fresh pollen. Fruit set was recorded as ripe fruit was harvested ~3–4 weeks after hand pollination.

Ten commercial cultivars and 15 lines of *L. camara* that represented five ploidy levels (2*x* to 6*x*) and a wide range of pollen stainability (1.5% to 79.1%) were selected as the pollen source for pollinating *L. depressa* and assessing *L. camara*'s ability to cause fruit (seed) set on the native species. Hand pollinations were conducted in early to late fall 2009 (the first season) and repeated in mid to late spring 2010 (the second

season). More than 100 flowers were pollinated per cross in a season with one exception when flowers were not available.

Pollen Staining

Pollen staining was done with cotton blue vital stain similar to chapter 2 on one plant of each line of *L. camara* upon completion of the first season of pollinations. *L. depressa* stainability was assessed using three randomly selected plants from the crosses. The pollen stainability from Chapter 2 was averaged with those data collected during this study.

Seed Germination

Ripe fruit was collected and stored under ambient (22.2°C) conditions until seed extraction. All pulp was removed from the seed and soaked in water overnight (Heit 1946), cleaned and sown on 8 February and 16 July 2010 for the first and second seasons of pollinations respectively. Seed were sown in a greenhouse on the surface of Fafard® 2B potting soil under intermittent mist. After germination seedling phenotypes were examined to determine if their phenotypes were intermediate between the parents.

Progeny Ploidy Analysis

Analysis was performed using fully expanded young leaves and the Partec PA I ploidy analyzer and the CyStain UV Ploidy Precise P dye (Partec, Germany). The manufacturer recommended ploidy analysis procedure was followed with minor modifications (supplemented with 2% w/v PVP and 0.01% β-mercaptoethanol) with dye mixture kept on ice. The ploidy level of progeny was determined by comparing to one or more commercial cultivars (reference cultivars) with known ploidy levels that were included in the same analysis.

Results and Discussion

L. camara as the Pollen Source

The highest fruit set was 28.3% ('Cream') in the first season and 25.2% ('Lola') in the second season (Table 6-1). Seasonal effects seemed to be evident in a number of crosses, especially those with 'Lola', 'Pink Caprice', and 'Cajun Pink' as the pollen source. For example in 'Lola' x *L. depressa*, the fruit set was 28.3% in the first season, but it dropped to 14.5% in the second season. When 'Canjun Pink' was the pollen source, the fruit set was 9.0% and 2.4% in the first and the second season, respectively. With 'Pink Caprice' as the pollen source, fruit set was 1.6% in the first season and increased to 16.1% in the second season. Seasonal changes might be due to the difference in temperature between the two seasons, or plant response to temperature fluctuations, etc.

Correlation analysis indicates that pollen stainability of *L. camara* is the most important factor determining the percentage of fruit set on *L. depressa*. An R^2 value of 0.9405 was obtained by fitting the observed pollen stainability to the observed fruit set with a polynomial equation: $y = 0.3832 – 0.096x + 0.0089x^2 – 0.0002x^3 + 0.000002x^4$, (Figure 6-1), where y is fruit set (%) and x the pollen stainability (%). This relationship suggests that as the pollen stainability decreases, fruit set can drop more rapidly. Figure 6-1 suggests that when pollen stainability is below 10%, pollinated flowers would generally not set fruit.

L. depressa seeds resulting from *L. camara* pollination had low germination (0 to 33.3 %), similar to the germination of *L. depressa*'s open-pollinated seeds (S. Wilson, personal communication) (Table 6-2).

As described in Chapter 2, the main factor determining *L. camara*'s pollen stainability is its ploidy level. Thus, the following discussion of the various cultivars/breeding lines' ability to cause fruit set on *L. depressa* will be by ploidy level.

Diploid *L. camara* cultivars

This group consisted of two cultivars, 'Cream' and 'Lola'. When pollinated with their pollen, 14.5% to 28.3% of *L. depressa* flowers set fruit, indicating that 'Cream' and 'Lola' were the most compatible with *L. depressa*. Nevertheless, *L. depressa* seeds from crosses with the two cultivars had a low germination rate (5.5%) (Table 6-2).

Triploid *L. camara* cultivars/breeding lines

Three commercial triploid cultivars and five triploid lines were used as pollen sources to pollinate nearly 1,300 *L. depressa* flowers. Four triploids with pollen stainability below 10% ('Miss Huff', 'New Gold', GDOP-4, and 605-35) did not cause any fruit set in either season. Of the four triploids with 11.5 to 14.9% pollen stainability, one (623-5) did not produce fruit and three ('Athens Rose', 605-43, and 617-1) did cause fruit set on *L. depressa*, ranging from 0.3% to 3.2%. However, none of the seed extracted from the fruit germinated. These results show that triploid *L. camara* with low pollen stainability (<10%, even <15%) had little potential to cross-pollinate *L. depressa*.

Tetraploid *L. camara* cultivars/breeding lines

This group consisted of four commercial cultivars and six breeding lines. When pollinated with these cultivars/lines, *L. depressa* had an average fruit set of 3.1%, only 14% of the pollination success rate of diploid *L. camara*, but about 10 fold of that of the triploid *L. camara*.

As pollen stainability varied among tetraploids, so did the pollination success rate. In one pollination season, 'Pink Caprice' caused 16.1% of the flowers to set fruit on *L.*

depressa, close to the pollination success rate of diploid *L. camara*. In most crosses, however, the observed fruit set was between 0.6% and 6.0% (Table 6-1). Two tetraploid lines, CAOP-73 and 604-5, did not cause any fruit set on *L. depressa*. This result seems to be understandable for 604-5, as its pollen stainability was low (12.1%), but it was unexpected for CAOP-73, which had 40.7% pollen stainability. There might be other mechanism(s) in CAOP-73 that made CAOP-73 and *L. depressa* less compatible. On the other hand, CAOP-88, even with only 3.2% pollen stainability, was able to cause some fruit set in one season.

L. camara pentaploids and hexaploids

Two pentaploids ('Cajun Pink' and 629-1) and three hexaploids (PIT-2, PIT-20, and 620-1) were available as the pollen source for pollinating *L. depressa*. 'Cajun Pink' and two *L. camara* lines affected 0.5% to 5.7% fruit set, and two lines did not, even though they had similar pollen stainability (24.6% to 39.1%) (Table 6-1). On average, pentaploids and hexaploids appeared to be less crossable with *L. depressa* than tetraploids. None of the seeds (15) extracted from fruit of *L. depressa* × pentaploid *L. camara* germinated, while the only seed from the *L. depressa* × hexaploid *L. camara* cross (620-1) germinated.

L. camara as the Female

The same 10 commercial cultivars and 15 lines were used as female parents and pollinated with *L. depressa*, which had an average pollen stainability of 63% (from two greenhouse tests) and an average of 67.3% from the plants used for pollinations. A total of 104 to 270 flowers were pollinated per cross in Season one (September–November 2009), and 140 to 304 flowers were pollinated per cross in Season two (April–May 2010). *L. camara's* fruit set varied between 0 and 45.9% in Season one and

between 0 and 30.0% (Table 6-3) in Season two among cultivars/lines. Seasonal effects seem to be evident in crosses involving ‘Cream’, ‘Lola’, ‘Carlos’, and ‘Pink Caprice’ (plus or minus 6.2% to 7.0%), and particularly large in crosses involving ‘Miss Huff’ and 611-7 (plus or minus 28.0% to 28.8%). When pollinated with *L. depressa*, ‘Athens Rose’ and 604-5 did not set any fruit in both seasons, suggesting that they are highly female-sterile.

It has been shown that ploidy level (Chapter 3) and reproductive mode (chapter 5) strongly affect *L. camara*’s fruit set in open pollination (OP) and OP seed germination. Table 6-4 indicates that these two factors played a similarly important role in *L. camara’*s fruit (or seed) production in hand pollination with *L. depressa* as well as seed germination.

Diploid *L. camara*

The average fruit set of ‘Cream’ and ‘Lola’ over two seasons was 11.4% and 21.7%, respectively, being the fifth and second highest among the average fruit set data for all 25 *L. camara* cultivars/lines evaluated. As a ploidy group, the average fruit set was 16.5%, representing the highest among the five ploidy levels. Seeds resulting from ‘Cream’ or ‘Lola’ × *L. depressa* germinated, with 22.3% germination percentage. This germination rate was similar to that from chapter 3 (24.1%). All germinated plants were found to be the result of successful pollinations based on intermediate phenotypes.

Triploid *L. camara*

Fruit set of three triploid cultivars and five triploid lines varied, with the two-season average ranging from 0 to 31.4% (Table 6-3). Three of the triploids, ‘Miss Huff’, ‘New Gold’, and GDOP-4, are UFG producers, and their two-season average fruit set was 31.4%, 1.4%, and 7.8%, respectively. Among the five non-UFG-producing triploids, 605-

35, 605-43, 617-1 and 623-5 had 0.3% to 1.7% fruit set, and ‘Athens Rose’ did not set any fruit in both seasons. The average fruit set of these non-UFG producing triploids was 0.4% (from 1,396 flowers pollinated), in contrast with the 13.5% average fruit set of the UFG producing triploids (33.8-fold higher).

Because of the low fruit set on non-UFG-producing triploid cultivars/lines, only 11 seeds were obtained (Table 6-4). The germination percentage of these seeds was 27.2% compared to 11.1% germination in Chapter 3. A total of 145 seeds were obtained from the three UFG-producing *L. camara* triploids, and the seed germination was 47.5% (29.3% chapter 3), approximately 75% higher than that of non-UFG-producing triploids.

These results show that certain triploid *L. camara* lines were highly crossable as a female with *L. depressa* and could produce a large number of fruit. Seeds from these fruit could germinate at high percentages. UFG production played a very important role in the high fruit set and seed germination of these triploids. In contrast, non-UFG-producing triploid *L. camara* were the least crossable as a female with with *L. depressa* among *L. camara* polyploids and these triploids set only a very low percentage of fruit.

Tetraploid *L. camara*

Fruit set varied considerably among *L. camara* tetraploid cultivars and lines. Line 604-5 did not set any fruit on 176 flowers pollinated, while ‘Pink Caprice’, CAOP-88, and 611-7 had 11.3%, 12.7%, and 16.0% fruit set, respectively. Fruit set of ‘Dallas Red’ and ‘Carlos’ was much lower, only 1.7% and 4.1%, respectively (Table 6-3).

Three of the 10 tetraploids were known to produce UFGs and seven were not. When data were pooled and averaged, the UFG-producing tetraploids had 7.9% fruit set, and the non-UFG-producing tetraploids had 9.1% fruit set. It seems that the UFG production trait did not make any difference in changing fruit set in tetraploid *L. camara*

× *L. depressa* crosses. The role of UFG production seems to be very obvious in seed germination (Table 6-4). Seeds from non-UFG-producing *L. camara* tetraploids had 18.5% germination, similar to those from non-UFG producing diploid or triploid *L. camara*, but seeds from UFG-producing *L. camara* tetraploids had 54.4% germination, nearly 2-fold increase compared to the non-UFG-producing tetraploids.

Pentaploid and hexaploid *L. camara*

The average fruit set of the two pentaploids ('Cajun Pink' and 629-1) over the two seasons was between 2.2% and 4.0%, which was relatively low. A total of 22 seeds were obtained from the 690 flowers pollinated, of which only two germinated, resulting 9.1% germination, lower than that of diploids, triploids and tetraploids (Table 6-4).

The average fruit set of three hexaploids (PIT-2, PIT-20 and 620-1) over the two seasons was 0.3 to 1.2% (Table 6-4). As a ploidy level, the average fruit set was 0.8%, only slightly higher than that of non-UFG-producing triploids (0.3%), suggesting that hexaploids were overall highly female-sterile and rarely crossable with *L. depressa*. None of the six seeds obtained from hexaploid *L. camara* × *L. depressa* crosses germinated.

Ploidy analysis of progeny from *L. camara* and *L. depressa* crosses

Ten of the 25 crosses between *L. camara* and *L. depressa* resulted in a total of 150 progeny. The number of progeny per cross varied from one to 63. The most successful crosses involved two *L. camara* diploids, one triploid, and three tetraploids. The cross between diploid *L. camara* 'Cream' and *L. depressa* produced 12 progeny, and all of them were triploids. This was unexpected, because 'Cream' was a diploid known not to produce UFGs and the *L. depressa* accession was expected to be a diploid from previous reports (Sanders, 1987a). The *L. depressa* accession provided for

this experiment was non-distinguishable morphologically from typical *L. depressa*, but further analysis showed that the *L. depressa* accession was a tetraploid. The discovery of this tetraploid suggests that natural polyploidization had occurred in *L. depressa*. Similarly, the cross between diploid 'Lola' and *L. depressa* produced triploids.

