

Use of Crape Myrtle, Lagerstroemia (Myrtales: Lythraceae), Cultivars as a Pollen Source by Native and Non-Native Bees (Hymenoptera: Apidae) in Quincy, Florida

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Use of crape myrtle, *Lagerstroemia* (Myrtales: Lythraceae), cultivars as a pollen source by native and non-native bees (Hymenoptera: Apidae) in Quincy, Florida

T. Charles Riddle and Russell F. Mizell, III*

Abstract

Crape myrtle, Lagerstroemia species (Myrtales: Lythraceae), has become a dominant flowering plant in the ecosystems of the southeastern USA. Examination of flower records for bees shows few records of pollinators visiting these species even though they produce dimorphic pollen. Sampling of bees from a multi-cultivar crape myrtle planting at the University of Florida's Institute of Food and Agricultural Sciences, North Florida Research & Education Center in Quincy, Florida, via established transect walks in 2009 and 2010, and intensive net collecting in 2011, indicated that bee species from several functional groups (based on taxonomy, body size, and sociality) visited crape myrtle. Results also indicated that crape myrtle cultivars were used differently by the following major bee species (Hymenoptera: Apidae): the honey bee, Apis mellifera L.; the bumble bees Bombus impatiens Cresson and Bombus fraternus (Smith); and the carpenter bees Xylocopa micans Lepeletier and Xylocopa virginica (L.). Numbers of the native bumble bee species varied significantly between years whereas those of honey bees did not. All bee species displayed a marked preference for specific cultivars through time. Bombus impatiens exhibited a very patchy distribution related to the availability of bahiagrass flowering in the understory; these bees used bahiagrass but quickly returned to crape myrtle when bahiagrass was mowed. This suggests that the relationship between this pollinator and the non-native crape myrtle is a weak interaction and a number of unstudied factors may be affecting it. The presence of artificial colonies of B. impatiens resulted in a patchy distribution of these bees nearest the colonies. In contrast, the presence of a honey bee colony near the plot had no effect on honey bee numbers or distribution within the plot. Crape myrtle appears to provide a pollen source for several native bee species as well as for honey bees. Evidence suggests that certain combinations of crape myrtle cultivars could provide additional spatial and temporal support for a diversity of functional groups of pollinators and may augment pollinator species richness. Moreover, as crape myrtle blooms during summer months when other pollen sources are scarce, it has great potential to alleviate stress on pollinators due to food shortages. This work is congruent with previous research demonstrating that crape myrtle supports a large number of beneficial insects, and it further defines the importance of this non-native plant species in impacting several regulating ecosystem services.

Key Words: native pollinator; Apis mellifera; Bombus; Xylocopa; behavior

Resumen

Las especies del árbol de Júpiter, Lagerstroemia (Myrtales: Lythraceae), se ha convertido en una planta con flores dominante en los ecosistemas del sureste de EE.UU. Una examinación de los registros de las abejas en las flores reveló pocos registros de polinizadores que visitan estas especies a pesar de que producen polen dimorfico. El muestreo de las abejas de una siembra multi-cultivo de árbol de Júpiter en el Instituto de Alimentos y Ciencias Agrícolas de la Universidad de Florida, del Centro de Investigación y Educación del Norte de la Florida en Quincy, Florida, a través de veredas con transectos establecidos en el 2009 y el 2010, y la recolección intensiva usando redes en el 2011, indicaron que las especies de abejas de varios grupos funcionales (basado en la taxonomía, el tamaño del cuerpo, y la sociabilidad) visitaron los árboles de Júpiter. Los resultados también indican que los cultivares de árboles de Júpiter se utilizaron de manera diferente por las siguientes especies principales de abejas (Hymenoptera: Apidae): la abeja de la miel, Apis mellifera L.; los abejorros Bombus impatiens Cresson y Bombus fraternus (Smith); y las abejas carpinteras Xylocopa micans Lepeletier y Xylocopa virginica (L.). Los números de las especies de abejorros nativos variaron significativamente entre los años mientras que los de las abejas no. Todas las especies de abejas muestran una marcada preferencia por los cultivares específicos a través del tiempo. Bombus impatiens mostró una distribución muy desigual en relación con la disponibilidad de las flores de pasto bahía en el sotobosque; estas abejas utilizan pasto bahía pero volvieron rápidamente a los árboles de Júpiter cuando el pasto de bahia fue cortado. Esto sugiere que la relación entre este polinizador y los árboles de Júpiter no nativos es una interacción débil y una serie de factores no estudiados puede estar afectando a la misma. La presencia de colonias artificiales de B. impatiens dio lugar a una distribución irregular de estas abejas cercanas a las colonias. Por el contrario, la presencia de una colonia de abejas cerca de la parcela no tuvo efecto sobre el número de abejas de miel o distribución dentro de la parcela. Los árboles de Júpiter parece proveer una fuente de polen para varias especies de abejas nativas, así como para las abejas de miel. La evidencia sugiere que ciertas combinaciones de cultivares de árboles de Júpiter podrían proveer apoyo espacial y temporal adicional para una diversidad de grupos funcionales de los polinizadores y pueden aumentar la riqueza de especies de polinizadores. Además, como árboles de Júpiter aflorecen durante los meses de verano cuando otras fuentes de polen son escasas, estos tienen un gran potencial para aliviar la presión sobre los polinizadores, debido a la escasez de alimentos. Este trabajo es congruente con investigaciones previas que demuestran que el árbol de Júpiter es compatible con una gran cantidad de insectos beneficiosos, y define además la importancia de esta especie de plantas no nativas en el impacto de varios servicios de los ecosistemas que regulan.

