Evaluation of Interspecific Hybrids between *Lagerstroemia indica* and *L. speciosa*

Cecil Pounders^{1,3}, Tim Rinehart², and Hamidou Sakhanokho²

Southern Horticultural Laboratory, Agricultural Research Service, United States Department of Agriculture, P.O. Box 287, 810 Hwy 26 W, Poplarville, MS 39470

Additional index words. sterility, SSR markers, ‘Princess’ crape myrtle, ‘Monia’ crape myrtle, pollen fertility

Abstract. Production of viable interspecific seedlings from a cross between *Lagerstroemia indica* L. ‘Tonto’ × *L. speciosa* (L.) Pers. was confirmed by comparison of morphological traits and genetic markers. Traits such as plant height and width showed marked variation within the seedling population whereas variation in other traits such as flower size and color was very limited. Seedlings were found to be functionally sterile as either male or female parents. Observed sterility prevents the maximum introgression of important complex traits such as cold hardiness by sib mating or backcrossing into clones derived from this parental combination. ‘Princess’ was confirmed to be a sterile hybrid of *L. indica* and *L. speciosa* whereas ‘Monia’ was indicated to have *L. indica* in its ancestry but not *L. speciosa*.

Most of the 56 (Furtado and Srisuko, 1969) to 80 (Cabrera, 2004) species within the genus *Lagerstroemia* are tropical plants with little cold hardiness. They generally have small white to lavender flowers with little ornamental appeal and are used commercially, primarily for lumber. Only *L. indica*, *L. fauriei* Koehne, *L. subcostata* Koehne, and *L. limii* Merr. (*L. chekiangensis* Cheng) have sufficient cold hardiness to be grown in temperate regions. The two most popular ornamental species are *L. indica*, the Indian crape myrtle, and *L. speciosa*, the Queen’s crape myrtle. The ornamental appeal of the two species is somewhat different, with *L. speciosa* being more robust in all traits but displaying a more limited range of flower colors (lavender, pink, white) and growth habits than *L. indica*. In the United States, crape myrtles (*L. indica* and *L. indica* × *L. fauriei* hybrids) are widely planted in the South as small flowering trees. The use of *L. speciosa*, which forms a large tree, is limited to southern Florida, coastal California, and Hawaii.

In 1963, a chance interspecific seedling (‘Basham’s Party Pink’) between *L. fauriei* and *L. indica* was discovered in Conroe, TX (Egolf and Andrick, 1978). This discovery, in combination with research at the U.S.

National Arboretum indicating *L. fauriei* was resistant to powdery mildew, served as the impetus for a *Lagerstroemia* interspecific breeding program. This work resulted in the release of more than 20 interspecific hybrid cultivars (Egolf, 1981a, 1981b, 1986a, 1986b, 1987, 1990; Pooler, 2006a; Pooler and Dix, 1999) that successfully combined the powdery mildew resistance of *L. fauriei* with other desirable horticultural traits from *L. indica*. A high degree of fertility was apparently maintained throughout initial crosses between the two species and various combinations of progeny. Other interspecific crosses incorporated into the *L. indica* × *L. fauriei* breeding program include *L. subcostata* (‘Yuma’) (Egolf, 1987) and *L. limii* (‘Arapaho’, ‘Cheyenne’) (Pooler, 2006a). Several of the U.S. National Arboretum interspecific releases have also demonstrated increased resistance to *Cercospora* leaf spot (Hagan et al., 1998), flea beetles (*Altica* spp.), and Japanese beetle (*Popillia japonica* Newman) (Pettis et al., 2004).

A combination of complementary traits from *L. indica* and *L. speciosa* would appear to have great potential for expanding genetic diversity, for improving pest resistance, and for introducing novel ornamental traits in commercial cultivars. A review of the list of recognized *Lagerstroemia* cultivars (Dix, 1999) indicates a long history of crosses between the two species. There are, however, less than 10 listed cultivars that resulted from hybridization of *L. indica* and *L. speciosa*, and only two of these, ‘Maiden Blush’ and ‘Monia’, were hybridized in the United States (Dix, 1999). Review of the patent for ‘Maiden Blush’ (Spring, 1965) indicates the cultivar is a hybrid between *L. indica* and *L. reginae* Roxb. rather than with *L. speciosa*. ‘Princess’, a cultivar of unknown origin

marketed for more than 40 years by Florida nurseries, appears to be a sterile interspecific cross with *L. speciosa*.