When *L. depressa* (4*x*) was pollinated with 'Cream' (2*x*), the progeny were not triploids as expected; instead they were pentaploids (Table 6-5). This type of 2*n* + *n* progeny were also observed in the cross between *L. depressa* and 'Lola' (2*x*), between *L. depressa* and breeding line 611-7. These results suggest that the *L. depressa* accession used in this study carried the UFG production trait like some *L. camara* cultivars.

Apomixis seems to have occurred in this *L. depressa* accession as well. The cross between *L. depressa* and 'Pink Caprice' resulted in one tetraploid (Table 6-5). It could be either a *n* + *n* or 2*n* + 0 progeny. Morphologically it was nearly identical to *L. depressa* and did not express any of the paternal parent's flower pigmentation (pink). Thus, the descendant is more likely to be apomictic than zygotic.

The reciprocal cross ('Pink Caprice' x *L. depressa*) produced 26 progeny, 20 of which were tetraploids and six hexaploids. These hexaploids would be 2*n* + *n* type, but the tetraploids could be 2*n* + 0 or *n* +*n* type, because 'Pink Caprice' was known to produce UFGs and apomictic seed. Thus, their origin remains to be determined. Morphological and/or molecular marker characterization could help address this question.

The crosses between DROP-25 or 611-7 and *L. depressa* also produced tetraploid progeny (Table 6-5). As DROP-25 and 611-7 were known not to produce UFGs or

apomictic seeds, these tetraploid progeny would be more likely to be *n* + *n* type than 2*n* + 0 type.

The cross between 'Miss Huff' and *L. depressa* yielded 63 progeny. A great majority of the progeny (61 out of 63) were triploids resembling 'Miss Huff' morphologically, indicating that very likely these had resulted from 'Miss Huff's UFGs and apomixis (Table 6-5). Two progeny were pentaploids, likely produced from 'Miss Huff's UFGs fertilized with *L. depressa* (2*n* + *n*).

Summary

Hand pollinations were made between 25 *L. camara* cultivars/breeding lines and one *L. depressa* accession to assess hybridization potential between the two species. The *L. camara* cultivars/breeding lines used represented five ploidy levels (diploid to pentaploid), a wide range of pollen stainability (1.5% to 79.1%) and fruit production capacity (0.006 to 7.137 fruit per peduncle from Chapter 3). Results indicate that pollen stainability of *L. camara* was the most important factor determining the potential of *L. camara* as a male parent to hybridize *L. depressa* and cause fruit production. As *L. camara*'s pollen stainability reduced, fruit set *L. camara* could cause on *L. depressa* dropped rapidly. As *L. camara* pollen stainability dropped to below 10%, it caused very little fruit set on *L. depressa*. Among the five ploidy levels, diploid *L. camara* cultivars ('Cream' and 'Lola') seemed to be most compatible with *L. depressa,* followed by tetraploids. Triploid *L. camara* with low pollen stainability (<10%, even <15%) had little potential to cross-pollinate *L. depressa*. Pentaploids and hexaploids appeared to be less crossable with *L. depressa* than tetraploids.

The same *L. camara* cultivars/breeding lines were used as female parents and pollinated with *L. depressa*. Results reveal that *L. camara*'s ploidy level and mode of

reproduction played important roles in fruit (or seed) production after hand pollination with *L. depressa*. Diploid *L. camara* produced the highest number of fruit among the five ploidy levels. UFG-producing triploid *L. camara* lines were highly crossable as a female with *L. depressa* and produced large numbers of fruit and seeds, whereas non-UFG-producing triploid *L. camara* were the least crossable as female with *L. depressa.* The UFG production trait made no difference in fruit set in tetraploid *L. camara* × *L. depressa* crosses but caused large differences in seed germination.

Ploidy analysis results revealed that natural polyploidization had occurred in *L. depressa*. Ploidy level distribution among the progeny of crosses between *L. camara* and *L. depressa* suggest that the *L. depressa* accession used in this study carried the UFG production and apomixis traits like some *L. camara* cultivars did. This is the first report of such traits in *L. depressa*. Further analyses of morphological and molecular markers are needed to confirm the presence of UFGs and apomixis in *L. depressa*.

Collectively, results from this chapter suggest that hybridization potential was more prevalent when *L. depressa* was the pollen donor and *L. camara* the pollen recipient. This may be due to the fact that the male fertility of many *L. camara* cultivars/breeding lines have been much reduced but their female fertility hasn't been reduced as much because of the UFG formation and apomixis traits. Thus, eliminating UFG formation and apomixis is critical for developing highly sterile *L. camara* cultivars.

Table 6-1. Fruit set on *L. depressa* flowers pollinated with *L. camara*. Hand pollination was performed in greenhouse conditions in fall 2009 (Season 1) and repeated spring 2010 (Season 2).

L. camara cultivar/line	Ploidy	Stainable pollen (%)[Z]	*L. depressa* flowers pollinated (no.)		Fruit set (%)		Average fruit set (%)
			Season 1	Season 2	Season 1	Season 2	
'Cream'	2*x*	79.1	106	129	20.6	25.2	22.9
'Lola'	2*x*	74.5	107	49	28.3	14.5	21.4
'Athens Rose'	3*x*	14.9	117	80	3.2	0.0	1.6
'Miss Huff'	3*x*	1.8	125	119	0.0	0.0	0.0
'New Gold'	3*x*	1.5	18	100	0.0	0.0	0.0
GDOP-4	3*x*	6.0	99	111	0.0	0.0	0.0
605-35	3*x*	7.4	104	105	0.0	0.0	0.0
605-43	3*x*	14.8	105	109	1.0	0.0	0.5
617-1	3*x*	11.5	110	-	0.3	-	0.3
623-5	3*x*	14.6	111	125	0.0	0.0	0.0
'Bandana Cherry'	4*x*	37.4	95	93	4.4	2.3	3.3
'Carlos'	4*x*	51.2	109	114	2.7	6.0	4.3
'Dallas Red'	4*x*	29.4	102	105	0.9	0.0	0.5
'Pink Caprice'	4*x*	68.7	305	93	1.6	16.1	8.9
CAOP-73	4*x*	40.7	126	-	0.0	-	0.0
CAOP-88	4*x*	3.2	109	71	1.0	0.0	0.5
DROP-25	4*x*	37.4	338	-	0.6	-	0.6
PCOP-6	4*x*	39.0	113	-	1.6	-	1.6
604-5	4*x*	12.1	118	-	0.0	-	0.0
611-7	4*x*	47.6	111	94	6.3	1.1	3.7
'Cajun Pink'	5*x*	39.1	123	122	9.0	2.4	5.7
629-1	5*x*	32.8	102	112	0.0	0.0	0.0
PIT-2	6*x*	26.1	106	79	1.0	0.0	0.5
PIT-20	6*x*	24.6	101	129	2.0	0.0	1.0
620-1	6*x*	31.5	78	-	0.0	-	0.0

[Z]Pollen staining rates from Chapter 2 were combined with another pollen sample during the first season of pollinations.

Table 6-2. Differences among *L. camara* ploidy levels in causing fruit set on *L. depressa* flowers and seed germination.

Ploidy level of *L. camara* as ♂	Fruit set (%)			Seeds sown (no.)	Seeds germinated (no.)	Seed germination (%)
	Season 1	Season 2	Average			
2*x*	24.4	19.9	22.2	91	5	5.5
3*x*	0.6	0.0	0.3	3	0	0.0
4*x*	1.9	4.3	3.1	47	4	8.5
5*x*	4.5	1.2	2.8	15	0	0.0
6*x*	1.0	0.0	0.5	3	1	33.3

Table 6-3. Fruit set of *L. camara* flowers pollinated with *L. depressa*. Hand pollination in greenhouse conditions was performed fall 2009 (Season 1) and repeated spring 2010 (Season 2).

L. camara cultivar/line	Ploidy	UFG producer	*L. camara* flowers pollinated		Fruit set (%)		Average fruit set (%)
			Season 1	Season 2	Season 1	Season 2	
'Cream'	2*x*	No	270	249	14.8	7.9	11.4
'Lola'	2*x*	No	224	304	18.5	24.9	21.7
'Athens Rose'	3*x*	No	165	169	0.0	0.0	0.0
'Miss Huff'	3*x*	Yes	191	158	45.9	17.0	31.4
'New Gold'	3*x*	Yes	124	140	1.5	1.3	1.4
GDOP-4	3*x*	Yes	149	204	7.1	8.4	7.8
605-35	3*x*	No	184	188	0.5	0.4	0.5
605-43	3*x*	No	191	280	0.5	0.3	0.4
617-1	3*x*	No	219	-	1.7	-	1.7
623-5	3*x*	No	200	221	0.0	0.5	0.3
'Bandana Cherry'	4*x*	No	104	162	9.6	8.8	9.2
'Carlos'	4*x*	No	182	197	0.6	7.6	4.1
'Dallas Red'	4*x*	No	162	151	1.5	1.8	1.7
'Pink Caprice'	4*x*	Yes	235	239	14.4	8.2	11.3
CAOP-73	4*x*	No	128	-	8.7	-	8.7
CAOP-88	4*x*	No	182	197	14.5	10.9	12.7
DROP-25	4*x*	No	148	-	8.1	-	8.1
PCOP-6	4*x*	Yes	178	-	8.2	-	8.2
604-5	4*x*	Yes	176	-	0.0	-	0.0
611-7	4*x*	No	149	189	2.0	30.0	16.0
'Cajun Pink'	5*x*	Yes	141	191	5.4	2.6	4.0
629-1	5*x*	Yes	202	156	4.4	0.0	2.2
PIT-2	6*x*	Yes	161	147	0.6	0.0	0.3
PIT-20	6*x*	Yes	140	166	0.0	1.9	1.0
620-1	6*x*	Yes	153	-	1.2	-	1.2

Table 6-4. Total seed set and germination rates when *L. camara* was used as a female for *L. depressa* pollen over two seasons.

L. camara ploidy level	Gamete type	Fruit set (%) Season 1	Season 2	Average	Seed sown (no)	Seed germ.	Germ. %
2*x*	Non-UFG	16.7	16.4	16.5	179	40	22.3
3*x*	Non-UFG	0.6	0.3	0.4	11	3	27.2
3*x*	UFG	18.2	8.9	13.5	145	69	47.5
4*x*	Non-UFG	6.4	11.8	9.1	168	31	18.5
4*x*	UFG	7.5	8.2	7.9	68	37	54.4
5*x*	UFG	4.9	1.3	3.1	22	2	9.1
6*x*	UFG	0.6	1.0	0.8	6	0	0.0

Table 6-5. Ploidy level distribution of crosses between *L. camara* and *L. depressa*.

Seed parent (ploidy)	Pollen parent (ploidy)	Progeny available (no.)	Ploidy level distribution in progeny 3*x*	4*x*	5*x*	6*x*	Possible modes of reproduction
'Cream' (2*x*)	*L. depressa* (4*x*)	12	12				*n* + *n*
L. depressa (4*x*)	'Cream' (2*x*)	3			3		2*n* + *n*
'Lola' (2*x*)	*L. depressa* (4*x*)	24	24				*n* + *n*
L. depressa	'Lola' (2*x*)	1			1		2*n* + *n*
'Miss Huff' (3*x*)	*L. depressa* (4*x*)	63	61		2		2*n* + 0, 2*n* + *n*
'Pink Caprice' (4*x*)	*L. depressa* (4*x*)	26		20		6	2*n* + 0, 2*n* + *n*
L. depressa (4*x*)	'Pink Caprice' (4*x*)	1		1			*n* + *n* or 2*n* + 0
DROP-25 (4*x*)	*L. depressa* (4*x*)	6		6			*n* + *n*
611-7 (4*x*)	*L. depressa* (4*x*)	13		13			*n* + *n*
L. depressa (4*x*)	611-7 (4*x*)	1				1	2*n* + *n*

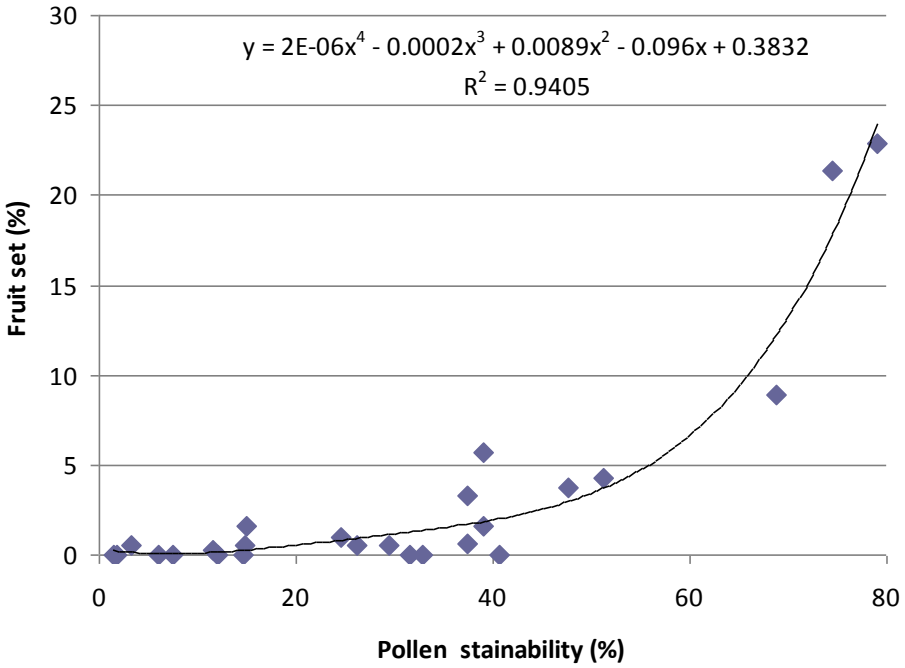

Figure 6-1. Polynomial relationship between *L. camara* pollen stainability and fruit set of *L. depressa* flowers pollinated with *L. camara*.