Palabras Clave: polinizadores nativos; Apis mellifera; Bombus; Xylocopa; comportamiento

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Riddle & Mizell: Use of crape myrtle cultivars by bees

"Pollinators are vital to agriculture because most fruit, vegetable, seed crops and other crops that provide fiber, drugs, and fuel are pollinated by animals. Over and above its direct economic value to humans, pollination by animals provides essential maintenance of the structure and function of a wide range of natural communities in North America, and it enhances aesthetic, recreational, and cultural aspects of human activity" (Anonymous 2007). Although only poor statistics are available to document the phenomenon, insect pollinators, native bees and the non-native honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), appear to have been declining in the U.S. at least sporadically since 1947 (Anonymous 2007) and likely much longer (Burkle et al. 2013). The following website contains results from the latest surveys and assessments: http://beeinformed.org/results-categories/winter-loss/.

Due to the importance of pollinators, there is great concern about their status (Winfree et al. 2009), current issues driving decline, and their future welfare (Kremen et al. 2007; Potts et al. 2010). Bees are mobile organisms and may travel long distances to collect nectar and pollen and in the process pollinate crop and non-crop plants (Vischer & Seeley 1982; Couvillon et al. 2014a). Thus, the resources acquired and services they provide during foraging are often found in, or delivered to, habitats some distance away from their nests or hives (Kremen et al. 2007). As a result, the issues of bee abundance and species richness surrounding pollinator services occur at the landscape level and are affected by many factors including habitat degradation, fragmentation and loss, invasive species, pesticides, and climate change that impact ecosystem structure, biodiversity, phenology, and stability (Kremen et al. 2007; Winfree et al. 2009; Potts et al. 2010; Burkle et al. 2013).

Kremen et al. (2002, 2004) reported that agricultural intensification in California reduced pollination services by 3 to 6 fold, and that the isolation from natural habitats was potentially more important in bee decline than management practices. They found that under organic production with nearby natural habitat, native bees without the help of honey bees could provide complete pollination of watermelon, a crop with high pollinator requirements. Other types of conventional, non-organic farms had reduced diversity and abundance of native bees.

Allsopp et al. (2008) indicated that both honey bee and native pollinator services are greatly undervalued. However, honey bees are used extensively as an agricultural input because they are excellent generalist pollinators (Allsopp et al. 2008). The need for pollinator diversity is emerging as important in the production of the more nutritious, higher value, pollinator-dependent crops. Body size represents a quantity/quality component of this diversity, with large bees delivering the pollen (quantity) and small bees spreading the pollen more evenly on the stigma (quality) (Aizen et al. 2009). However, flower visitor richness increases fruit set independently of honey bee visitation (Garibaldi et al. 2011, 2013). Current correlative evidence links this diversity, of which species richness is a component, to pollination success leading to enhanced crop yield without managed honeybees (Hoen et al. 2008). Increased emphasis on native pollinators and their native plant hosts has widened research, seeking to better understand native bee landscape-level behavior as well as discovery of mitigation methods to conserve and augment native as well as honey bees. Fruit set, a key to crop yield, has recently been shown to increase significantly with wild insect visitation in all studied cropping systems, and with honey bee visitation in only 14% of the systems (Kremen et al. 2002, 2004; Isaacs et al. 2008; Jakobsson et al. 2009).

Ecosystem services, processes that take place in the natural world which benefit mankind, are provided by the complex functional interactions between flora and fauna biodiversity and natural resources. These services contribute to the stability, productivity, and sustainability of landscapes. Pollination along with biological control is a regulating ecosystem service and highly valuable to mankind (Watson & Zakri 2005).

Both native and non-native species can provide important ecosystem services. Although a long-standing debate continues regarding the purposeful introduction of non-natives, their benefits are well documented (Knox & Mizell 1998, and literature therein). Isaacs et al. (2008) discussed the role of native plants in maximizing crop pollination and pest control. In this study, we used a non-native plant to determine its potential role in augmentation of both native bees and the non-native honey bee, *A. mellifera*.

Collectively known as crape myrtle, selections and interspecific hybrids of *Lagerstroemia indica* L. and *L. fauriei* (L.) (Myrtales: Lythraceae) comprise the majority of cultivated, ornamental flowering types in the world (Wang et al. 2007; Pounders et al. 2010). The approximately 56 species of *Lagerstroemia* are native to Southeast Asia (Furtado & Srisuko 1969). Characteristics of high tolerance of a wide range of abiotic conditions, few host-specific insect and disease pests, attractive bark, variable flower colors including red, pinks, white, lavender, and purple, a long showy summer flowering period, bright and showy autumn leaf coloration, and variable size from small shrubs to larger trees have made *L. indica* × *fauriei* crape myrtles very common and important woody landscape plants in the southern U.S. (Mizell & Knox 1993; Chappell et al. 2012).

Lagerstroemia species have several interesting morphological and physiological characteristics. Six alkaloids have been isolated from *L. indica*, mainly in the seed pods, with only trace amounts in the leaves and stems (Ferris et al. 1971; Nepi et al. 2003; Odintsova 2008). The flowers do not produce nectar. Flowers of 82% of all *Lagerstroemia* species have dimorphic stamens, with dimorphic pollen within multiple flowers composing a large terminal panicle (Kim et al. 1994). The dimorphic pollen occurs in 2 spatially and morphologically distinct staminal whorls with one type, the lower antepetalous whorl opposite the petals, functioning as food for visiting insects, and the other, higher antesepalous whorl, for fertilization (Muller 1981; Kim et al. 1994; Nepi et al. 2003).