At least three epithets have been used to designate crosses between *L. indica* and *L. speciosa*. First, *Lagerstroemia* × *matthewsii* Searl is indicated to refer to crape myrtles hybridized by C. Matthews in Queensland, Australia, in the 1880s and offered by Searl & Sons, Sydney, Australia, circa 1905 (Egolf and Andrick, 1978). Second, *Lagerstroemia* × *eavesii* Eaves (Ali, 1977) refers to a hybrid (*L. matthewsii* × *L. indica*) produced by S.H. Eaves, Brisbane, Australia, and sold by F. Ferguson & Son, Hurstville, NSW, Australia, in 1913 and later hybrids sold by Hazlewood Bros., Epping, NSW, Australia, in the 1960s (Dix, 1999). Finally, *Lagerstroemia* × *lancesteri* Hort is reported to be a hybrid between *L. indica* ‘Candida’ and *L. matthewsii* (Percy-Lancaster, 1921) marketed by Hobbie & Co., Calcutta, India, in the 1940s (Dix, 1999). None of the three epithets has been found to be validly published and are illegitimate taxonomically.

Interspecific hybrids between *L. indica* and *L. speciosa* have never been studied in detail. More information on the effects of various mating combinations on fertility and the interchange of traits including pest resistance, cold hardiness, and various ornamental attributes is necessary to optimize *Lagerstroemia* breeding programs. The first objective of this study was to determine fertility and initial variation in progeny for traits such as plant height, plant width, leaf size, and flower size from controlled crosses between ‘Tonto’ crape myrtle, descended from both *L. indica* and *L. fauriei*, and *L. speciosa*. The second objective was to test simple sequence repeat (SSR) markers to verify the interspecific hybrid status of progeny from the controlled mating and to evaluate the parentage of two commercial cultivars reported to have originated from crosses between *L. indica* and *L. speciosa*.

Materials and Methods

Plant materials. The following plant material was used in this study: *L. speciosa*, ‘Tonto’, ‘Arapaho’, ‘Monia’, ‘Princess’, ‘Rosa Nova’, and ‘Whit IV’. ‘Tonto’ is a complex hybrid between *L. indica* and *L. fauriei* (Egolf, 1990), whereas ‘Arapaho’ is a hybrid with *L. indica*, *L. fauriei*, and *L. limii* in its parentage (Pooler, 2006a). ‘Monia’ and ‘Princess’ are reported to have originated from crosses between *L. indica* and *L. speciosa* (Dix, 1999), whereas ‘Rosa Nova’ and ‘Whit IV’ are cultivars of *L. indica*.

Pollen of *L. speciosa* used in 2004 to generate the interspecific seedlings evaluated during this study was collected from lavender-flowered accessions 78500B and 2000378A at Montgomery Botanical Center, Miami, FL. Foliage and flower samples of *L. speciosa* for morphological comparison as well as pollen used in fertility evaluations in 2006 were collected from two lavender-flowered specimens at The Liner Farm, St.

Received for publication 12 Feb. 2007. Accepted for publication 16 Mar. 2007.

Mention of trade names of commercial products in the publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

¹Research Geneticist.

²Research Molecular Geneticist.

³To whom reprint requests should be addressed; e-mail cpounders@msa-stoneville.ars.usda.gov

Fig. 1. Flower of 'Tonto' $\times$ *Lagerstroemia speciosa* seedling showing (A) antepetalous stamen, (B) antepetalous stamens, and (C) petal ruffle typical of *L. indica*.

Table 1. Comparison of eight morphological traits for 'Tonto', *Lagerstroemia speciosa*, 'Tonto' $\times$ *L. speciosa* seedlings (T $\times$ S), 'Princess', and 'Monia'.

	Monia	Tonto	T $\times$ S	<i>Lagerstroemia speciosa</i>	Princess
Leaf width (cm) ^a	3.5 d	3.0 e	5.7 b	7.8 a	5.0 c
Leaf length (cm)	4.5 c	4.3 c	10.4 b	14.7 a	10.5 b
Flower diameter (cm)	4.0 d	3.8 e	5.0 c	6.3 a	5.5 b
Petal width (mm)	14 c	10 d	20 b	23 a	20 b
Petal length (mm)	14 d	11 e	21 c	24 a	22 b
Petal crinkle	Yes	Yes	Yes	No	Yes
Dimorphic stamens	Yes	Yes	Yes	No	Yes
Flower color ^b	Red purple 70B	Red purple 58A	Red purple 72B	Violet G 84C	Purple 76B

^aAll measurements reported are the means of 10 random representative specimens.

^bMeans followed by the same symbol within a row are not statistically different based on Duncan's Multiple Range Test (MRT) ($P \leq 0.05$).

^cColor was determined using the Royal Horticultural Society Colour Chart.

T $\times$ S, 'Tonto' $\times$ *L. speciosa* seedlings.