CHAPTER 7
DEVELOPING STERILE TRIPLOIDS IN *LANTANA CAMARA*

Rationale

The ornamental plant industry is a major contributor to Florida's economy with an annual wholesale value of nearly $700 million (USDA report, 2010) (Hodges and Haydu 2006; www.usda.gov). *L. camara* is a very important crop of this industry, contributing to more than 1% (~$40 million) of the total annual wholesale value. According to a survey of the Florida nurseries, 19% of the responding nurseries produced *L. camara* (Wirth et al., 2004). Yet, *L. camara* can hybridize with *L. depressa*, a native lantana species endemic to southern Florida (Sanders 1987a). Because of this, *L. camara* is listed as a Category I invasive species in Florida (FLEPPC, 2009). Sterile cultivars are needed to protect native lantana and meet the industry's need for commercial production.

A breeding program was initiated in 2004 at the University of Florida's Gulf Coast Research and Education Center to develop new sterile *L. camara* cultivars. The polyploid nature of the species (Sanders, 2006) was thought to provide numerous opportunities and avenues for breeding (Eigsti, 1957). Initial efforts produced a number of highly sterile triploids, but they had poor growth habits for ornamental use. To overcome this difficulty, additional tetraploids were used as breeding parents in interploid crosses. Triploids produced from these crosses had mounding or semi-spreading growth habits but produced more fruit (seed) than desired.

To improve the effectiveness and efficiency in genetic sterilization of *L. camara*, the male and female fertility of 26 *L. camara* cultivars was investigated (Chapters 2 and 3). The majority of the triploid cultivars had pollen stainability below 10%, even as low

as 1.8%. Thus, high levels of male sterility can be achieved in *L. camara* through ploidy manipulation, particularly triploid production. However, most triploid cultivars produced as much fruit (or seed) as diploids or tetraploids did. Very few of the existing commercial *L. camara* cultivars have the desirable levels of male and female sterility.

Only a weak correlation was found between male and female fertility in *L. camara* polyploids (Chapter 3; Khoshoo and Mahal, 1967; and Spies, 1984c). To understand this phenomenon, ploidy and molecular marker analysis were performed on *L. camara* progeny from controlled as well as open pollinations. Results have shown that *L. camara* has evolved the ability to produce unreduced female gametes (UFG) and to develop seed through apomixis (Czarnecki and Deng, 2009; Chapter 5). These traits have enabled *L. camara* triploids to produce fruit (seed). Many *L. camara* cultivars can reproduce through multiple pathways. Therefore, it is necessary to identify diploids and tetraploids that do not have these traits to develop highly sterile cultivars in *L. camara*,.

The objectives of this study were to 1) produce new triploids through interploid crosses, 2) evaluate the effects of parental combination, direction of pollination, and environmental factors on pollination success, 3) select promising sterile triploids, 4) assess their male and female sterility, 5) determine their hybridization potential with *L. depressa*, 6) evaluate their plant performance, and 7) select the best triploids as candidates for release.

Materials and Methods

Parental Plant Materials

Three diploid cultivars (‘Cream’, ‘Denholm White’, ‘Lola’), one diploid breeding line (LAOP-9), and five tetraploid cultivars (‘Carlos’, ‘Dallas Red’, ‘Gold’, ‘Irene’, and ‘Pink Caprice’) were selected as parents for interploid crosses to produce triploids (Table 7-

1). Parental plants were propagated by cuttings and grown in 30.5-cm plastic containers filled with a commercial soilless mix, VerGro® container mix A (Verlite Co., Tampa, FL) amended with controlled release fertilizer, Osmocote® (15N-3.9P-10K, 8-9 months release at 21°C; The Scotts Company, Marysville, OH) at 7.12 kg · m^{-3}. Containerized parental plants were grown in the greenhouseset to 29/21°C day/night temperature under natural light and ranged from 32 to 15°C.

Parental plants used for growth chamber experiments were grown in 15.2-cm plastic containers in the soil mix previously described from 23 April 2007 to 7 August 2007. Three Percival Scientific growth chambers (model E 30B, Boone, IA) set to 16 hour days and held at 21.1, 26.7, and 32.2°C were used. After pollinations were completed and seed were collected, the temperatures were changed in rotation across the three chambers. Plants were given two weeks to acclimate at each temperature before pollinations were started again.

Hand Pollination

Flowers were emasculated by removing the corolla prior to opening. Emasculated flowers were pollinated by transferring fresh pollen from a newly opened flower with a small paint bush. The brush was cleaned in 100% ethanol and dried before it was used to pollinate another flower. When lantana fruit turned dark purple to black, they were collected for seed extraction. Crosses were made in 20 April 2006–28 June 2006, 20 September 2006–30 January 2007, and 15 February 2007–31 July 2007.

Progeny Growing and Evaluation

Seeds were extracted and cleaned within 1 to 2 weeks after fruit was harvested. Seeds were sown on the surface of a peat/vermiculite mix (VerGro® container mix A,

Verlite, Tampa, FL) and germinated under intermittent mist in a greenhouse. Temperatures in the greenhouse ranged from 16°C (night) to 30°C (day), and no artificial lighting was used. The majority of seeds germinated within four months after sowing, but some took as long as a year to germinate. After young seedlings had developed true leaves, they were transferred to 10.2-cm plastic containers filled with VerGro® container mix. Plants were fertilized by incorporating Osmocote® (15N-3.9P-10K) at 7.12 kg m-3 in the soilless mix.

When seedlings were 2 to 4-month old, ploidy analysis was performed using fully expanded young leaves and the Partec PA I ploidy analyzer and the CyStain UV Ploidy Precise P dye (Partec, Germany). The manufacturer recommended ploidy analysis procedure was followed with minor modifications (supplemented with 2% w/v PVP and 0.01% β-mercaptoethanol) with dye mixture kept on ice.

Identified triploids were first evaluated in the greenhouse for their growth habit, leaf characteristics, and flower characteristics. Plants were grown in 10.2-cm pots with the potting soil as described above. Those that had weak vigor, erratic growth habits, few leaves, and/or undesirable flowers were eliminated. The selected triploids were then planted in the ground beds mulched with white plastic. Plants in the ground beds were irrigated biweekly and fertilized with 29.7 cm^3 Osmocote® (15N-3.9P-10K, 8-9 months release, The Scotts Company). Again, triploids that had weak vigor, erratic growth habits, few leaves, and/or undesirable flowers were eliminated.

Statewide trials of promising triploids

Mature cuttings were taken on 14 – 16 March 2009, treated with 1:7 ratio of Dip'n Grow® (Dip'n Grow Inc. Clackamas, Oregon) rooting hormone, and rooted in 128 cell

speedling trays in Fafard® 3B (Anderson, SC) potting soil. Rooted cuttings were grown in 10.2-cm pots with the potting soil as described above.

When plants were 2-month-old, they were distributed to three locations. The two northern sites were pure plantings of only *L. camara* lines. Northern sites were located at the North Florida Research and Education Center (NFREC) Quincy, Florida and at the University of Florida Plant Science Research and Education Unit located near Citra, Florida. The two locations in central Florida were mixed plantings of *L. camara* and *L. depressa* (the Florida native lantana) at Balm, Florida at the Gulf Coast Research and Education Center (GCREC) and Ft. Pierce, Florida at the Indian River Research and Education Center (IRREC).

Planting was completed the week of 5 May 2009. All the trial sites included the commercial cultivars 'Pink Caprice' and 'New Gold' and triploid lines developed at GCREC. To prevent fertile pollen drift 'Pink Caprice' was planted in a sequestered location at least 150 feet away from any other lantana plants. Cultivar 'New Gold' was planted in the main plot with the other trialed triploid lines. The plants were grown in raised beds under artificial white on black plastic mulch on 1.8 m centers and fertilized with two 29.7 cm^3 of 15-9-12 slow release 12-14 month Osmocote® (The Scotts company, Marysville Ohio). To provide adequate spacing for growth plants were planted in 2 plant plots separated by the native lantana for the mixed planting sites across three blocks at each site providing a 3.6 m separation from the experimental lines in each plot. Watering durations and rates were similar across locations (two hours twice a week from drip irrigation).

Evaluating male sterility

Ten UFBLs and two commercial cultivars (‘Pink Caprice’ and ‘New Gold’) and the Florida native *L. depressa* planted in raised beds at the GCREC and IRREC were screened. Collection of anthers was done on three intervals, 24 September, 6 October, and 16 November of 2009. Pollen was stained with cotton blue vital stain (Eng. Scientific, Inc. Product No. 6730, Clifton, NJ). Anthers were collected from predehiscent flower clusters. Four anthers from three flower clusters were collected from each plant sample into 1.5-mL eppendorf tubes with approximately 100-µL of stain. A total of 8–12 anthers were placed in stain were then allowed to soak in the stain overnight at 65°C. Anthers were triple rinsed with distilled water, placed onto microscope slides and fixed with a 4:1 glycerol to water ratio. Slides were observed at a 400x magnification and pictures were taken for manual scoring later.

Evaluating female sterility

Determining the female fertility of the triploid and commercial cultivars was done by randomly collecting 20 fresh peduncles with entirely spent flowers from each plant in a plot (similar to Chapter 3). Peduncles were bulked by plots then stripped of any seed present and an average seed per peduncle was calculated.

Evaluating plant performance

Plants were evaluated at four week intervals starting at planting (5 May 2009) until the conclusion of the study on 14 December 2009. The two characteristics considered at all four sites were plant quality and flowering intensity. Plant quality (PQ) was rated on a scale of 1 to 5. A rating of 1 indicated that the plant was growing poorly with few shoots, sparse foliage, and/or severe diseases, while a rating of 5 indicated that the plant grew well with dense foliage. A rating of 5 would be a very desirable

plant. Flowering intensity (FI) was a visual rating of the percentage of maximum bloom. A categorical scale from 1–5 representing 0–20%, 21–40%, 41–60%, 61–80% and 81–100 of the maximum flower coverage of the plant.

Statistical Analysis

Data were analyzed using PROC GLM in SAS® for Windows 9.2 (SAS® Institute, Cary, NC) to determine the significance of differences among ploidy levels, lantana lines, and seasons. Percentage data were transformed using the arcsine square root function when necessary. Differences were determined among evaluation weeks, locations, and lines using Tukey's W procedure for mean separation in SAS®.

Results

Triploid Generation

Selecting parents for interploid crosses

Thirty crosses were designed among three diploid cultivars, 'Cream', 'Denholm White', and 'Lola', and five tetraploid cultivars, 'Carlos', 'Dallas Red', 'Gold', 'Irene', and 'Pink Caprice'. These crosses were made in spring 2006 and repeated in fall 2006. Pollination success rates ranged from 0–18.3%, with an average of 2.5% (data not shown). Crosses involving 'Denholm White' did not result in much fruit as it was later found to be highly female sterile (Chapter 3). Progeny of 'Cream' had erratic growth habits and light flower colors (personal observations). 'Lola' was the best parent among the three diploid cultivars in terms of pollination success rate. When hand-pollinated, 0.8–3.22% of 'Lola's flowers set fruit.

Most of the progeny of 'Gold' and 'Pink Caprice' produced from these crosses were tetraploids or hexaploids, rather than triploids, because these two tetraploid cultivars formed unreduced female gametes and apomictic seeds (Chapters 4 and 5).