Many insect species including members of the orders Diptera, Coleoptera, Hemiptera, Hymenoptera, Lepidoptera, and Neuroptera have commonly been observed visiting crape myrtle flowers (R. F. Mizell and T. C. Riddle, personal observations 1982–2014), including *Lagerstroemia speciosa* L. in Brazil (Vitali-Veiga et al. 1999). Depending on latitude and cultivar, crape myrtles bloom from late spring to early fall and provide a pollen source during hot summer months, when few other such resources are available (Bolques & Knox 1997; Vitali-Veiga et al. 1999; Pounders et al. 2010; Couvillon et al. 2014b; R. F. Mizell and T. C. Riddle, personal observations 1982–2014).

Our previous research on crape myrtle documented the role and interactions of this important non-native plant species with a number of insects and ecological phenomena. For example, the host-specific, non-native crapemyrtle aphid, *Tinocallis kahawaluokalani* (Kirkaldy) (Hemiptera: Aphididae), is used as prey by an array of native beneficial insects (Mizell & Schiffhauer 1987). Crape myrtles are also important hosts of native leafhopper vectors of the Pierce's disease bacterium, *Xylella fastidiosa* Wells et al. (Xanthomonadales: Xanthomonadaceae), and vector feeding behavior and nutrition research have been reported in detail (Andersen et al. 1989; Redak et al. 2004; Mizell et al. 2008, 2012).

The differential susceptibility of 33 cultivars to the crapemyrtle aphid has been determined (Mizell & Knox 1993). There is an apparent lack worldwide of hymenopteran parasitoids affecting crapemyrtle aphids (Mizell et al. 2002). The impact of the non-native Asian ladybeetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), disrupting native beneficial insects feeding on crapemyrtle aphids has been reported (Mizell 2007). The host preference and suitability of known crape myrtle germplasm lines to crapemyrtle aphids has been compared (Herbert et al. 2009). In addition, controlled feeding experiments using crapemyrtle aphids have shown that crape myrtle germplasms differentially affect the physiology and mortality of predacious insects at higher trophic levels, specifically the green lacewing *Chrysoperla ru-filabris* (Bermeister) (Neuroptera: Chrysopidae) (Herbert 2009).

Here, we report the use of crape myrtle cultivars by native and honey bee pollinators. The objectives of this study were 1) to determine the bee fauna associated with *L. indica* and its hybrid cultivars from crosses with *L. fauriei*, 2) to determine the relative preference of individual bee species for crape myrtle cultivars, which will lead to recommendations for pollinator augmentation, and 3) to determine potential interactions among non-native and native pollinators on this widespread, non-native plant species. Also, the fortuitous presence of flowering-seed formation bahiagrass (*Paspalum notatum* Flüggé; Poales: Poaceae) in the plot understory and the addition of commercial colonies of *Bombus impatiens* Cresson (Hymenoptera: Apidae) were used to advantage to investigate the spatial distribution and response behavior of *B. impatiens* to the 2 host plant species.

Materials and Methods

This study was conducted at the University of Florida's Institute of Food and Agricultural Sciences (UF/IFAS), North Florida Research & Education Center (NFREC-Quincy) in Quincy, Florida (30.5427833°N, 84.5956833°W) and used an existing 0.5 ha planting of crape myrtle. The original crape myrtle planting contained 4 replicates each with 4 plants of 12 hybrids (*L. indica ' L. fauriei*) plus 2 *L. indica* selections, planted in a randomized complete block design. The germplasm by flower color for the 14 cultivars represented in the planting were as follows: white 'Acoma', 'Byer's Wonderful White', and 'Natchez'; red 'Carolina Beauty' and 'Tonto'; lavender 'Apalachee', 'Lipan', and 'Yuma'; and pink: 'Biloxi', 'Miami', 'Osage', Sioux', 'Tuscarora', and 'Tuskegee'. Land adjacent to the study plot consisted of a hardwood forest, another large planting of crape myrtle, areas planted with small grains, and tilled plots with miscellaneous other vegetation over the course of the experiment.

For this experiment, the block of 224 crape myrtles was divided into 56 quadrats, each of which contained 4 crape myrtles of the same cultivar. Quadrats were arranged within 8 rows running east to west, and each row contained 7 quadrats for a total of 28 plants per row (Fig. 1).

Plant spacing was 6 m between and 5 m within the rows. Areas within the row approximately 2 m wide under the crape myrtle were maintained as bare soil by using herbicides. Vegetation in the row centers consisted of bahiagrass, *P. notatum*, which was mowed as needed but was allowed to reach the flowering stage during the study in 2010 (Fig. 2). Crape myrtles were pruned annually by topping to about 1 m height in early Feb and provided a maintenance application of 10:10:10 (N:P:K) fertilizer to promote flowering.

Ward et al. (2014) found that only 2 simple 15 min surveys of native bee abundance on flowers are all that is required to provide "good estimates" of bee abundance and diversity at a particular site. To quantify bee occurrence, transect walks (Kjohl et al. 2011; Ward et al. 2014) were made through all quadrats in the same serpentine fashion on several dates. The entire block of crape myrtle was monitored for pollinator activity as the flowers and pollens became available. Once bees were observed foraging on crape myrtle flowers, transect walks and counts were initiated. The geographical coordinate of the 3rd crape myrtle from the east was used in the analysis to indicate guadrat position (Fig. 1). As bees were identified, each was recorded by quadrat. In 2009 (year 1), 3 transect walks were conducted on 10, 21, and 30 Jul. In 2010 (year 2), walks were conducted on 14, 21, and 27 Jul. The intent was to generate a spatio-temporal snapshot of bee abundance by species and crape myrtle cultivar. Bee collections via netting were made from the entire planting to assess accuracy of identifications. Percentage bloom was estimated visually for each cultivar on each date. In 2009, independent of this study, 1 honey bee hive was placed 15 m south of the field between the 10th and 11th crape myrtle from the west end. The adjoining plot about 25 m south of the crape myrtle planting was <0.25 ha in size and planted with cantaloupe and watermelon. The honey bee hive was for the pollination of this plot. Several other active hives were also on the NFREC-Quincy lands.