Cloud, FL. Plants of 'Rosa Nova' were obtained from Antonio Grassi Nursery, Pistoia, Italy. 'Monia' plants were propagated from plants obtained from Monrovia Nursery, Azusa, CA. 'Tonto' and 'Whit IV' plants were obtained from Byers Nursery, Huntsville, AL. 'Princess' and 'Arapaho' plants were obtained from The Liner Farm.

Seeds from the 2004 interspecific cross were germinated in 5-cm (220-mL) cells in a greenhouse maintained at a minimum temperature of 18 °C during Mar. 2005. In late May, plants of the clones obtained from the various nurseries and 102 interspecific seedlings were shifted into no. 3 containers (9 L) in a pine bark substrate top-dressed with 6.6 kg·m⁻³ 19N-2.1P-7.4K Osmocote Pro

fertilizer (Scotts-Sierra Horticultural Products Co., Maryville, OH). Plants were grown in full sun with supplemental irrigation until late October, when the interspecific seedlings and plants of 'Princess' were moved to a greenhouse maintained at a minimum temperature of 2 °C. Named cultivars were exposed to ambient winter conditions. In Mar. 2006, all plants were consolidated back on the container pad, top-dressed again with fertilizer, and grown as in 2005.

Pollinations. Pollen was collected in late June in early morning before anthesis. Anthers were extracted onto white paper and pollen was allowed to dehisce at room temperature (21 °C), then it was stored in vials at -5 °C. One day before beginning

pollinations, seed pods and open flowers were removed from panicles of plants to be used as female parents. These plants were kept in mesh-covered cages throughout pollinations to exclude pollinators. The following morning before anther dehiscence, all newly opened flowers on cultivars in isolation cages were emasculated and flower petals removed. Pollen was applied to stigmas using a fine-tip brush. When pollinations for a study were completed, all remaining flower buds were removed. Plants were left in isolation cages for an additional week until pod development was evident or flowers had senesced. The number of seed pods set for a particular cross was counted after 3 weeks, divided by the number of attempted pollinations, then multiplied by 100 to determine the percentage of pod set.

Pollen staining. Five flowering 'Tonto' $\times$ *L. speciosa* seedlings were randomly selected to study pollen viability. Three flowers were collected from the seedlings and from plants of 'Tonto', 'Princess', and 'Monia'. Flowers of the seedlings and three cultivars had dimorphic stamens (antepetalous and antepetalous) (Kim et al., 1994) that were extracted separately for staining. Freshly dehiscent pollen was placed on a microscope slide in a drop of 1% acetocarmine stain and covered with a coverslip. The slide was heated for 15 s over steam. Specimens were examined using a light microscope, and pollen was scored as stained (viable) or unstained (nonviable). Three fields of 100 grains each were counted, and the percentage of stainable pollen was calculated for each flower and stamen type. Frozen samples of pollen from the monomorphic stamens of *L. speciosa* were also evaluated using this procedure.

Simple sequence repeat development and sample processing. Enriched libraries were created by Genetic Information Services (Chatsworth, CA) with the microsatellite motifs GA, AAG, ATG, and CAG using 'Tonto' and 'Whit IV' genomic DNA. Sequencing 1152 clones produced 1765 high-quality sequences that assembled into 250 contigs and 445 singletons. Automated analysis identified 502 sequences containing microsatellite regions where primers could be designed. Some sequences contained multiple microsatellites for a total of 684 potential SSR loci.

DNA was extracted from 1-cm² pieces of fresh leaf tissue using the Qiagen Plant Mini Kit (Qiagen, Valencia, CA) and quantified using a NanoDrop Spectrophotometer (Nanodrop Technologies, Wilmington, DE). Simple sequence repeat amplification was performed using a modified 3-primer protocol (Rinehart et al., 2006). Fluorescence-labeled polymerase chain reaction (PCR) fragments were visualized by automated capillary gel electrophoresis on an ABI3100-Avant using ROX-500 size standard (Applied Biosystems, Foster City, CA). GeneMapper version 3.7 was used to recognize and size peaks (ABI, Foster City, CA).