Thus, they were not desirable parents for producing sterile triploids. When crossed with diploid cultivars, ‘Carlos’, ‘Dallas Red’, and ‘Irene’ produced triploids (Chapters 4 and 5). ‘Carlos’ and ‘Irene’ were very similar in growth habit, and leaf and flower characteristics, but ‘Carlos’ set higher percentages of fruit (1.5 to 18.3%), thus ‘Carlos’ seemed to be a more desirable tetraploid parent. The majority of ‘Dallas Red’s progeny expressed an erratic growth habit, but this tetraploid cultivar did not produce unreduced female gametes or apomictic seeds and was one of only a few cultivars available at the time with a red flower color, thus it was selected as a tetraploid parent.

In evaluating ‘Lola’s open-pollination progeny in early 2007, one line with a more compact growth habit and darker yellow flower than ‘Lola’ was identified. It was named as LAOP-9 and used to replace ‘Denholm White’ as a compact mounding type.

Effects of parental combination on pollination success

Two tetraploid cultivars (‘Carlos’ and ‘Dallas Red’) and two diploids (‘Lola’ and LAOP-9) were paired in eight interploid crosses to evaluate the effects of parental combinations on pollination success. As controls for the interploid crosses, the two tetraploid parents were paired in two crosses, and the two diploid parents were paired in another two crosses. Thus, a total of 12 crosses were designed from the four parents. Without considering the ploidy level differences, these crosses resembled a full diallel mating design. Hand pollinations were performed in February through July 2007. Fruit set percentages of pollinated flowers were recorded as pollination success rates. The number of flowers pollinated over the 6-month period for each interploid cross ranged from 1204 to 1894 (Table 7-2).

The observed pollination success rates varied considerably among the interploid crosses, from 0.1% to 9.1% during the Feb.-May pollination period and 1.2% to 38.2%

during the June-July pollination period (Table 7-2). Overall, pollination success rates were higher when the tetraploid parent was ‘Carlos’ than it was ‘Dallas Red’, and when the diploid parent was LAOP-9 than it was ‘Lola’.

A strong seasonal effect on pollination success seemed to exist (to be discussed separately later). The direction of crossing and the level of compatibility between parents seemed to affect pollination success to a large extent. Taking the June-July pollination period’s data for example, 4.3% of pollinated flowers set fruit when ‘Lola’ was the maternal parent. The parents were reciprocally crossed (‘Carlos’ x ‘Lola’), and 20.5% of the pollinated flowers set fruit, which is 3.8-fold higher (Table 7-3). A similar effect was observed in the cross between ‘Carlos’ and LAOP-9 and in the cross between ‘Dallas Red’ and LAOP-9. In these crosses, the pollination success rate was 3 or 1.5-fold higher when the maternal parent was a tetraploid than when it was a diploid (38.2% vs. 9.5%, or 25.3% vs. 10.1%). A similar effect was also evident in the Feb.-May pollination period’s data in these crosses, except for the cross between ‘Dallas Red’ and LAOP-9. Using tetraploids as female parents when crossed with diploids is likely to be more productive than the reciprocal.

The crosses between ‘Dallas Red’ and ‘Lola’ had the lowest pollination success rates (0.3% and 0.1% for the Feb-.May pollination period and 1.2% and 4.1% for the June-July pollination period), regardless of the direction of crossing (Table 7-1), in contrast to the results when LAOP-9 was the diploid parent. As described above, when LAOP-9 was crossed with ‘Dallas Red’, the pollination success rates were 1.8% to 2.1% for the Feb.-May pollination period and 10.1% to 25.3% for the June-July pollination period (Table 7-2). As described above, LAOP-9 was an OP progeny of ‘Lola’. The

cause of the lower level of cross-compatibility between ‘Dallas Red’ and ‘Lola’ remains to be elucidated.

Seasonal effects on pollination success rates

The monthly pollination success rate of each of the eight interploid crosses was similar among February, March, and April (thereafter referred to as the first pollination period) and similar between June and July (referred to as the second pollination period) (data not shown). Thus the pollination data were combined and averaged within each period. The average pollination success rates were very different between the two periods. The difference in pollination success rates ranged from 2.3-fold (‘Carlos’ and ‘Lola’) to as great as 14.1-fold (‘Dallas Red’ and LAOP-9). Similar differences were observed in two 2*x* x 2*x* crosses (3.0% to 7.2% in the first period vs. 41.0% to 28.3% in the second period) and two 4*x* x 4*x* crosses (1.1% to 1.7% in the first period vs. 15.8% to 14.7% in the second period). These differences indicated a strong seasonal effect on pollination success, as the fertilization and irrigation regime was held consistent in the greenhouse from February to July. Several environmental factors were different between the two pollination periods, including temperature, light level, photoperiod, humidity, etc. As shown below, experimental results indicated that temperature was a factor causing this seasonal effect on pollination success.

Effects of growing temperatures

Significant differences ($F = 31.08$, $P=<0.0001$) were observed in pollination success between different pollination periods. It was hypothesized that one of the main environmental factors for such differences was growing temperatures. To test this hypothesis, one of the interploid crosses, ‘Carlos’ x ‘Lola’, was selected, and the parental plants were subjected to three treatments: growing at 21.1°C, 26.7°C, or

32.2°C. The average pollination success rate was the highest (25.0%) when parental plants were grown at 26.7°C (Figure 7-2). The average success rate was 9.1% for plants subjected 21.1°C. These success rates were similar to the results from the first and second pollination periods (Table 7-1). For the 32.2°C treatment, more than 230 flowers were pollinated, but none of them set any fruit. Additionally, the parental plants grown at 32.2°C seemed to produce ~60% fewer flowers compared to those grown at 21.1°C or 26.7°C (~230 vs. ~540).

Selection of new triploids

Over the course of hand pollinations in 2006 and 2007, 393 triploids were generated. They were first evaluated in the greenhouse and then in the field for growth habit and leaf and flower characteristics. Fourteen of them received high scores and were selected for propagation tests. Four of them did not root well or grow well in containers. The remaining 10 triploids rooted well and grew well in containers, and they were selected for further evaluation of male sterility, female sterility, and plant performance.

Male Sterility of Promising Triploid Selections

Pollen staining

Three experiments were conducted to assess the pollen stainability of the 10 promising triploids. In each experiment, more than 2000, up to 5141 (Table 7-4), pollen grains were examined per triploid, except for T8, which produced less pollen. Similar pollen stainabilitiy was observed for each triploid among the experiments. Thus, the average pollen stainability will be used for discussion.

The commercial triploid cultivar included in the experiments (‘New Gold’) had a pollen stainability of 0.3%. The male-fertile control included in the experiments, ‘Pink

Caprice', had a pollen stainability of 65.5% (Table 7-4). Two triploids had pollen stainability slightly above 10% (12.2% for T6 and 10.4% for T7). The pollen stainability of the remaining eight triploids was below 10.0%, ranging from 2.8% (T8) to 9.7% (T2).

Hand pollination

Two hand pollination experiments were performed in fall 2009 and spring 2010 to assess these triploids' ability to cause fruit set on *L. depressa* to confirm that low pollen staining lines will not cause seed set at seen in Chapter 7. In each experiment, fresh pollen was collected from unopen flowers and immediately applied onto emasculated *L. depressa* flowers. Of the two triploid controls, 'New Gold' did not cause any fruit set in both experiments (Table 7-5). 'Athens Rose's caused 3.2% fruit set in the first experiment and no fruit set in the second experiment. 'Pink Caprice' caused fruit set in both experiments: 1.6% in the first one and 16.1% in the second one, resulting in an average of 8.9% fruit set. These results were similar to the results described in Chapter 6. Seven triploids, T1, T2, T4, T5, T6, T8, and T9, did not cause fruit set in both experiments. T3's pollen caused 2.8% fruit set in the first experiment, but none in the second one. Similarly, hand-pollination with T7's pollen caused a low percentage of fruit set on *L. depressa*.

Female sterility of promising triploid selections

'Pink Caprice' was very prolific at all four sites throughout the 6-month period of evaluation, producing 3 to as many as 18 fruit per peduncle (Table 7-5). Interestingly it set more fruit when grown in Quincy and Citra without *L. depressa* planted in the plots (pure planting) than when grown in Balm and Ft. Pierce with *L. depressa* planted in the plots (mixed planting) (14.118 vs. 6.783 on average). The female-sterile control included in the study, 'New Gold', was highly sterile, producing less than 0.1 fruit per

peduncle in the six evaluations from 30 July to 14 December 2009 in Quincy and Citra when *L. depressa* was not present. However, when 'New Gold' was inter-planted with *L. depressa* and grown in Balm and Ft. Pierce, this triploid cultivar set, on average across two sites and over six evaluations, 10.5-fold more fruit (0.540 per peduncle). The average fruit set of the new triploids across four sites over 6 months was below 0.100 fruit per peduncle, except for T1 grown in Quincy and Citra. Thus these triploids were highly sterile. Compared to 'Pink Caprice', the fruit production capacity or female fertility of these triploids was reduced by ~137 fold. Compared to 'New Gold' (0.17 FPP), the new triploids maintained their low level of fruit set (40% of 'New Gold') and high level of female sterility even when *L. depressa* was inter-planted and viable pollen was available from *L. depressa* plants.

Plant performance of promising triploid selections

Controls. Within 8 weeks post planting (WPP), plants of 'New Gold' reached a quality rating of 3.0 or above at all four sites. Its plant quality ratings remained at or above 3.0 through 28 WPP. 'New Gold' plants grown in Quincy, Citra, and Balm received a quality rating between 3.0 and 5.0 and had an average PQ rating per site over the six evaluations of 4.1 (Citra), 4.3 (Balm), and 4.5 (Quincy) (Table 7-7). When planted in Ft. Pierce, 'New Gold' did not grow as well as it did in the other three sites and received an average PQ rating of 3.4. With all four sites and all six evaluations considered, 'New Gold' average plant quality rating was 4.1, the highest among all the entries in the experiments.

'New Gold' planted in Quincy and Citra received a flowering intensity rating at 8 WPP of 3.3 and 4.8, and maintained its rating between 4.0 and 5.0 (Table 7-7). 'New Gold' grown in Balm received a FI rating of 2.7 at 8 WPP and 3.2 to 5.0 thereafter until

the end of the evaluation. The average FI rating per site over the 6-month period was 4.7 for Quincy, 4.8 for Citra, and 4.3 for Balm. 'New Gold' grown in Ft. Pierce had lower FI ratings (2.0 to 3.5 vs. 3.2 to 5.0) in five out of the six evaluations and a lower average rating (2.9 vs. 4.3 to 4.8). 'New Gold's average flowering intensity rating across four sites over six evaluations was 4.2, the highest among all entries.

A similar site effect was observed in 'Pink Caprice' (and all triploid selections). Plants grew better and opened more flowers in Quincy, Citra, and Balm than in Ft. Pierce. 'Pink Caprice's average plant quality rating in Quincy, Citra, and Balm was 3.3, 2.7, and 3.4, respectively, while this rating in Ft. Pierce was 2.3. 'Pink Caprice's average FI rating in Quincy, Citra, and Balm was 3.7, 3.3, and 3.4, respectively, but it was 1.3 in Ft. Pierce. 'Pink Caprice's overall PQ and FI ratings were 2.9 and 2.8, respectively (Tables 7-7 and 7-8).

To determine if the time period and site influenced PQ evaluations were analyzed by evaluation week and site. PQ evaluations were found to be influenced by both evaluation week and site. Evaluations of plant quality revealed that weeks were found to be significantly different at $P < 0.0001$ (F value 10.41, DF 5) and sites were significantly different at $P < 0.0001$ (F value 80.14, DF 3). The evaluation periods when separated indicated WPP 12 through 24 to be the highest two statistical groupings. WPP eight and 28 were the lowest evaluations of PQ. When separating sites Quincy was rated the highest rated followed by Balm and Citra, and Ft. Pierce was found to be the lowest rated site statistically.

Analysis of FI data were done similar to PQ for evaluation weeks and sites. Flowering intensity was also found to be influenced by the evaluation WPP and site.

Statistical groups did not follow PQ groups completely. Evaluations of FI revealed that WPP were significantly different at P <0.0001 (F value 21.17, DF 5) and sites were significantly different at P <0.0001 (F value 233.51, DF 3). The WPP evaluations when separated indicated WPP 12 through 28 to be the highest two statistical groupings. Only the initial evaluation eight WPP was in the lowest statistical group. Site separation indicated that Citra and Quincy were in the highest evaluation group followed by Balm and lastly Ft. Pierce in the lowest statistically rated site.

T1 selection. When grown in Quincy, Citra, and Balm, plants of this triploid received a quality rating between 3.0 and 5.0 in 17 out of 18 evaluations and a FI rating between 3.5 and 5.0 in 15 out of 18 evaluations (Table 7-7 and 7-8). The average PQ in these locations was 4.4, 3.9, and 3.4, respectively, and the average FI rating in these locations was 4.5, 4.3, and 3.3, respectively. T1's PQ rating in Ft. Pierce was 2.5 to 3.5, with an average of 2.8, and its FI rating was 1.2 to 2.5, with an average of 2.0. The overall average PQ rating and FI rating of T1 was 3.3 and 3.5, respectively.