0	Acoma	Osage	0	Biloxi		Tonto	۰	0	°Cpean	•	0	o Tuske o o	0	۰	Miami	٥
۰ ۰	Apalach	Sioux	• •	Bwhite	3 4	Tuska	0	0	Lipan	0	\$, Yuma _♦	٥	0	Natch	٥
۰ ۰	Biloxi	, s ^{Tonto}	00	Cbeau] [Tuske	0	0	, Miami	0	\$	Acoma	٥	٥	osage	0
۰ ٥	Bwhite	, sTuska	00	Lipan] [·	Yuma	0	0	Natch	0	\$	Apalach	0	0	Sioux	0
0	Cbeau o	o o Tuske	•	Miamj		Acoma	0	0	osage	0	\$	° Biloxi	0	0	Tonto	٥
	Lipan	S Yuma		Natch		。 Apalach	0	0	Sioux	0	\$	Swhite	0	٥	Tuska ◇ ◇	0
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Fig. 1. Cultivar positions in crape myrtle experimental block. Each small circle is 1 crape myrtle. Each quadrat has 4 crape myrtle plants of the same cultivar. Legend for cultivar abbreviations: Apalach = 'Apalachee', Bwhite = 'Byers Wonderful White', Cbeau = 'Carolina Beauty', Natch = 'Natchez', and Tuske = 'Tuskegee'.

1 2 3 4 5 6 7 8 9 10 11 12 13 14 14	15 16 17 18 19 20 21 22 23 24 25 26 27 28	29 30 31 32 33 34 35 36 37 38 39 40 41 42	43 44 45 46 47 48 49 50 51 52 53 54 55 56	57 58 60 61 62 63 64 65 66 67 68 9 70	71 72 73 74 75 76 77 78 79 80 81 82 83 84	85 86 87 88 90 91 92 93 94 95 96 97 98	99 100 101 102 103 104 105 106 107 108 109 110 111 112	113 114 115 116 117 118 119 120 121 122 123 124 125 126	127 128 129 130 131 132 133 134 135 136 137 138 139 140	141 142 143 144 145 146 147 148 149 150 151 152 153 154	155 156 157 158 159 160 161 162 163 164 165 166 167 168	169 170 171 172 173 174 175 176 177 178 179 180 181 182	183 184 185 186 187 188 189 190 191 192 193 194 195 196	
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Fig. 2. Bahiagrass quadrat positions.

In 2010 (year 2), a substantial number of *B. impatiens* workers were noticed in early morning visiting bahiagrass in the row centers. This bee may well be the most abundant native bumble bee and as such is the only other bee available commercially for pollination augmentation. This raises the question, as the honey bee declines, are the high costs for this bee justified, or is habitat manipulation a viable alternative? Therefore, these areas were divided into 196, 16 m² guadrats with flagging to establish transects (Fig. 2) for determination of their spatial distribution in response to bahiagrass in the presence of crape myrtle (Figs. 3 and 4). In 2010, although *B. impatiens* is an abundant native bumble bee, 2 B. impatiens colonies (Koppert B.V., P.O. Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands) were placed independent of this study on the NFREC-Quincy property for pollination of cucurbit crops. One hive with 4 colonies each was about 425 m northwest and the other about 700 m southeast of the crape myrtle block. In an attempt to ascertain the spatial distribution, the contribution of the commercial colonies and the effect of changes in forage distribution on bees, 2 additional transect walks were made through these quadrats on 16 and 21 Jul to count bumble bees that were visiting the bahiagrass. A 3rd transect was made on 23 Jul as part of the study after all B. impatiens colony doors were closed well before daylight. The bahiagrass was mowed on 2 Aug, and a subsequent transect walk counted bees visiting crape myrtle on 3 Aug. Also in 2010, 4 transect walks on 16 Jun, 6, 21, and 27 Jul were conducted to count bees on crape myrtle. Before opening the colony doors, and after the transect

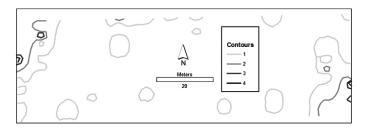


Fig. 3. Isolines depicting *Bombus impatiens* aggregations and gaps in bahiagrass on 23 Jul 2010.



Fig. 4. Isolines depicting *Bombus impatiens* distributions in crape myrtle on 21 Jul 2010.

walk through bahiagrass, an additional walk through crape myrtle on 23 Jul recorded *B. impatiens* during the morning foraging period. Date, time, and weather conditions were recorded. Again, supplementary net collections were made to validate identification accuracy.

In 2011 (year 3), only net collections of bees were made from the study block. We collected as many different bees as possible that were observed foraging on crape myrtle. This was done without regard to time or cultivar. Species were identified using a Leica MZ12.5 scope equipped with a Leica DFC 295 camera and Discover Life online keys (http://www.discoverlife.org/mp/20q?guide=Bee_genera). Where identifications were in question, key characters were imaged. The images were posted to the Bee Monitoring Network (https://groups. yahoo.com/neo/groups/beemonitoring/info) for verification. Voucher specimens are housed at the NFREC-Quincy. Transect walks, and bee counts on flowers, were discontinued in year 3 because intensive net collecting was not only time consuming, but might also potentially bias results.