Data analysis. Initial testing of 103 primer pairs, using a modified M13-tailed method, against 'Tonto' and *L. speciosa* resulted in 43 polymorphic loci. Of these, 15 trinucleotide SSRs were used to verify hybrids. Data were compiled for the seven taxa (*L. speciosa*, 'Tonto', 'Princess', 'Monia', and three 'Tonto' × *L. speciosa* seedlings) and analyzed for shared allele frequencies. All alleles were represented as diploid. Populations version 1.2.28 was used for phenetic analyses (Langella, 2002). Genetic distances between individual samples were calculated using allele sharing distance to create a distance matrix (Jin and Chakraborty, 1994; Stephens et al., 1992). Principle coordinates analysis (PCoA) plots were based on the allele sharing distance matrix, which included missing data as null alleles. Principle coordinates analysis was performed using NTSys software (version

1.70; Exeter Software, Setauket, NY). Computation of means, ses, coefficients of variation, analysis of variation, and mean separations were performed with SAS 9.1 (SAS, Cary, NC).

Results and Discussion

Initial pollinations between 'Tonto' and *L. speciosa* generated 102 seedlings. Plant growth of the seedlings was vigorous, with a mean height of 124 cm and width of 88.2 cm after 2 years growth in no. 3 nursery containers. Coefficients of variation for the two traits were 35.2% and 27.4% respectively. During the first summer, 10% of the seedlings flowered, with flowering increasing to 39% during the second summer. Under similar conditions, seedlings grown from the *L. speciosa* parents as breeding stock have not flowered in 5 years. All flowering seedlings

had deep-purple flowers (Red Purple 72B by Royal Horticultural Colour Chart, 2001. Royal Horticultural Society, London). Flowers had the petal ruffle and dimorphic stamens characteristic of 'Tonto' and *L. indica* (Fig. 1). Leaf length, leaf width, flower diameter, petal width, and petal length were all intermediate between the parents (Table 1) (Fig. 2). Characterization of the same traits for 'Princess' and 'Monia' found 'Princess' was most similar to the interspecific seedling population, with the exception of having lavender-pink (Purple 76B) flowers. 'Monia' morphological traits were intermediate between 'Tonto' and the seedling population.

Three random F₁ plants were chosen for genetic characterization using SSR markers (Table 2). Allele size sharing between progeny and parents confirms that plants are interspecific hybrids between 'Tonto' and *L. speciosa* (Table 3). With the exception of CRAPE165_166 locus in hybrid #3, all loci are represented by one maternal and one paternal allele. The lack of maternal, or 'Tonto', allele for this sample is likely the result of failure during PCR amplification. Average genetic similarity between the three interspecific hybrids was ≈90%, whereas 'Tonto' and *L. speciosa* are only ≈10% similar. 'Princess' and 'Monia' were also tested with the same SSR markers (Table 2). Thirteen of the 15 loci for 'Princess' contain one allele size corresponding to *L. speciosa*, suggesting it is an interspecific hybrid between *L. speciosa* and *L. indica*. In contrast, 'Monia' is missing data for one locus, but none of the other 14 loci contain allele sizes observed in the *L. speciosa* sample. This result casts doubt on *L. speciosa* being involved in the parentage of 'Monia'. The PCoA plot shows 'Monia' and 'Tonto' separate from all other samples, and 'Princess' and the three interspecific hybrids intermingled and intermediate to *L. speciosa* and *L. indica* samples (Fig. 3). 'Tonto' and 'Princess' share 62% genetic similarity, which is comparable to the similarity between 'Tonto' and 'Monia' at 68%. However, the average genetic similarity between 'Princess' and the F₁ hybrids is 86% whereas

Fig. 2. Visual comparison of leaf and petal size between *Lagerstroemia speciosa*, 'Princess', a 'Tonto' × *L. speciosa* seedling, 'Monia', and 'Tonto'.

Table 2. Simple sequence repeat loci used to verify interspecific hybrid status.