T2 selection. This line is a sibling of the T1 selection. They were similar in 16 out of 18 and 13 out of 18 evaluations, with ratings between 3.0 and 5.0 for PQ and FI (Table 7-7 and 7-8). The highest PQ ratings were from Balm (3.8) and Quincy (4.2). The other two sites had PQ ratings of 3.1 (Citra) and 3.6 (Quincy). The FI ratings were 3.4 and 4.0 for Balm and Citra, respectively. Evaluations from Ft. Pierce only exceeded 3.0 for week 24 (PQ) and week 28 (FI), with averages of 2.7 and 2.2 for PQ and FI respectively.

T3 selection . T3 was rated the highest in Quincy (4.1) for PQ and the same in Citra and Quincy for FI (4.2) (Table 7-7 and 7-8). From Balm and Citra 15 observations

were above a 3.0 rating with averages of 3.2 (Balm) and 3.8 (Citra). PQ of T3 rated well with 16 observations over 3.0. Balm had an average FI of 3.1. Ft. Pierce was again lower than the other sites with PQ and FI averages of 2.6 and 2.3. The collective averages of PQ and FI across all sites were 3.4 and 3.5 respectively.

T4 selection . This selection had the highest PQ and the lowest FI of all the triploid selections (Table 7-7 and 7-8). Looking more closely at this line 16 PQ and 13 FI observations were at or above 3.0 for the sites excluding Ft. Pierce. For this particular line the averages were affected largely by Ft. Pierce as it was the lowest rated FI of all triploids at that site. The other sites averaged 2.5 (Balm), 3.4 (Citra) and 3.8 (Quincy). The best attribute of this plant is its PQ as it was the highest rated of the triploid selections at 3.7. At Ft. Pierce this plant was tied for the highest rated PQ of all the triploids with an average of 2.8. The other sites average 3.9, 3.8, and 4.4 for Balm, Citra, and Quincy respectively.

T5 selection. This selection was rated a little higher on average and had 17 observations for PQ and FI were above the 3.0 rating (Table 7-7 and 7-8). The three site averages for PQ were 3.5, 3.6, and 4.2 for Balm, Citra and Quincy respectively. The FI rating for the previously mentioned sites was 3.3, 4.2, and 4.4 respectively. Ft. Pierce averaged 2.7 (PQ) and 1.9 (FI). The overall averages were 3.5 for PQ and 3.4 for FI.

T6 selection . Plants of this triploid did not grow well at the four test sites, with a quality rating of below 3.0 in 15 out of 24 evaluations (Table 7-7). Its FI rating ranged from 1.7 to 5.0 when grown in Quincy, Citra, and Balm, and from 1.2 to 2.8 in Ft. Pierce (Table 7-8). Its overall average PQ and FI ratings were 2.4 and 3.3, respectively.

T7 selection. Plants of this triploid seemed to be weak, not growing or flowering well at all test sites. Its overall PQ and FI ratings were 2.4 and 2.9, respectively, below the acceptable level for commercial production (Table 7-7 and 7-8).

T8 selection. This selection was similar to T6: Plants did not grow well, but they flowered acceptably. Its average PQ rating per site was below 3.0 for three out of the four sites (Table 7-7 and 7-8). Its average FI per site was 4.1 for Quincy, 4.3 for Citra, 3.6 for Balm, and 1.9 for Ft. Pierce.

T9 selection . Plants of this selection received a quality rating of 3.0 or above (3.0 to 4.7) and a FI rating of 3.0 or above (3.3 to 5.0) in 15 out of the 18 evaluations in Quincy, Cita, and Balm (Table 7-7 and 7-8). The average PQ and FI ratings per site over 6 months were 4.1 and 4.1 for Quincy, 3.8 and 4.3 for Citra, and 3.0 and 3.1 for Balm. Its PQ rating in Ft. Pierce was between 2.0 and 2.8, averaged to 2.6. Its FI was 1.3 to 2.7, averaged to 1.9.

T10 selection. Plants of this triploid did not grow well at all four sites, and its PQ rating was below 3.0 in 13 out of 24 evaluations (Table 7-7). Its FI rating was 3.7 or 3.8 in Quincy and Citra, but was 2.7 and 1.8 in Balm and Ft. Pierce (Table 7-8). Thus the ornamental value of this selection was lower than other triploids.

Discussion

Breeding *L. camara* For Sterile Cultivar Release

Optimizing environment

Developing sterile *L. camara* cultivars is a multi-step, lengthy process. Increasing the efficiency in each step is very important. Each step should be optimized as information becomes available. This study has revealed that environmental conditions, especially growing season and temperature, had significant effects on pollination

success. The genetic by environemental interactions are extensive and important when breeding. These discoveries could lead to much improved rates of seed set. In the case of difficult crosses, it may be possible to manipulate the growth conditions to enhance seed production.

Optimizing crosses

As discussed in Chapters 4 and 5, the selection of tetraploids that do not produce unreduced female gametes (UFGs) and apomictic seeds is critical for producing highly sterile triploids. However, this critical piece of information was not available when the first 30 crosses were undertaken to produce triploids. Serendipitously three non-UFG producing cultivars were included in those crosses. As the information became available, pollination efforts were focused on these non-UFG-producing tetraploids. Results from this study showed that triploid selections resulting from non-UFG-producing tetraploids were both highly male- and female-sterile. Currently the number of non-UFG-producing tetraploids is limited. More non-UFG-producing tetraploids need to be identified or developed.

The results from this study (Tables 7-3) also indicate that the direction of crossing played a critical role in successful triploid production. Diploid plants were much more successful at causing seed production on tetraploid plants than the reverse. This may be due to the violation of the endosperm balance number (EBN) theory. This theory states that when the maternal contribution to the endosperm to paternal contribution exceeds a 2:1, embryo abortion may occur (Burton and Husband, 2000; Carputo et al., 2003; Poehlman and Sleper, 1995). In the case of interploid crosses, some tolerance may exist if the higher ploidy level plant is used as the maternal source so as not to exceed this ratio. The other explanation for the lack of seed set is that some parents

could have very low pollen fertility levels. As seen in table 7-3 the diploid plants have on average nearly double the pollen staining rates of the tetraploids. Given that the diploids have higher pollen staining and crosses of the 4*x* x 2*x* nature are expected to be more effective these factors are likely not mutually exclusive.

Another factor that needs to be considered in lantana interploid crosses is cultivar incompatibility. Out of the 12 crosses made in 2007, 'Dallas Red' by 'Lola' (4*x* x 2*x*) had the lowest fruit setting cross. This cross normally would be expected to have high seed set rates based on the high pollen stainability of 'Lola' and the high set rates on 'Carlos'. 'Dallas Red' set a relatively high percentage of fruit when pollinated with LAOP-9, a direct descendant of 'Lola'. Thus it would be expected that a cross between 'Dallas Red' and 'Lola' should have seeded readily. The observed low fruit set in this cross indicated strong incompatibility between the two cultivars.

Selecting triploids as candidates for releasing

When male and female sterility, leaf and flower characteristics, and plant performance were considered, four triploids, T2, T3, T4, and T9, seemed to have the best potential to be released as new cultivars. Their pollen stainability was below 10% (some similar to sterile control 'New Gold'), a safe level from controlled pollinations in Chapter 6. These triploids all had extremely low fruit production (below one seed for every 16 flower clusters), comparable to 'New Gold', the industry standard sterile cultivar. As a matter of fact, the new triploids seemed to be more sterile than 'New Gold'. When inter-planted with lantana plants producing viable pollen, 'New Gold' produced much more fruit (over 1 fruit per flower cluster), while the four new triploids remained highly sterile.

Summary

A productive breeding program working with *L. camara* will include a number of factors that have been explained from multiple experiments over several years of study. For the purpose of releasing sterile cultivars of *L. camara* it was necessary to determine the maximum levels of male sterility possible to achieve (Chapter 2). Similarly information about the the level of seed production and seed viability (Chapter 3) present in commercial cultivars was needed. This provided a basis for comparison for these new selections. After the discovery of UFG production and other reproductive modes (Chapters 4 and 5) it became necessary to ensure the parents and progeny did not contain any of the genes related to those traits. For a plant that is valued primarily for its ornamental attributes the last stage of selection pressure was exerted on PQ and FI.

Collection of clear fertility data and using it in conjunction with performance analyses greatly assisted plant selection. It was clear that the site a plant is grown can drastically affect its performance characteristics. However, these plants were generally consistent across sites. This made the selection process straightforward in most cases. In one particular incident with T1 and T2 sibling plants, T2 was selected over T1 even though the plant performance was just a little lower. The reason for this was that T1 had slightly higher seed production rates than T2. The statewide evaluation of these triploids successfully demonstrated the selection of ornamentally desirable, highly male and female sterile lines across several months indicating a high level of landscape performace and durable supression of sexual reproduction.

Table 7-1. Parental lines used in interploid cross pollinations.

Line	Ploidy	Form	Foliage density	Opening flower color	Finishing flower color
'Carlos'	4*x*	Mounded	High	Yellow	Magenta
'Dallas Red'	4*x*	Erratic	Low	Yellow	Red
'Denholm White'	2*x*	Mounded	High	Cream	White with yellow center
'Irene'	4*x*	Mounded	High	Yellow	Magenta
LAOP-9	2*x*	Mounded	High	Dark Yellow	Dark Yellow
'Lola'	2*x*	Erratic	Medium	Yellow	Yellow

Table 7-2. Pollination success rates for eight interploid crosses between two tetraploids and two diploids for triploid generation. Two tetraploid by tetraploid and two diploid by diploid crosses were included as controls.

Seed parent	Pollen parent	Nature of Cross	Flowers pollinated		Pollination success rate (%)	
			Feb. - May	June - July	Feb. - May	June - July
'Carlos'	'Lola'	4*x* x 2*x*	715	489	9.1	20.5
'Lola'	'Carlos'	2*x* x 4*x*	875	541	1.4	4.3
'Carlos'	LAOP-9	4*x* x 2*x*	783	476	6.6	38.2
LAOP-9	'Carlos'	2*x* x 4*x*	936	579	1.0	9.5
'Dallas Red'	'Lola'	4*x* x 2*x*	1298	541	0.3	1.2
'Lola'	'Dallas Red'	2*x* x 4*x*	975	460	0.1	4.1
'Dallas Red'	LAOP-9	4*x* x 2*x*	1149	504	1.8	25.3
LAOP-9	'Dallas Red'	2*x* x 4*x*	702	624	2.1	10.1
'Carlos'	'Dallas Red'	4*x* x 4*x*	837	398	1.1	15.8
'Dallas Red'	'Carlos'	4*x* x 4*x*	931	491	1.7	14.7
'Lola'	LAOP-9	2*x* x 2*x*	1433	461	7.2	41.0
LAOP-9	'Lola'	2*x* x 2*x*	860	668	3.0	28.3

Table 7-3. Results of full diallel crosses of four lines of *Lantana camara* indicating the rates of seed production compared to the rates of pollen stainability.

Parent	'Carlos' (49.4[Z])	'Dallas Red' (31.7)	'Lola' (81.1)	LAOP-9 (79)
'Carlos' (4x, 1.9[Y])	-	15.8	20.5	38.2
'Dallas Red' (4x, 0.6)	14.7	-	1.2	25.3
'Lola' (2x, 0.9)	4.3	4.1	-	41.0
LAOP-9 (2x, 0.4)	9.5	10.1	28.3	-

[Z]Pollen stainability (%) with cotton blue stain along the top of the table from chapter 2.
[Y]Ploidy level followed by the average seed production per seed cluster in open pollination from chapter 3.

Table 7-4. Pollen stainability of 10 new triploid lines, three commercial cultivars and *L. depressa*.