Statistical Analyses. All analyses, unless otherwise stated, were conducted using SAS (SAS Institute, Inc. 2011) or ArcGIS (ESRI 2013). The factors that remained constant throughout the study were, in order of significance, cultivar and color. Single sample dates in 2009 did not provide enough degrees of freedom, nor did they have the numbers of individuals, necessary to run multifactorial models to gauge the significance of each factor to be analyzed; therefore, dates were combined. Different numbers of levels of percentage bloom occurred

as bloom progressed. These were nonrecurring. This precluded combination of dates for estimation of least squares means for this factor. In 2009, there were 3 sample weeks. A reduced model containing cultivar, week, and replication was constructed and analyzed by species for this factor. For the count data from study years 1 and 2, least squares means (LSMeans), with Tukey adjustment for multiple comparisons at $P \le 0.05$, was used in SAS PROC GLM to analyze the effects of cultivar, for counts of B. impatiens workers, Bombus fraternus (Smith), Xylocopa micans Lepeletier (females), Xylocopa virginica (L.), and the honey bee, A. mellifera. Color was input for analysis using the standard published by the Crape Myrtle Society of America (http://www.guidestar. org/organizations/75-2957884/crape-myrtle-society-america.aspx). Spatial pattern was evaluated for all species and dates by constructing abundance-by-location maps by species and date using inverse distance weighting in ESRI ArcGIS 10.2 (ESRI 2013). Isolines drawn within the GIS indicated possible aggregations on 23 Jul 2010 in bahiagrass, and on 21 Jul 2010, point pattern analysis was conducted using SADIE red-blue methodologies (Spatial Analysis of Distance IndicEs, version 3) (Perry & Conrad 2007) to determine significance.

For 3 consecutive sets of similar dates within the 2 years of the counts study, LSMeans tests, with replication included in the model, were run for counts of *B. impatiens*, *B. fraternus*, and *A. mellifera* to assess the contributions of the honey bee hives. A single degree of freedom was available for these tests based on the 2 available dates. In order to determine the contribution of *B. impatiens* colonies to field abundance, the count on 2 Aug with the colony doors closed was compared with the count with the colony doors open on 3 Aug. LSMeans tests with replication included in the model were used. Daily maximum and minimum temperatures were obtained from the weather network FAWN (http://fawn.ifas.ufl.edu/) for 20 Oct 2008 to 15 May 2009 and for the same dates in 2009–2010. Spectral reflectance patterns were recorded for the freshly opened flowers of each crape myrtle cultivar in the study by using Ocean Optics USB2000 (Ocean Optics, Inc., 830 Douglas Avenue, Dunedin, Florida 34698).

Results

Honey bee counts were similar in both years; however, counts of all other bee species were substantially higher in 2010 than in 2009. For 2009, the highest number of native bees was observed on 21 Jul and included 59 native bees and 48 honey bees. Honey bee numbers peaked for 2009 on 30 Jul. On that date, 174 honey bees and 31 native bees were counted. For 2010, the highest number of native bees was observed on 6 Jul and included 433 native bees and 60 honey bees. The highest number of honey bees in 2010 occurred on 21 Jul. On that date, 171 honey bees and 311 native bees were counted. This level of bee visitation allowed analysis as indicated in the methods. Cultivar was the most significant effect, but combining sample dates in 2009 improved cultivar significance, which was at least an order of magnitude greater than other factors. Color had minimal significance. Replication always contained 4 levels but never approached significance. The notable exception was for honey bees, where higher amounts of percentage bloom of individual cultivars were significant on 6 dates across 2 years (Table 1). Exceptions for "week" occurred for X. micans (females) (F = 4.42; P = 0.014), X. virginica (F = 3.67; P = 0.014), and the honey bee, A. mellifera (F = 6.42; P = 0.002). Although there was some significance on all dates, cultivar preference was different for each species of native bee on each date, indicating variable spatio-temporal preference at the landscape scale. Honey bees and B. impatiens had the same cultivar preference only on 30 Jul 2009. On the remaining 5 survey dates, honey bee cultivar preference differed from that of the native bees (Table 2).

 $\ensuremath{\textbf{Table 1.}}$ Significance of bloom volume to honey bee visitation in crape myrtle cultivars.

Date	Factors in model	df	F	Р
10-VII-2009	Bloom, replication	5	9.92	<0.0001
21-VII-2009	Bloom, replication	5	3.26	0.0131
30-VII-2009	Bloom, replication	2	11.09	0.0001
16-VI-2010	Bloom, replication	3	4.52	0.0071
24-VI-2010	Cultivar, color, bloom, replication	13	2.19	0.0278
15-VII-2010	Cultivar, color, bloom, replication	12	4.70	0.0001

Degrees of freedom are given for the factor "bloom."