Locus	GenBank accession no.	Repeat motif	Left primer	Right primer
CRAPE9_10	EF143815	(CAG)8	AGCTTCACAGTTTGATCAGTCCCT	CTTCAGGAGTAAATAAGAGGCGCA
CRAPE17_18	EF143816	(GAA)5	GAGAAGAAGATCTCCAAGGACGG	CTTCCTTGCTCGAGATACCAATGT
CRAPE21_22	EF143817	(TCT)6	CACATCCACAAAGCTGTCGTAGTC	CTTCGAGAAGGTCTTCATGATGCT
CRAPE37_38	EF143818	(GAA)12	CTCCATTTCAAAACTCTCCCTCT	CTTCTGCTTGAGAGGAATCTCTG
CRAPE71_72	EF143819	(GAT)7	GAGAACTCGAATGGGTGTTGTCT	TGGGAAAATGGAGAACAAAGATA
CRAPE89_90	EF143820	(CTG)4	GAATAGGATGATTCTCCGCTTCT	AAAGCAGAGAGGCTGAAATTAACG
CRAPE99_100	EF143821	(CAG)7	CGCACGGATCTAAGAAAAGAAGAA	TTATAGAAGCAAACCTCTGCAGCC
CRAPE105_106	EF143822	(GCA)8	CACATCCACTGGAATTGAAACAGA	CTTCATTGCCAGGAAGAACATGAG
CRAPE113_114	EF143823	(TGC)6	GACCTCAACTCGGAAGAAGACGTT	ATTGGGATTCTGAGTACATACGG
CRAPE117_118	EF143824	(CAG)6	GGAGTAATGACTACTTCAGCCCGA	ATTGTCAGAGTACCCATCTGGGAG
CRAPE119_120	EF143825	(CAG)7	GGAGTAATGACTACTTCAGCCCGA	TAGAGTACCCATCTGGGAGACGAA
CRAPE143_144	EF143826	(GAA)7	TGTGTTGTGTTGTGCTCTTTGAGA	GATGGACTAATGGCTGTCCCTAAA
CRAPE165_166	EF143827	(AGA)8	TCCATCAGAACATCAAGATTCTCT	ATGCAAGCTTACCACAACAGGTA
CRAPE191_192	EF143828	(TCT)8	TAGGGTTGGAGTGGAACAGAAAG	AGAGATCAGATGAGGAGGAGGAG
CRAPE205_206	EF143829	(AGA)16	TTCGTATTTAACTGCCATTGACGA	CGTGATAAGGACCGACTAGCCAT

Complete DNA sequence information is available from GenBank (www.ncbi.nlm.nih.gov) under the accession numbers listed. Repeat motif and number of repeat units are listed next to primer sequences. Amplified products ranged from 84 to 157-bp long depending on the locus.

Table 3. Allele size variation observed for interspecific hybrids.

Locus	Actual diploid allele sizes in base pairs (allele:allele)						
	<i>Lagerstroemia speciosa</i>	Tonto	Monia	Princess	Hybrid 1	Hybrid 2	Hybrid 3
CRAPE9_10	148:148	160:166	145:166	148:160	148:160	148:166	148:160
CRAPE17_18	159:159	156:156	156:156	156:159	156:159	156:159	156:159
CRAPE21_22	140:140	143:143	143:143	140:143	140:143	140:143	140:143
CRAPE37_38	132:132	135:157	154:154	135:147	132:135	132:135	132:157
CRAPE71_72	147:147	149:149	149:149	147:149	147:149	147:149	147:149
CRAPE89_90	171:171	168:168	168:168	168:171	168:171	168:171	168:171
CRAPE99_100	138:138	144:144	148:148	138:148	138:144	138:144	138:144
CRAPE105_106	124:124	122:143	137:143	124:143	122:124	122:124	124:143
CRAPE113_114	168:168	180:180	180:180	168:171	168:180	168:180	168:180
CRAPE117_118	168:168	162:165	162:162	162:168	162:168	162:168	165:168
CRAPE119_120	162:162	157:160	157:157	157:162	157:162	157:162	160:162
CRAPE143_144	121:121	128:128	134:134	121:131	121:128	121:128	121:128
CRAPE165_166	110:110	107:107	000:000 ^a	110:115	107:110	107:110	110:110
CRAPE191_192	151:151	156:166	166:169	151:166	151:166	151:166	151:156
CRAPE205_206	156:156	159:191	149:152	145:159	156:191	156:159	156:159

^aDenotes missing data.

Allele sizes reflect the addition of 23 nucleotides that are incorporated during the 3-primer polymerase chain reaction protocol. All samples were assumed to be diploid. As expected from trinucleotide repeats, allele sizes for each locus varied by multiples of 3 bp.

Fig. 3. Principal coordinates analysis of all taxa used to derive a genetic distance matrix. Each axis represents a key variable that explains variation within the data, simplifying the data set to three dimensions but describing as much variation as possible. Samples that cluster together are most likely to share ancestry. Principal coordinates C1, C2, and C3 represent 19%, 73%, and 8% of the variation respectively.

the average genetic similarity between 'Monia' and the F₁ hybrids is 57%.

Preliminary evaluation of morphological traits and genetic markers indicates there is ample opportunity to select superior cultivars from the interspecific population. Variation in height and width among the interspecific seedlings was more than twice that reported for a population of open-pollinated *L. speciosa* seedlings generated from a single mother tree (Jamaludheen et al., 1995). Another trait that appeared to be highly variable in the seedlings was panicle size, which varied in length and floral density. The trait was not measured because development was hampered by plant size and cultural conditions. Variation within the seedlings for other traits of ornamental interest, such as cold hardiness and pest resistance, can also

be determined as the population ages and is exposed to more diverse environmental conditions. Flower size and color were both very uniform among the seedlings, offering little or no prospect for selection.