Line	Gulf Coast (REC) 1		Gulf Coast (REC) 2		Ft. Pierce (REC) 1		Pollen counted total[Z]	Grand avg.[X]
	Pollen counted	Avg.[Z]	Pollen counted	Avg.[Z]	Pollen counted	Avg.[Z]		
T1 - 624-1	4171	9.6	3598	7.1	2770	6.6	10539	7.8def
T2 - 624-4	3417	12.3	2919	8.7	2412	8.3	8748	9.7cde
T3 - 705b-3	5141	6.5	3752	6.4	4025	2.4	12918	5.1gh
T4 - 702a-3	3992	3.1	2983	4.6	3808	1.9	10783	3.2ih
T5 - 605-16	3500	9.7	2099	10.3	4202	6.1	9801	8.7def
T6 - 625-2	2429	17.8	2517	11.8	3693	6.9	8639	12.2c
T7 - 603-8	2826	13.7	2417	9.3	2828	8.1	8071	10.4cd
T8 - 605-7	471	3.3	1813	2.5	933	2.6	3217	2.8i
T9 - 613-3	3679	6.8	2237	8.5	4846	2.8	10762	6.1gf
T10 - 603-17	3381	11.0	2936	8.7	3835	3.7	10152	7.8efg
'Athens Rose'							6042	20.6*
'New Gold'	2245	0.9	1816	0.3	2550	0.1	6611	0.4j
'Pink Caprice'	2211	62.0	2030	65.1	1752	69.9	5993	65.6a
L. depressa[Y]	722	42.3	780	45.7	1006	33.4	2508	40.5b

[Z]Indicates average pollen staining (%) with cotton blue from plants in field conditions at the University of Florida Gulf Coast Research and Education Center, Balm, Florida (GC) across two time periods and plants at the Indian River Research and Education Center, Ft. Pierce, Florida (FP).

[Y]Three *L. depressa* plants were sampled from each field.

[X] Letters indicate mean separation by Tukey's W procedure.

[*]Pollen staining from two replicated garden trials in 2009.

Table 7-5. Fruit set of *Lantana depressa* when nine *Lantana camara* triploids and three commercial cultivars were used as a pollen source.

L. camara line used	*L. depressa* flowers pollinated		Fruit set (%)		Average fruit set (%)[Z]
	Fall 2010	Spring 2011	Fall 2010	Spring 2011	
T1 - 624-1	107	116	0.0	0.0	0.0a
T2 - 624-4	114	119	0.0	0.0	0.0a
T3 - 705b-3	64	114	2.8	0.0	1.4ab
T4 - 702a-3	133	107	0.0	0.0	0.0a
T5 - 605-16	209	85	0.0	0.0	0.0a
T6 - 625-2	79	114	0.0	0.0	0.0a
T7 - 603-8	317	-	1.4	-	1.4ab
T8 - 605-7	112	-	0.0	-	0.0a
T9 - 613-3	114	97	0.0	0.0	0.0a
‘Athens Rose’	117	80	3.2	0.0	1.6ab
‘New Gold’	18	100	0.0	0.0	0.0a
‘Pink Caprice’	305	93	1.6	16.1	8.9b

[Z]Letters indicate mean separation by Tukey’s W procedure.

Table 7-6. Fruit production per peduncle of 10 *Lantana camara* triploids and two commercial cultivars with *Lantana depressa* planted at Quincy, Citra, Balm, and Ft. Pierce in Florida. Lines were found to be significantly different at P <0.0001 (F value 19.41, DF 10).

Line	Site	Type of planting	WPP 8	WPP 12	WPP 16	WPP 20	WPP 24	WPP 28	Average	Line average[X]
T1	Quincy	Pure[Z]	0.008	0.000	0.092	0.217	0.225	0.592	0.189	0.117b
	Citra	Pure	0.017	0.033	0.025	0.000	0.700	0.208	0.164	
	Balm	Mixed[Y]	0.025	0.059	0.149	0.017	0.017	0.016	0.047	
	Ft. Pierce	Mixed	0.067	0.133	0.017	0.058	0.100	0.033	0.068	
T2	Quincy	Pure	0.025	0.000	0.017	0.017	0.008	0.000	0.011	0.062ab
	Citra	Pure	0.008	0.042	0.025	0.000	0.167	0.033	0.046	
	Balm	Mixed	0.000	0.127	0.308	0.103	0.075	0.025	0.106	
	Ft. Pierce	Mixed	0.117	0.167	0.075	0.017	0.075	0.025	0.079	
T3	Quincy	Pure	0.008	0.000	0.008	0.000	0.000	0.033	0.008	0.019a
	Citra	Pure	0.025	0.008	0.000	0.000	0.000	0.017	0.008	
	Balm	Mixed	0.000	0.017	0.074	0.033	0.017	0.016	0.026	
	Ft. Pierce	Mixed	0.025	0.033	0.025	0.025	0.042	0.033	0.031	
T4	Quincy	Pure	0.000	0.000	0.000	0.125	0.000	0.358	0.081	0.023a
	Citra	Pure	0.000	0.017	0.000	0.000	0.000	0.000	0.003	
	Balm	Mixed	0.000	0.017	0.016	0.008	0.000	0.000	0.007	
	Ft. Pierce	Mixed	0.008	0.008	0.000	0.000	0.000	0.000	0.003	
T5	Quincy	Pure	0.025	0.000	0.000	0.008	0.000	0.008	0.007	0.023a
	Citra	Pure	0.000	0.008	0.000	0.000	0.083	0.033	0.021	
	Balm	Mixed	0.000	0.017	0.058	0.000	0.016	0.016	0.018	
	Ft. Pierce	Mixed	0.017	0.108	0.058	0.017	0.042	0.008	0.042	
T6	Quincy	Pure	0.008	0.000	0.008	0.008	0.000	0.008	0.006	0.007a
	Citra	Pure	0.017	0.008	0.000	0.000	0.017	0.000	0.007	
	Balm	Mixed	0.000	0.017	0.008	0.000	0.000	0.009	0.006	
	Ft. Pierce	Mixed	0.008	0.000	0.000	0.008	0.008	0.017	0.007	

Table 7-6 Continued.

Line	Site	Type of Planting	WPP 8	WPP 12	WPP 16	WPP 20	WPP 24	WPP 28	Average	Line Average[X]
T7	Quincy	Pure[Z]	0.025	0.000	0.108	0.167	0.133	0.483	0.153	0.062ab
	Citra	Pure	0.017	0.008	0.000	0.000	0.017	0.000	0.007	
	Balm	Mixed[Y]	0.008	0.009	0.008	0.025	0.016	0.049	0.019	
	Ft. Pierce	Mixed	0.050	0.075	0.058	0.017	0.033	0.017	0.042	
T8	Quincy	Pure	0.000	0.000	0.033	0.100	0.025	0.008	0.028	0.036ab
	Citra	Pure	0.000	0.017	0.008	0.000	0.083	0.033	0.024	
	Balm	Mixed	0.000	0.025	0.093	0.066	0.042	0.007	0.039	
	Ft. Pierce	Mixed	0.033	0.067	0.067	0.017	0.050	0.083	0.053	
T9	Quincy	Pure	0.008	0.000	0.017	0.100	0.008	0.150	0.047	0.025a
	Citra	Pure	0.017	0.000	0.025	0.000	0.000	0.117	0.026	
	Balm	Mixed	0.000	0.020	0.058	0.000	0.000	0.026	0.017	
	Ft. Pierce	Mixed	0.008	0.033	0.000	0.000	0.008	0.008	0.010	
T10	Quincy	Pure	0.008	0.000	0.075	0.008	0.058	0.150	0.050	0.021a
	Citra	Pure	0.008	0.008	0.008	0.000	0.017	0.000	0.007	
	Balm	Mixed	0.017	0.000	0.067	0.008	0.000	0.000	0.015	
	Ft. Pierce	Mixed	0.000	0.025	0.000	0.008	0.033	0.008	0.013	
‘New Gold’	Quincy	Pure	0.017	0.017	0.125	0.017	0.008	0.075	0.043	0.294c
	Citra	Pure	0.175	0.025	0.017	0.000	0.033	0.058	0.051	
	Balm	Mixed	0.430	0.581	0.833	0.746	0.673	0.227	0.582	
	Ft. Pierce	Mixed	0.208	0.250	1.142	0.408	0.675	0.308	0.499	
‘Pink Caprice’	Quincy	Pure	7.150	22.838	20.825	17.000	11.138	11.275	15.038	10.451
	Citra	Pure	15.808	10.867	16.092	9.175	12.783	14.467	13.199	
	Balm	Mixed	1.143	10.683	12.416	4.226	8.883	7.532	7.481	
	Ft. Pierce	Mixed	5.067	6.608	9.525	8.000	4.583	2.733	6.086	
L. depressa	Quincy	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1.918
	Citra	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	
	Balm	Mixed	-	3.805	2.349	1.159	1.810	-	2.281	
	Ft. Pierce	Mixed	1.367	3.517	2.092	1.105	0.800	0.444	1.554	

[Z]Only *Lantana camara* lines were planted at this location.
[Y]*Lantana camara* and *Lantana depressa* lines were planted at this location.
[X]Superscript letters indicate mean separation by Tukey’s W procedure (α 0.05).

Table 7-7. Plant quality ratings of new triploid lines and commercial cultivars with *Lantana depressa* planted at four sites: Quincy, Citra, Balm, and Ft. Pierce in Florida. Plants were planted on 5 May 2009. Lines were found to be significantly different at P <0.0001 (F value 29.64, DF 11). Plants were evaluated in a scale of 1–5. Increasing evaluations indicated more compact and healthy plants.

Line	Site	WPP 8	WPP 12	WPP 16	WPP 20	WPP 24	WPP 28	Site avg.	Grand avg.[z]
T1	Balm	2.5	3.2	4.2	4.2	3.2	3.2	3.4	3.6ab
	Citra	3.2	4.5	4.8	4.0	4.0	3.0	3.9	
	Ft. Pierce	2.2	2.5	2.5	2.8	3.5	3.3	2.8	
	Quincy	3.3	4.8	5.0	4.2	4.3	4.8	4.4	
T2	Balm	3.5	3.5	3.8	4.0	3.8	3.8	3.8	3.3bcd
	Citra	2.2	3.3	4.2	3.3	3.0	2.7	3.1	
	Ft. Pierce	2.2	2.7	2.5	2.8	3.2	2.8	2.7	
	Quincy	3.3	4.0	4.0	3.0	3.8	3.7	3.6	
T3	Balm	2.7	2.8	3.5	3.7	3.3	3.3	3.2	3.4bc
	Citra	3.3	4.0	4.5	4.2	3.5	3.5	3.8	
	Ft. Pierce	2.0	2.3	2.7	2.8	3.2	2.7	2.6	
	Quincy	2.7	4.8	5.0	3.8	4.2	4.2	4.1	
T4	Balm	3.2	4.0	4.2	4.3	4.0	3.7	3.9	3.7ab
	Citra	4.7	5.0	3.5	4.0	3.5	2.3	3.8	
	Ft. Pierce	2.7	3.2	2.7	2.8	2.7	2.5	2.8	
	Quincy	2.2	5.0	5.0	4.5	4.8	5.0	4.4	
T5	Balm	3.8	3.7	4.0	3.5	3.2	3.0	3.5	3.5bc
	Citra	3.7	4.0	4.3	3.8	3.2	2.7	3.6	
	Ft. Pierce	2.8	3.0	2.8	2.8	2.5	2.0	2.7	
	Quincy	3.5	4.8	4.3	4.3	3.8	4.5	4.2	
T6	Balm	3.7	3.3	4.0	3.5	2.7	2.8	3.3	3.1cd
	Citra	4.3	3.7	3.5	3.2	2.7	2.2	3.3	
	Ft. Pierce	2.8	2.8	2.0	2.2	2.3	2.2	2.4	
	Quincy	3.7	3.5	3.5	3.0	2.7	3.2	3.3	

Table 7-7 Continued.

Line	Site	WPP 8	WPP 12	WPP 16	WPP 20	WPP 24	WPP 28	Site avg.	Grand avg.[z]
T7	Balm	2.2	2.8	3.3	3.2	3.0	2.8	2.9	2.4f
	Citra	1.0	2.5	2.7	2.3	2.7	2.3	2.3	
	Ft. Pierce	1.7	1.8	1.7	2.0	2.2	1.8	1.9	
	Quincy	3.5	2.7	2.5	2.3	1.8	2.8	2.6	
T8	Balm	2.5	2.5	3.0	3.0	2.8	1.8	2.6	2.6ef
	Citra	2.5	3.0	4.0	3.2	2.8	1.8	2.9	
	Ft. Pierce	1.3	1.8	2.0	1.5	1.3	1.2	1.5	
	Quincy	2.5	3.2	3.5	3.0	3.3	3.7	3.2	
T9	Balm	2.5	2.7	3.7	3.3	3.0	3.0	3.0	3.4bc
	Citra	4.2	4.3	4.0	4.2	3.5	2.7	3.8	
	Ft. Pierce	2.8	2.7	2.5	2.3	2.5	2.5	2.6	
	Quincy	3.0	4.7	4.5	3.8	4.3	4.2	4.1	
T10	Balm	2.0	2.2	2.2	3.0	2.2	2.0	2.3	2.4f
	Citra	1.0	3.0	3.0	3.0	2.3	2.0	2.4	
	Ft. Pierce	1.2	2.0	2.2	2.2	2.2	2.3	2.0	
	Quincy	3.5	3.5	3.0	3.0	2.3	2.7	3.0	
‘New Gold’	Balm	4.0	4.7	4.7	5.0	3.8	3.5	4.3	4.1a
	Citra	4.5	5.0	4.0	4.0	4.0	3.0	4.1	
	Ft. Pierce	3.5	3.2	3.3	3.0	3.8	3.8	3.4	
	Quincy	3.2	5.0	5.0	5.0	4.5	4.3	4.5	
‘Pink Caprice’	Balm	3.3	3.7	4.2	3.7	3.0	2.7	3.4	2.9de
	Citra	4.2	3.0	3.2	2.0	2.0	2.0	2.7	
	Ft. Pierce	1.7	2.5	3.2	2.7	2.2	1.5	2.3	
	Quincy	2.5	3.5	3.0	3.0	3.3	4.8	3.3	

[z]Letters indicate mean separation by Tukey’s W procedure (α 0.05).