Honey bee abundance was not significantly increased by a proximal colony based on models run for honey bees for 3 pairs of similar dates from 2009 and 2010. No significant aggregation was noted in either year, nor was abundance related to distance from the hive. On 21 Jul 2010, counts of B. impatiens on crape myrtle and bahiagrass were 130 and 144 bees, respectively. Two days later (23 Jul 2010) with all artificial colony doors closed, the count for B. impatiens bees in crape myrtle was 137 and in bahiagrass 154. The difference among these counts was not statistically significant. For the 3 consecutive and similar dates examined, counts for B. impatiens were significantly higher in 2010 than 2009 (F = 10.68, P = 0.001; F = 1.33, P = 0.026; F= 4.91, P = 0.029). For B. fraternus, for 3 similar dates in each of the 2 years, there were significantly more bumble bees on all dates in 2010 than in 2009 (F = 14.18, P < 0.001; F = 7.82, P = 0.006; F = 17.23, P < 0.001) when no artificial colonies were present. Whereas year was always a significant factor for both species, the significance of crape myrtle cultivar often eclipsed year when included in the model.

Isolines produced using ArcGIS on 2010 data led to further point pattern analysis (Figs. 3 and 4). The aggregations were located on the corners of the block closest to the colonies, as expected (Fig. 4) (Sadie $P_a = 0.0097$) (Perry & Conrad 2007). Although the pattern observed in crape myrtle was not significant (Fig. 3), point pattern analysis showed that there were significant gaps and aggregations in counts of *B. impatiens* foraging on 23 Jul 2010 in bahiagrass. When counts were made following mowing of the flowering bahiagrass, the abundance of *B. impatiens* in crape myrtle increased by 40%. However, this numerical increase was not statistically significant.

Although spectral patterns were measured in this study, they were not included because Funderburk et al. (2015) published representative spectral graphs that were taken from the same plants with the same equipment used in this study.

Discussion

Pruning can affect the phenology of crape myrtle flowering (Gilman et al. 2008). For this study, spring pruning served to facilitate the observations on flowers by reducing plant size, stimulating growth and flowering on new growth, and changing the relative time of flowering by a few days such that the observed plants flowered closer together in time than under natural conditions and to some degree out of synchrony with unpruned plants (Bolques & Knox 1997; Gilman et al. 2008). Thus, the pruning enabled comparisons and detections of the frequency of cultivar use by the bee species with less confounding of the results due to flowering that would naturally be less synchronized if not pruned (Bolques & Knox 1997; Pounders et al. 2010). Additionally, the pruning treatment resulted in the conclusion that specific crape myrtle cultivars might be managed to change their phenologies (estimate of 7 to 10 d) to favor preferred pollinator species for augmenta
 Table 2. Bee preference for crape myrtle cultivars by year.

	Year 2	.009		Year 2010		
Bee species	Cultivar preferred	ferred <i>F P</i> ^a Cultivar preferred		Cultivar preferred	F	P^{a}
	10-VII-2009			6-VII-2010		
Bombus impatiens Cresson		0.96	0.507		1.55	0.142
Bombus fraternus (Smith)		1.89	0.065	'Natchez'	5.74	< 0.001
Apis mellifera L.	'Miami'	4.25	< 0.001		1.19	0.321
Xylocopa virginica (L.)		1.60	0.130		0.63	0.815
Xylocopa micans Lepeletier	'W. White'	4.22	<0.001		1.25	0.282
	21-VII-2009			21-VII-2010		
Bombus impatiens Cresson		1.08	0.401	'Osage'	73.35	< 0.001
Bombus fraternus (Smith)	'Osage'	2.75	0.007	'Apalachee'	12.99	< 0.001
Apis mellifera L.		1.77	0.083	'Sioux'	2.07	0.038
Xylocopa virginica (L.)		_	_		_	_
Xylocopa micans Lepeletier		1.41	0.198		1.65	0.113
	30-VII-2009			27-VII-2010		
Bombus impatiens Cresson	'Osage'	3.62	0.001		_	_
Bombus fraternus (Smith)	C C	1.32	0.243	'Natchez'	6.75	< 0.001
Apis mellifera L.	'Osage'	5.20	< 0.001	'Sioux'	6.24	< 0.001
Xylocopa virginica (L.)	-	_	_		2.96	0.004
<i>Xylocopa micans</i> Lepeletier		_	_	'Yuma'	6.11	< 0.001

"Values from LSMeans with Tukey adjustment for multiple comparisons. F and P are for Type III sum of squares for cultivar. For the 14 cultivars in the study, degrees of freedom (df) are 13 for all species on all sampling dates.

tion. Cogent to facilitating this potential practice, crape myrtles will re-flower a 2nd time if the developing seed heads are removed as the 1st bloom ends. Moreover, sporadic repeat blooming also occurs at moderate levels naturally (Chappell et al. 2012). A full season management strategy to augment bee species for continued crop pollination would necessarily contain many components. However, crape myrtle is readily used, widely available, and provides an abundant pollen resource at an opportune time, especially for honey bees (Couvillon et al. 2014b). Crape myrtle blooms as early as May in the Deep South and summer–fall months elsewhere (Chappell et al. 2012). Focused selections, accounting for native bee preferences, would heighten crape myrtle's contribution to pollinator support. Concurrently, selection of plants to promote aphids for augmentation of natural enemies could also be addressed (Mizell & Schiffhauer 1987).