No open-pollinated seed pods were observed on the interspecific seedlings or 'Princess', whereas many pods were present on 'Tonto' and 'Monia' plants grown in the same environment. Examination of pollen after staining with 1% acetocarmine found no fertile pollen in the samples collected from the 'Tonto' × *L. speciosa* seedlings and only 0.2% stainable pollen from 'Princess' (Table 4). 'Tonto' had ≈28% stainable pollen. Stainable pollen for 'Monia' and *L. speciosa* was 69.8% and 97.8% respectively. A similar study in India found 82.2% stainable pollen for *L. indica* but only 6.1% for the interspe-

cific clone 'Lancasteri' [*L. indica* 'Candida' × (*L. indica* × *L. speciosa*)] (Datta and Jena, 1977).

Evaluation of fertility using five randomly selected 'Tonto' × *L. speciosa* seedlings or 'Princess' as female parents and 'Arapaho' or *L. speciosa* as male parents resulted in no pod set (Table 5), whereas remake of the 'Tonto' × *L. speciosa* cross resulted in a 13.5% pod set. Proficient pod set was also recorded when *L. speciosa* was crossed with 'Monia' (47.1%), 'Arapaho' (34.3%), and 'Rosa Nova' (30.8%).

A loss of fertility is often associated with crosses between different species (Poehlman, 1987). Taxonomically, *L. indica* is grouped in section *Sibia*, subsection *Sibia*, whereas *L. speciosa* is in section *Adambea*, subsection *Adambea* (Furtado and Srisuko, 1969). Fertile progeny have resulted from interspecific crosses of *L. indica* with *L. fauriei* (Egolf, 1981a), *L. subcostata* (Egolf, 1987) and *L. limii* (Pooler, 2006a), all of which are in section *Adambea*, subsection *Microcarpidium*. All 'Tonto' × *L. speciosa* progeny that have flowered are sterile, as is 'Princess', which we confirmed to be a hybrid between *L. indica* × *L. speciosa*. Sterility may be an asset if superior clones can be selected from the F₁ population, because it would prevent the possibility of the plants becoming invasive (Pooler, 2006b). However, sterility in the F₁ generation prevents the maximum introgression of important complex traits such as cold hardiness, which will severely limit the range of adaptation of selected clones.

The most apparent difference between the two groups of species within section *Adambea* that have been evaluated in interspecific crosses with *L. indica* is the presence of monomorphic stamens in *L. speciosa* and dimorphic stamens in *L. fauriei*, *L. subcostata*, and *L. limii*. Differences in stamen arrangement and pollen types within *Lagerstroemia* have been extensively studied (Kim et al., 1994). Species were categorized into six groups based on pollen size, shape, pseudocolpi, sculpture, and margo. Of the 42 species evaluated, three species including

Table 4. Percent stainable pollen in nine *Lagerstroemia* accessions.

Cultivar/hybrid	Pollen type	Stained pollen ^z (%)
<i>Lagerstroemia speciosa</i>	Monomorphic	97.0 ± 2.0
Monia	Antepetalous	69.8 ± 4.5
Monia	Antesepalous	91.2 ± 1.9
Tonto	Antepetalous	29.2 ± 2.1
Tonto	Antesepalous	27.3 ± 2.2
Princess	Antepetalous	0.2 ± 0.1
Princess	Antesepalous	0.2 ± 0.1
Tonto × <i>L. speciosa</i> #1	Antepetalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #1	Antesepalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #2	Antepetalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #2	Antesepalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #3	Antepetalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #3	Antesepalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #4	Antepetalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #4	Antesepalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #5	Antepetalous	0.0 ± 0.0
Tonto × <i>L. speciosa</i> #5	Antesepalous	0.0 ± 0.0

^zMean ± SE.Table 5. Comparison of pod set for five crape myrtle cultivars and F₁ interspecific seedlings.