Table 7-8. Flower intensity ratings of new triploid lined and commercial cultivars with *Lantana depressa* planted at four sites: Quincy, Citra, Balm, and Ft. Pierce in Florida. Plants were planted on 5 May 2009. Lines were found to be significantly different at P <0.0001 (F value 6.73, DF 11). Plants were evaluated in a scale of 1–5. Increasing evaluations indicated more open flowers.

Line	Site	WPP 8	WPP 12	WPP 16	WPP 20	WPP 24	WPP 28	Site avg.	Grand avg.[z]
T1	Balm	2.0	1.8	4.0	4.0	3.5	4.2	3.3	3.5ab
	Citra	3.5	4.5	4.8	5.0	4.0	4.0	4.3	
	Ft. Pierce	1.2	1.5	2.3	2.0	2.5	2.5	2.0	
	Quincy	2.8	5.0	5.0	4.3	4.8	5.0	4.5	
T2	Balm	3.3	1.3	3.0	3.8	4.0	4.7	3.4	3.4bc
	Citra	2.2	4.3	5.0	5.0	4.0	3.3	4.0	
	Ft. Pierce	1.2	2.0	2.2	1.8	2.5	3.3	2.2	
	Quincy	4.3	4.2	4.0	3.0	4.7	4.8	4.2	
T3	Balm	1.8	2.0	3.8	3.7	3.3	4.2	3.1	3.5b
	Citra	3.3	4.2	4.7	5.0	3.3	4.5	4.2	
	Ft. Pierce	1.3	2.2	3.2	1.7	2.2	3.2	2.3	
	Quincy	2.7	4.7	4.5	3.7	4.8	5.0	4.2	
T4	Balm	1.3	1.0	3.3	3.0	3.2	3.0	2.5	2.7c
	Citra	2.5	4.0	3.8	5.0	3.0	2.3	3.4	
	Ft. Pierce	1.0	1.3	1.7	1.2	1.2	1.0	1.2	
	Quincy	2.2	4.0	4.7	3.2	4.3	4.7	3.8	
T5	Balm	3.3	2.2	3.0	3.7	3.3	4.3	3.3	3.4b
	Citra	3.0	4.3	5.0	5.0	4.0	3.7	4.2	
	Ft. Pierce	1.7	1.8	3.0	1.2	1.8	1.8	1.9	
	Quincy	3.5	4.8	4.5	4.3	4.2	5.0	4.4	
T6	Balm	2.7	1.7	3.5	4.0	3.8	4.2	3.3	3.3bc
	Citra	3.7	4.0	4.0	5.0	3.8	3.5	4.0	
	Ft. Pierce	1.5	1.2	2.0	1.5	1.5	2.8	1.8	
	Quincy	3.8	5.0	4.2	3.7	3.8	4.5	4.2	

Table 7-8 Continued.

Line	Site	WPP 8	WPP 12	WPP 16	WPP 20	WPP 24	WPP 28	Site avg.	Grand avg.[Z]
T7	Balm	1.7	1.8	3.2	4.0	3.2	3.8	2.9	2.9bc
	Citra	1.0	3.7	3.5	4.3	3.8	3.2	3.3	
	Ft. Pierce	1.7	1.8	1.8	1.5	1.8	1.7	1.7	
	Quincy	3.0	3.2	3.5	3.5	4.0	4.8	3.7	
T8	Balm	2.5	2.3	4.3	4.2	4.0	4.0	3.6	3.5ab
	Citra	2.8	4.3	5.0	5.0	4.7	4.0	4.3	
	Ft. Pierce	1.3	1.7	2.7	1.5	2.0	2.5	1.9	
	Quincy	2.3	4.2	4.2	4.2	4.7	5.0	4.1	
T9	Balm	2.5	1.2	3.8	3.3	3.8	4.0	3.1	3.4bc
	Citra	4.2	4.7	4.7	5.0	3.7	3.7	4.3	
	Ft. Pierce	1.3	2.0	2.2	1.2	2.7	2.2	1.9	
	Quincy	2.7	5.0	4.3	3.7	4.5	4.7	4.1	
T10	Balm	1.3	1.8	2.7	4.0	3.2	3.2	2.7	3.0bc
	Citra	1.2	4.0	4.7	5.0	3.3	3.8	3.7	
	Ft. Pierce	1.0	1.7	2.2	1.7	1.8	2.3	1.8	
	Quincy	3.2	4.3	4.2	3.0	3.7	4.5	3.8	
‘New Gold’	Balm	2.7	3.2	4.7	5.0	5.0	5.0	4.3	4.2a
	Citra	4.8	5.0	5.0	5.0	5.0	4.0	4.8	
	Ft. Pierce	3.5	3.2	3.5	2.0	2.7	2.5	2.9	
	Quincy	3.3	5.0	5.0	5.0	5.0	4.7	4.7	
‘Pink Caprice’	Balm	2.8	1.7	4.5	4.3	3.3	3.5	3.4	2.9bc
	Citra	3.3	3.0	3.7	4.8	2.0	2.8	3.3	
	Ft. Pierce	1.0	1.8	2.0	1.0	1.0	1.0	1.3	
	Quincy	2.7	3.5	3.0	4.0	4.0	5.0	3.7	

[Z]Letters indicate mean separation by Tukey’s W procedure (α 0.05).

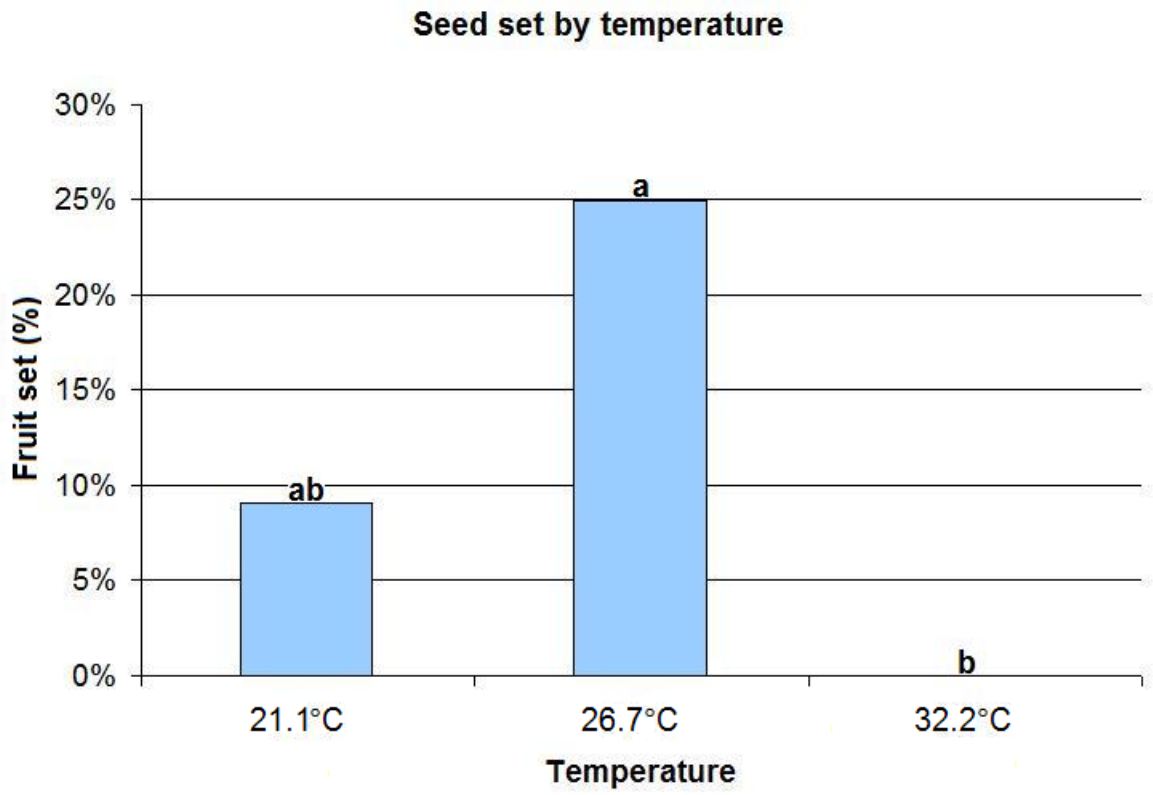

Figure. 7-1. Effect of three growth chamber temperatures on the percent fruit set of *Lantana camara* cultiar 'Carlos' pollinated with 'Lola'. Temperature effect was significantly different at P=0.0232 (F value 8.76, DF 2). Mean separation was performed using Tukey's W procedure; letters of different groups represent different statistical groups.

CHAPTER 8
CONCLUSIONS

Rationale

Lantana camara is a important ornamental and landscape plant in Florida. Yet, it is a Category I invasive species that can hybridize with the Florida native species *Lantana depressa*. Genetic sterilization has potential as an economical, preventive measure to control the invasiveness of *L. camara*. This study sought to identify the primary biological factors that affect *L. camara*'s male and female fertility, to assess *L. camara*'s hybridization potential with *L. depressa*, and to develop new sterile *L. camara* cultivars.

Male and Female Fertility of *L. camara*

Male fertility was assessed based on pollen stainability. Results revealed that pollen stainability varied from 1.8% to 81.1% among cultivars. Ploidy level was found to be the most important factor affecting male fertility. On average, diploids exhibited the highest male fertility, followed by tetraploids, pentaploids, hexaploids, and triploids.

There was significant pollen stainability variation within certain ploidy levels and genetic background. Pedigree may also play a significant role in determining *L. camara*'s male fertility. Pollen stainability variation was observed among triploids as well, ranging from 1.8% to 27.1%. Pollen stainability rates this high in triploids is unusual but not as high as some previously reported. Thus newly produced triploids need to be screened to identify triploids that can meet the required male sterility level.

The most important factors determining female fertility in plants are seed (or fruit) production and seed germination. Little information is available in the literature regarding fruit production capacity and seed germination of lantana cultivars. Results

showed that *L. camara* cultivars differed considerably in fruit production, ranging from 0.003 (‘Denholm White’) to 7.173 (in ‘Pink Caprice’) fruit per peduncle. Ploidy level, unreduced female gamete (UFG) production, and apomixis played significant roles in determining the fruit production capacity of *L. camara*. Results indicate that triploids without the UFG production and apomixis traits were most sterile. However, other genetic mechanisms might cause female sterility in *L. camara*. Principal component analysis based on female fertility index (product of fruit per peduncle and seed germination) and pollen stainability provided a useful way to visualize the reproductive characteristics of different *L. camara* cultivars/breeding lines.

Multiple Modes of Reproduction in *L. camara*

Ploidy analysis was performed on progeny from open and controlled pollinations and complimented with simple sequence repeat (SSR)-based molecular marker analysis. These analyses showed that *L. camara* could form three types of female gametes [reduced female gametes (RFGs), UFGs, and double UFGs (DUFGs)] and two types of male gametes [reduced male gametes (RMGs) and unreduced male gametes (UMGs)] and could develop seed through fertilization or apomixis, leading to six primary modes of reproduction in *L. camara*. These modes include: 1) RFGs and RMGs fertilized and developed into $n + n$ progeny, 2) RFGs undergoing apomixis ($n + 0$ progeny, or haploidization), 3) UFGs fertilized with RMGs producing $2n + n$ progeny, 4) UFGs developed via apomoxis directly into embryos and $2n + 0$ progeny, 5) DUFGs fertilized with RMGs, resulting in $4n + n$ progeny, and 6) DUFGs developed into $4n + 0$ progeny through apomixis. The UFG production trait was found to be controlled by nuclear gene(s). The primary reproductive pathway during microsporogenesis was the formation of RMGs. UMGs were observed, but they occurred at very low frequencies.