Sampling via established transect walks not only verified crape myrtle as a source of pollen for several abundant pollinators but also provided useful information regarding the spatio-temporal preferences of 5 common and larger native bee species. Net collecting of small to medium bee species visiting crape myrtle identified 5 species of Halictidae and an additional Apidoidae species. All of these bees have crop plant families in their flower visitation records (Discover Life 2014). An individual of the specialist species Habropoda laboriosa (F.) (Hymenoptera: Apidae) visiting blueberries is valued at between 20 and 75 dollars, depending on the value of the crop (Moisset & Buchmann 2011; Anonymous 2015). The bees in this study have the potential to pollinate multiple crops through the season, and therefore their value should be much greater (Table 3). Pollinator species in north Florida found on L. indica × L. faurieri cultivars are similar with respect to the bee genera on L. speciosa in Brazil, e.g., honey bees, 2 Bombus species, and 2 Xylocopa species (Vitali-Viega et al. 1999). Two recent bee surveys in Alachua County (Florida) natural areas and organic farms vouchered 146 species (Hall & Ascher 2010, 2011). Ten of these species accounted for 88.6% of passive captures. Of these, Lasioglossum pectorale (Smith) (Hymenoptera: Halictidae), Halictus poeyi Lepeletier (Hymenoptera: Halictidae), Augochlorella aurata (Smith) (Hymenoptera: Halictidae), Agapostemon splendens (Lepeletier) (Hymenoptera: Halictidae), and Melissodes bimaculata (Lepeletier) (Hymenoptera: Apidae) were collected directly from crape myrtle at NFREC-Quincy.

Table 3. Abundant full-seaso	n bees captured from crap	e myrtle in northern Florida.
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Scientific name	Туре	Body length (mm)	Cucurbitaceae ^a	Rosaceae ^b	Crape myrtle ^c
Bombus impatiens Cresson	Bumble bee	8.5-21	Х	Х	'Natchez', 'Osage'
Bombus fraternus (Smith)	Bumble bee	13–27	Х	Х	'Apalachee'
Xylocopa virginica (L.)	Carpenter bee	19–23		Х	'Natchez'
Xylocopa micans Lepeletier	Carpenter bee	15-19		Х	'Natchez'
Agapostemon splendens (Lepeletier)	Halictid	10	Х	Х	'Acoma'
Augochlorella aurata (Smith)	Halictid	5.5	Х	Х	'Acoma'
Halictus poeyi/ligatus Lepeletier	Halictid	8-10	Х	Х	'Acoma'
Lasioglossum pectorale (Smith)	Halictid	6		Х	'Acoma'
Melissodes bimaculata (Lepeletier)	Long horned	13–15	Х	Х	'Acoma'
Apis mellifera L.	Honey bee	9–20	Х	Х	'Miami'

^(a,b)An "X" marks a crop plant family to which the corresponding bee species is a known pollinator (Discover Life 2014). ^(c)Crape myrtle cultivar from which the same bee species was captured. 43

Further information about the bee species found in this study including the crop plant families from their flower visitation records are provided in Table 3.

Patch structure generally precludes ecological study of individual landscape components, but the artificial patch structure created by a randomized complete block design immersed in an agricultural landscape enabled observation of the behavior of several keystone bee species. Although only 2 families are represented in this study, the data show meaningful differences in their habits and abundances. Frequency of cultivar use by native bees was different among years and differed from honey bees (Table 2). The 10 species of bees recorded are present over a large geographical area throughout the pollinating season (Discover Life 2014). Spatio-temporal distribution, the several taxonomic affinities, the range in size, plus the wide range in host plant usage underscore functional differences and environmental importance.

Of the 2 L. indica cultivars in the design, 'Carolina Beauty' and 'Byer's Wonderful White', only the latter was ever preferred by any observed bee species, namely, by X. micans on 10 Jul 2009 (Table 2). This is consistent with differential crapemyrtle aphid populations by cultivar reported in previous work and perhaps indicates increased concentrations and higher alkaloid toxicity of not only leaves but also Lagerstroemia species pollen (Mizell & Knox 1993). The question of potential toxic effects from alkaloids and perhaps other defensive chemicals or primary nutrients in these Lagerstroemia species (Ferris et al. 1971) needs to be further addressed not just for the potential impact on pollinating bees but for beneficial insects and the other herbivores on crape myrtle. As examples, Herbert (2009) found that green lacewing pupa development time, mortality, and other life history parameters were affected by the crape myrtle cultivar fed upon by their crapemyrtle aphid prey. A worldwide search also indicated that the crapemyrtle aphid apparently has no parasitoids (Mizell et al. 2002).

Adler et al. (2006) found a positive correlation within *Nicotiana* (Solanales: Solanaceae) phenotypes between leaf alkaloids and nectar. They suggested that the physiologies of leaf and floral tissues are widely linked. Kempf et al. (2010) found that pyrrolizidine alkaloids could be detected in pollen and pollen products made by bees. These reports raise the possibility that crape myrtle pollen may also contain related chemicals. However, Wcislo & Cane (1996) stated that for the most part bees avoid visiting flowers of plants with toxic pollen or nectar. More research on the potential effects of pollen secondary compounds vs. nutrition is needed.

Nepi et al. (2003) compared the chemical composition of the "feeding" vs. "fertilizing" pollen of *L. indica*. They reported that total sugar concentrations are the same in the 2 pollen types, but found that the relative concentrations differ, with fertilizing pollen being sucrose rich and feeding pollen being richer in glucose and fructose. They also reported that fertilizing pollen contained on average 42% less water than feeding pollen and hypothesized that the characteristics of the individual pollen types correlated well with their individual functions. Moreover, the high fructose content of feeding pollen is consistent with the content of nectar that bees prefer, and its multisided morphology appears to improve digestion by bees (Nepi et al. 2003). This research documents the potential benefit to pollinators from gathering crape myrtle pollen.