Female	Male	Crosses (no.)	Pod set ^z (%)
Monia	<i>L. speciosa</i>	184	47.1 ± 13.9
Arapaho	<i>L. speciosa</i>	267	34.3 ± 6.7
Rosa Nova	<i>L. speciosa</i>	243	30.8 ± 5.0
Tonto	<i>L. speciosa</i>	244	13.5 ± 3.6
Princess	<i>L. speciosa</i>	64	0 ± 0.0
Tonto × <i>Lagerstroemia speciosa</i> #6	<i>L. speciosa</i>	45	0 ± 0.0
Tonto × <i>L. speciosa</i> #7	<i>L. speciosa</i>	47	0 ± 0.0
Tonto × <i>L. speciosa</i> #8	<i>L. speciosa</i>	35	0 ± 0.0
Tonto × <i>L. speciosa</i> #9	<i>L. speciosa</i>	29	0 ± 0.0
Tonto × <i>L. speciosa</i> #10	<i>L. speciosa</i>	23	0 ± 0.0
Princess	Arapaho	82	0 ± 0.0
Tonto × <i>L. speciosa</i> #6	Arapaho	60	0 ± 0.0
Tonto × <i>L. speciosa</i> #7	Arapaho	45	0 ± 0.0
Tonto × <i>L. speciosa</i> #8	Arapaho	47	0 ± 0.0
Tonto × <i>L. speciosa</i> #9	Arapaho	55	0 ± 0.0
Tonto × <i>L. speciosa</i> #10	Arapaho	49	0 ± 0.0

^zMean ± SE.

L. speciosa were found to have unique pollen and were not assigned to any of the five major groups, but were lumped into a sixth miscellaneous group. Group III included *L. subcostata*, *L. fauriei*, and *L. limii*, whereas *L. indica* was classified in group IV. *Lagerstroemia fauriei* pollen was indicated to have the greatest structural similarity with *L. indica* of the species in group III. The six pollen groups may be a better indicator of interspecific combinations most likely to retain fertility than the currently accepted sectional groupings.

Chromosome counts reported for *Lagerstroemia* species are often confusing. This is partly the result of small chromosome size and dysploidy within genera of the Lythraceae (Graham and Cavalcanti, 2001). Bowden (1945) reports 2n = 50 for both *L. indica* and *L. speciosa*, while Guha (1972) reports 2n = 50 for *L. indica* and 2n = 48 for *L. speciosa*. Meiosis was examined in pollen mother cells of 'Lancasteri', a clone with seed sterility and limited functional pollen that is reported to have resulted from a cross of *L. indica* 'Candida' with *L. indica* × *L. speciosa* (Ali, 1977; Datta and Jena, 1977). A high frequency of univalents and bivalents with abnormal meiotic behavior was

observed, indicating the two genomes involved in the parentage of the hybrid are cytogenetically well differentiated.

'Monia' is reported to be a hybrid between *L. indica* × *L. speciosa* made by Otto Spring, Okmulgee, OK (Dix, 1999). We have determined this parentage to be incorrect using SSR markers. Although it is possible that repeated backcrossing might bias the allele size variation such that our 15 markers were insufficient to detect the *L. speciosa* genetic background, it is unlikely given the lack of fertility we documented in the 'Tonto' × *L. speciosa* F₁ progeny and evidence that 'Monia' is both male and female fertile (Tables 4 and 5). Review of the patent for 'Maiden Blush' (Spring, 1965), another tropical hybrid crape myrtle produced by Spring (1965) and reported as an *L. speciosa* hybrid by Dix (1999), indicates the cultivar originated from a cross between *L. indica* and *L. reginae* (section *Adambea*, subsection *Adambea*) rather than *L. speciosa*. Our results indicate 'Monia' is probably an interspecific hybrid between *L. indica* and another species, but not *L. speciosa*.

In summary, seedling populations can be easily generated by crossing *L. indica* clones

with *L. speciosa*. Data recorded from the 'Tonto' × *L. speciosa* progeny display high variation for traits such as plant growth and form, indicating superior cultivars can be selected for such traits from the initial cross. The high level of sterility observed in 'Princess' and the interspecific progeny indicates that breeding programs requiring backcrossing or sib mating to improve traits such as flower size and cold hardiness will be difficult, if not impossible, to execute. The possibility of recovering fertile interspecific seedlings from other combinations with *L. speciosa* is being investigated. The SSR markers tested here should be useful tools for verifying interspecific hybrids and assessing genetic diversity among *Lagerstroemia* species.