These results indicate a strong need to screen breeding parents carefully and to avoid using those materials that can form UFGs or apomictic seed in crosses intended for developing sterile triploids. None of the diploids evaluated in this study showed tendency to produce UFGs or apomictic seeds, but three out of the six tetraploids studied showed such tendency. There is a dire need to identify or develop more tetraploids that do not carry the UFG production or apomixis trait for sterile triploid development.

L. camara's Hybridization Potential with *Lantana depressa*

Pollen stainability of *L. camara* was the most important factor determining the potential of *L. camara* as a male parent to hybridize with *L. depressa* and cause fruit production. As *L. camara*'s pollen stainability was reduced, its ability to cause fruit set on *L. depressa* dropped rapidly. When *L. camara*'s pollen stainability dropped to below 10%, it could hardly cause fruit set on *L. depressa*. Among the five ploidy levels, diploid *L. camara* cultivars seemed to be most compatible with tetraploid *L. depressa,* followed by tetraploids. Triploid *L. camara* with low pollen stainability (<10%) had very little potential to cross-pollinate *L. depressa*.

When *L. camara* was pollinated with *L. depressa,* ploidy level and mode of reproduction of *L. camara* were the primary factors determining fruit (or seed) production. Diploid *L. camara* produced the largest number of fruit among the five ploidy levels. UFG-producing triploid *L. camara* lines were highly crossable as a female with *L. depressa* and produced large numbers of fruit and seed, whereas non-UFG-producing triploid *L. camara* lines were the least crossable as a female with *L. depressa.*

Ploidy analysis results revealed that natural polyploidization had occurred in *L. depressa*. Ploidy level distribution among the progeny of crosses between *L. camara* and *L. depressa* suggest that the *L. depressa* accession used in this study carried the UFG production and apomixis traits like some *L. camara* cultivars did. This is the first report of such traits in *L. depressa*.

Developing Sterile Triploid Selections

Interploidy crosses were made among two diploid cultivars, one diploid breeding line, and three tetraploid cultivars that did not carry the UFG production and apomixis traits. These crosses resulted in 393 triploids, from which 10 triploids were selected. These selections showed high levels of male and female sterility when tested at four sites in southern, central and northern Florida. Four of the sterile triploid selections also performed and flowered well in these tests and have shown potential to be released as new cultivars.

Future Opportunities for Lantana Breeding

This study has demonstrated that high levels of male and female sterility can be achieved through selecting appropriate tetraploids and generating and selecting triploids. In the process of investigating male and female sterility of *L. camara*, one diploid cultivar and one tetraploid breeding line were found to have unusually low levels of fertility. 'Denholm White' is a diploid cultivar and has high pollen stainability. However, it set very little seed despite thousands of flowers that were hand- or open-pollinated (fruit per peduncle of 0.003). CAOP-88, a tetraploid individual, showed good female fertility but exceptionally low pollen stainability (3.2%). Although the exact genetic mechanisms causing the female sterility in 'Denholm White' and the male sterility in 'CAOP-88' remain to be elucidated, it is expected that the sterility should be

caused by genes rather than meiotic failure due to imbalanced chromosomes, since they are a diploid and tetraploid. These materials may offer additional genetic means to reduce the fertility of *L. camara* and to develop new sterile cultivars in this species.

APPENDIX A
FULL DATASET OF POLLEN STAINING OF ALL LANTAN A LINES STAINED

Table A-1. Pollen stainability of all lines screened in Seasons 1 and 2.

Line	Ploidy	Season 1	Season 2	Season 1[Y]	Season 2[Y]
'Cream'	2*x*	87.1 ± 1.1%	70.3 ± 0.3%	d-e	a-f
'Denholm White'	2*x*	68.3 ± 1.0%	72.6 ± 1%		a-f
GDGHOP-10	2*x*	N/A	0.0 ± 0%		8
GDGHOP-36	2*x*	N/A	1.3 ± 0.7%	n-o	5-8
GDOP-31	2*x*	N/A	0.0 ± 0%		8
'Landmark Flame'[Z]	2*x*	N/A	47.5 ± 1.8%		d-n
'Landmark White'	2*x*	N/A	45 ± 4.6%		e-o
LAOP-30	2*x*	54.4 ± 12.3%	70.2 ± 14.5%	e-f	a-f
LAOP-9	2*x*	88.7 ± 1.1%	69.3 ± 17.3%		a-g
'Lola'	2*x*	85.6 ± 1.8%	76.7 ± 3.3%		a-e
'Lucky Pot of Gold'	2*x*	N/A	87.6 ± 2%		a-c
Myst-107	2*x*	N/A	1.4 ± 0.8%		5-8
PKGHOP-1	2*x*	N/A	2.4 ± 1.5%	l-n	5-8
'Samantha'	2*x*	N/A	5.2 ± 2.9%	i-h	1-8
624-1	3*x*	N/A	17.2 ± 2.2%	i	q-1
712b-7	3*x*	N/A	10.1 ± 1.8%	k-l	t-5
713-1	3*x*	N/A	4.7 ± 0.5%		y-8
'Athens Rose'	3*x*	20.8 ± 3.1%	20.3 ± 3.5%	i-h	o-y
GDOP-4	3*x*	N/A	5.1 ± 0.9%	o	y-8
'Landmark Peach Sunrise'[Z]	3*x*	21.8 ± 2.8%	32.3 ± 0.9%		h-r
'Landmark Pink Dawn'	3*x*	8.9 ± 3.2%	4.0 ± 0.6%		z-8
'Lemon Drop'	3*x*	5.7 ± 0.9%	1.7 ± 0.6%	c-d	5-8
'Lucky Red Hot'[X]	3*x*	19.4 ± 0.9%	9.3 ± 3%	a	v-5
'Luscious Lemonade'	3*x*	N/A	4.2 ± 3%	a-b	3-8
'Miss Huff'	3*x*	2.0 ± 0.2%	1.9 ± 0.8%		4-8
'New Gold'	3*x*	0.8 ± 0.1%	2.7 ± 2%		5-8
'New Red Lantana'	3*x*	5.6 ± 0.8%	7.0 ± 1.7%		w-7

Table A-1 Continued.

Line	Ploidy	Season 1	Season 2	Season 1[Y]	Season 2[Y]
‘Patriot Fire Wagon’	3*x*	19.3 ± 1.0%	14.7 ± 2.5%		r-3
PCOP-12	3*x*	N/A	10.7 ± 0%		s-5
PCOP-2	3*x*	N/A	10.7 ± 1.8%		s-5
‘Red Butler’	3*x*	7.5 ± 1.8%	4 ± 1.7%		z-8
‘Red Spread Lantana’	3*x*	6.2 ± 0.7%	5.7 ± 0.3%		x-7
‘Samson Lantana’	3*x*	6.4 ± 0.4%	5.2 ± 0.7%		x-8
‘Silver Mound’	3*x*	N/A	0.5 ± 0.5%	n-o	6-8
‘Sunset Lantana’	3*x*	5.2 ± 0.7%	3.1 ± 1.2%		3-8
604-1	4*x*	N/A	49.7 ± 4.4%		c-m
604-5	4*x*	N/A	9.7 ± 1.3%		u-5
‘Anne Marie’	4*x*	N/A	43.2 ± 2.4%	d-e	e-o
‘Bandana Cherry Sunrise’	4*x*	N/A	35.1 ± 6%	a	g-q
‘Bandana Red’	4*x*	N/A	24.3 ± 2.2%	l-m	l-w
CAOP-88	4*x*	N/A	0.4 ± 0.4%	a	6-8
‘Carlos’	4*x*	54.5 ± 1.6%	44.2 ± 2.4%	f-h	e-o
‘Confetti’	4*x*	N/A	47.6 ± 3.1%		d-m
‘Dallas Red’	4*x*	34.5 ± 2.3%	29 ± 1%		j-u
DROP-25	4*x*	N/A	30.2 ± 2%	i-j	h-t
‘Gold’	4*x*	31.0 ± 9.6%	21.4 ± 8.2%		o-y
‘Irene’	4*x*	55.1 ± 1.2%	59.7 ± 3.2%	i-j	a-i
‘Landmark Gold’	4*x*	N/A	53.5 ± 4.7%		b-k
‘Lucky Peach’	4*x*	N/A	40 ± 6.3%		f-q
‘Patriot Cherry’	4*x*	N/A	16.3 ± 2.4%	l	q-2
‘Patriot Desert Sunset’	4*x*	N/A	60.2 ± 1.8%	l-m	a-h
‘Patriot Parasol’	4*x*	N/A	43.3 ± 4%		e-o
‘Patriot Passion’	4*x*	N/A	43.3 ± 2.2%		e-o
‘Patriot Rainbow’	4*x*	N/A	31.2 ± 3.2%	l	h-s
PCOP-6	4*x*	N/A	35.8 ± 2.1%	j-k	g-q
‘Pink Caprice’	4*x*	75.9 ± 2.3%	71.1 ± 1.9%	g-i	a-f
‘Radiation’	4*x*	40.7 ± 11.2%	23.9 ± 2.7%	c-d	l-w

Table A-1 Continued.

Line	Ploidy	Season 1	Season 2	Season 1[Y]	Season 2[Y]
629-1	5*x*	33.0 ± 3.1%	29.5 ± 3.8%		i-u
629-2	5*x*	52.9 ± 3%	43.7 ± 5.6%	k-l	e-o
'Cajun Pink'	5*x*	41.2 ± 2.5%	23.4 ± 2%		m-w
'Patriot Hallelujah'	5*x*	41.3 ± 4.3%	42.5 ± 3.1%		e-p
'Sonshine Lantana'	5*x*	13.6 ± 2%	21.7 ± 5%		n-x
'Spreading Sunset'	5*x*	N/A	25.4 ± 5.5%	m-o	k-v
620-1	6*x*	N/A	34.3 ± 1.8%		r-4
620-10*	6*x*	9.9 ± 0.9%	14 ± 4.1%		g-q
621-4	6*x*	24.5 ± 2.4%	17.9 ± 2.7%		p-z
'Tangerine'	6*x*	24.5 ± 5.2%	17.3 ± 3%		q-1
'Pale Blue'	*3x*	N/A	0.9 ± 0.9%	f	6-8
L. montevidensis (Lavender)	*3x*	1.2 ± 0%	0.2 ± 0.2%	f-g	7-8
L. montevidensis (White)	*3x*	2.4 ± 0.3%	0.6 ± 0.6%	b-c	6-8
L. canescens	N/A	N/A	87.9 ± 0.4%	l-m	a-b
L. involucrata	N/A	N/A	52 ± 3%		b-l
L. depressa var. *depressa*	N/A	N/A	58.6 ± 2.2%		a-j
L. depressa var. *floridana*	N/A	N/A	84.8 ± 2.3%	i-j	a-d
L. depressa	N/A	N/A	95.1 ± 3.1%	e-f	a
L. depressa (ft meyers)	3*x*	N/A	7.3 ± 2.2%		w-6
L. depressa (zugar)	3*x*	N/A	3.3 ± 1.4%		2-8

[Z]Removed improved from the cultivar name.
[Y]Letters A-Z followed by 1-8 indicates statistical groupings by Tukey's W procedure.
*Indicates significant difference between season 1 and 2.

APPENDIX B
BRANCH CUTTINGS FROM BALM, FLORIDA TRIAL

Table B-1. Quantitative data for potential analysis of ornamental characteristics of *L. camara* for evaluation and selection. Data was taken from 30 cm branch cuttings on 17 November 2009.

Sample	Flower clusters per node	St.er.	Nodes per dry weight index	St.er.	Flowers per dry weight index	St.er.	Lateral branches per dry weight	St.er.
T1	1.46	0.12	3.93	0.30	5.53	0.23	0.91	0.07
T2	1.35	0.05	4.56	0.18	6.00	0.02	1.19	0.05
T3	1.48	0.27	4.04	0.64	5.59	0.32	1.04	0.12
T4	0.93	0.17	4.89	0.77	4.27	0.28	0.97	0.12
T5	1.38	0.10	4.35	0.28	5.84	0.27	0.98	0.04
T6	1.32	0.14	5.87	0.31	7.34	0.60	1.12	0.15
T7	1.41	0.24	5.43	0.70	7.15	0.72	1.21	0.16
T8	1.73	0.39	4.72	1.13	7.12	0.37	1.26	0.09
T9	1.49	0.31	3.98	0.96	5.27	0.20	0.89	0.07
T10	1.54	0.26	4.54	0.74	6.32	0.33	0.77	0.06
‘New Gold’	1.08	0.06	10.86	0.50	11.49	0.21	2.70	0.18
‘Pink Caprice’	1.18	0.14	4.96	1.15	5.21	0.35	0.74	0.11
Lantana depressa var. *depressa*	0.83	0.05	15.04	0.50	12.32	0.88	2.05	0.32

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BIOGRAPHICAL SKETCH

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