Abundance of native bee species was markedly higher in year 2 than year 1, whereas counts of honey bees remained similar. Examination of weather records indicates a similar number of frost-free days in each year. However, date of first frost was 6 Dec 2008 and 29 Oct 2009, and date of last frost was on 8 Apr 2009 and 7 Mar 2010. This wide difference in weather among years may explain the higher seasonal abundance of native bees and indicate the need for developing climate-based models to determine native pollinator efficacy. The similar

seasonal abundance for honey bees among years and the difference in frequency of cultivar use indicate that honey bees are not a good proxy for native bee populations or their feeding parameters. Native bees appear to have tremendous potential for pollination in "good" years, but the importance of honey bees is accentuated because their abundance does not seem to be as adversely affected by temperature extremes.

Significant interactions among the pollinator species were not detected either visually or by analyses of cultivar preferences. No 2 bee species preferred (statistically) the same cultivars at the same time with the exception of 'Osage' on 30 Jul 2009 by both B. impatiens and A. mellifera (Table 2). Honey bees use ephemeral scents to mark flowers during feeding visits to enable siblings to avoid recently visited flowers. Bumble bees and honey bees can also scent mark flowers to promote congener feeding (Goulson et al. 1998). Crape myrtle flowers only provide pollen, and the flower structures are such that the yellow food pollen is concentrated and readily visible in the middle of the flowers surrounded by the colored petals that occur in large numbers on the flowers. Flowers occur on the current year's growth and most branches produce flowers, especially when exposed to full sunlight. Therefore, each crape myrtle will contain large numbers of flowering panicles with even larger numbers of flowers per panicle. Given the volume of flowers available at any one time during bloom on an individual tree, it is unlikely that bee scent marking behavior affected subsequent visits. Moreover, percentage bloom was measured but significantly affected cultivar choice only for honey bees (Table 1).

The crape myrtle cultivars in the study represent a wide range of flower colors, and based on spectral reflectance patterns, there is a great deal of variation in spectra (Funderburk et al. 2015, data were recorded from the same plots). Thus, visual cues for the bees, even among flowers from cultivars of ostensibly the same color, such as white (data not shown), vary accordingly. Flower color did not appear to significantly affect the choice of cultivar for any of the observed bee species, as each species never selected more than 1 cultivar from any color group. Nevertheless, crape myrtle flowers offer a wide range of color options to other would-be insect visitors (Mizell & Schiffhauer 1987; Vitali-Veiga et al. 1999). Reflected wavelengths in the ultraviolet range often associated with flowers as bee nectar guides do not appear at high intensities in the cultivars studied, possibly due to sampling error in reflectance, which is measured over very small areas (Funderburk et al. 2015).

The manipulation of bahiagrass to determine the impact on bumble bee behavior demonstrated that grass pollen can be used heavily by the native *B. impatiens* and further showed that this species can change its foraging habits rapidly. The rapid return of *B. impatiens* from foraging on bahiagrass to crape myrtle was also documented by removal of the grass resource. The presence of artificial colonies of *B. impatiens* resulted in a detectable aggregation of these bees at the end areas of the study block, nearest the colony locations. Furthermore, the distribution of feeding on bahiagrass in the plot led to significant gaps and aggregations in the foraging patterns (Fig. 4). Patches and gaps in *B. impatiens* foraging behavior have important implications for colony placement as this native bee finds increased use as an agricultural input (Artz & Nault 2011).

Mosaics of natural, urban, and rural habitats are the current norm for most landscapes other than large tracks of government-controlled parks and forests. This has significant impact on landscape-level community processes, with many practical implications relative to natural functions as well as augmentation and delivery of regulating ecosystem services (Lovell & Johnson 2009). Bee distribution and abundance are negatively affected by habitat disturbance, habitat loss, and fragmentation (Winfree et al. 2009). Organisms operate within habitat mosaics at various scales, and this is affected by the composition and quality of

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the habitat resources necessary to sustain individuals (Williams & Kremen 2007). Kremen et al. (2004, 2007) found that, for pollinators, the proximity of natural habitats was a determinant factor in their survival and efficacy in agriculture of any type because bees continually collected pollen from native plants. Williams & Kremen (2007) indicated that habitat connectivity was critical for bee reproduction.

Vasquez et al. (2012) discussed the importance of plant-pollinator interactions within mutualistic networks and reported that the strengths and impacts of these interactions were unevenly distributed, with few strong and many weak associations, similar to patterns in food webs. Interaction strengths were a strong predictor of the sign of species impacts. This study documented that crape myrtle is used as a pollen source by honey bees and a number of common native bees at a time of year when such resources are naturally scarce. The experiment with bahiagrass and B. impatiens demonstrated that the association between pollinators and crape myrtle is likely a weak one. A similar relationship was found with native predacious insects and crapemyrtle aphids in north Florida and was viewed as a positive behavior that stimulated movement of beneficials from crape myrtle to pecan (Mizell & Schiffhauer 1987). Current and potential use of crape myrtle in the urban and rural landscape to augment regulatory ecological services appears to be highly important (Lovell & Johnson 2009), but the potential alkaloid issue remains for study along with potential impacts of use of neonictinoid insecticides for control of pests of Lagerstroemia species (Mizell et al. 2015).

Lagerstroemia indica × L. fauriei plants have steadily increased in numbers and have become prominently distributed throughout the landscapes of the southeastern U.S. and elsewhere since the original importation hundreds of years ago. This study and previous research document the unusual functional importance of this plant species/ cultivars to the ecology of the region. Moreover, this "landscape-level experiment" with a non-native species has been running virtually undocumented since at least the 1700s. Accompanying the prized colorful flowers are ecological mechanisms with profound environmental impacts (possibly negative as well as positive) and usefulness to mankind. Much research remains to fully understand the true ecological roles and value of *Lagerstroemia* species, and the present study may serve as a baseline of comparison for future bee studies or augmentation activities.

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