Literature Cited

- Ali, R. 1977. Chromosome numbers in some species of *Lagerstroemia*. *Curr. Sci.* 46:579–580.
- Bowden, W.M. 1945. A list of chromosome numbers in higher plants I. *Acanthaceae* to *Myrtaceae*. *Amer. J. Bot.* 32:81–92.
- Cabrera, R.I. 2004. Evaluating and promoting the cosmopolitan and multipurpose *Lagerstroemia*. *Acta Hort.* 630:177–184.
- Datta, R.M. and P.K. Jena. 1977. Preliminary meiotic studies in three species of garden *Lagerstroemia* (*L. flos-reginae* Retz., *L. Lancasteri*, a new horticultural variety and *L. indica* Linn. Var *Rose Colour*). *Indian Agriculturist* 21:87–89.
- Dix, R.L. 1999. Cultivars and names of *Lagerstroemia*. U.S. National Arboretum. 2 Nov. 2006. <www.usna.usda.gov/Research/Herbarium/Lagerstroemia/index.html>.
- Egolf, D.R. 1981a. 'Muskogee' and 'Natchez' *Lagerstroemia*. *HortScience* 16:576–577.
- Egolf, D.R. 1981b. 'Tuscarora' *Lagerstroemia*. *HortScience* 16:788–789.
- Egolf, D.R. 1986a. 'Acoma', 'Hopi', 'Pecos', and 'Zuni' *Lagerstroemia*. *HortScience* 21:1250–1252.
- Egolf, D.R. 1986b. 'Tuskegee' *Lagerstroemia*. *HortScience* 21:1078–1080.
- Egolf, D.R. 1987. 'Apalachee', 'Comanche', 'Lipan', 'Osage', 'Sioux', and 'Yuma' *Lagerstroemia*. *HortScience* 22:674–677.
- Egolf, D.R. 1990. 'Caddo' and 'Tonto' *Lagerstroemia*. *HortScience* 25:585–587.
- Egolf, D.R. and A.O. Andrick. 1978. The *Lagerstroemia* handbook/checklist. American Association of Botanical Gardens and Arboreta, Inc., Wilmington, DE.
- Furtado, C.X. and M. Srisuko. 1969. A revision of *Lagerstroemia* L. (Lythraceae). *Garden Bul.* (Singapore) 24:185–334.
- Graham, S.A. and T.B. Cavalcanti. 2001. New chromosome counts in Lythraceae and a review of chromosome numbers in the family. *Syst. Bot.* 26:445–458.
- Guha, S. 1972. Cytotaxonomic studies on the family Lythraceae. *Pro. Indian Academy of Science Congress Assoc.* 59:344–345.
- Hagan, A.K., G.J. Keever, C.H. Gilliam, J.D. Williams, and G. Creech. 1998. Susceptibility of crape myrtle cultivars to powdery mildew and Cercospora leaf spot in Alabama. *J. Environ. Hort.* 16:143–147.
- Jamaludheen, V., K. Gopikumar, and K. Sudhakar. 1995. Variability studies in *Lagerstroemia* (*Lagerstroemia speciosa* Pers.). *Indian Forester* 121:137–142.

- Jin, L. and R. Chakraborty. 1994. Estimation of genetic distance and coefficient of gene diversity from single-probe multilocus DNA fingerprinting data. *Mol. Biol. Evol.* 11: 120–127.
- Kim, S.C., S. Graham, and A. Graham. 1994. Palynology and pollen dimorphism in the genus *Lagerstroemia* (Lythraceae). *Grana* 33:1–20.
- Langella, O. 2002. Populations: A free population genetics software. 29 Feb. 2006. <www.pge.cnrs-gif.fr/bioinfo/populations/index.php?lang=en&doc=populations>
- Percy-Lancaster, S. 1921. *Lagerstroemia*. *Proc. J. Agr. Hort. Soc. India* 20–21.
- Pettis, G.V., D. Boyd, S. Braman, and C. Pounders. 2004. Potential resistance of crape myrtle cultivars to flea beetle (Coleoptera: Chrysomelidae) and Japanese beetle (Coleoptera: Scarabaeidae) damage. *J. Econ. Entomol.* 97:981–992.
- Poehlman, J.M. 1987. *Breeding field crops*. Van Nostrand Reinhold, New York.
- Pooler, M.R. 2006a. ‘Arapaho’ and ‘Cheyenne’ *Lagerstroemia*. *HortScience* 41:855–856.
- Pooler, M.R. 2006b. Crape myrtle: *Lagerstroemia indica*, p. 428–449. In: N.O. Anderson (ed.). *Flower breeding and genetics: Issues, challenges, and opportunities for the 21st century*. Vol. 2. Springer, New York.
- Pooler, M.R. and R.L. Dix. 1999. ‘Chickasaw’, ‘Kiowa’, and ‘Pocomoke’ *Lagerstroemia*. *HortScience* 34:361–363.
- Rinehart, T.A., B.E. Scheffler, and S.M. Reed. 2006. Genetic diversity estimates for the genus *Hydrangea* and development of a molecular key based on SSR. *J. Amer. Soc. Hort Sci.* 131:787–797.
- Spring, O. 1965. Variety of crepe myrtle. U.S. Patent and Trademark Office. PP 2,551.
- Stephens, J.C., D.A. Gilbert, N. Yuhki, and S.J. O’Brien. 1992. Estimation of heterozygosity for single-probe multilocus DNA fingerprints. *Mol. Biol. Evol.* 9:729–743.