Influence and implications of the reproductive variability and visitation of insects in four cultivars of *Mangifera indica* in Puerto Rico.

by

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Dedication to my family ~

My husband and daughter, thanks for their love, support and understanding.

My parents, for showing me the way to overcoming.

My sister, for teaching me nobility.

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ABSTRACT

In this study, I explored the possible vulnerabilities of mango *Mangifera indica* L. (Anacardiaceae) crops related to pollination processes. A main question that was asked was what are the potential vulnerabilities of mango yield at the pollination stage and what are the implications of these influences to mango production in Puerto Rico? To address these questions, I studied three mango cultivars of Floridian origin (Keitt, Kent and Tommy Atkins) and West Indies (Julie) origin all of which are part of a larger mango germplasm managed by the Juana Diaz Agricultural Experimental Station of the University of Puerto Rico in Mayagüez. Through various studies and building upon a rich literature on mango reproduction, this work tackled four main objectives:1) Evaluate the variation of floral and inflorescences characteristics of these cultivars, 2) Conduct experimental studies to evaluate the dependency of these cultivars on local pollinators and the mating systems these cultivars. 3) Monitor pollinator communities and fruit yields over three flowering seasons evaluating the relationships between pollinator diversity and abundance with fruit yields, 4) Conduct field experiments and observations to estimate the pollination efficiency and effectiveness of dominant pollinators. A minor objective was to compare estimates of pollinator diversity and abundance using different field methods (net sweeping and video cameras). Results showed that there is significant difference across cultivars in variables related to inflorescence size (width, length), architecture, flower production and flower sex ratios but that these differences were not necessarily consistent across years, nor across cultivars. Flowers of *M. mangifera*, reflect color in the UV region, but these patterns are qualitatively different in Keitt which also shows qualitative differences between hermaphroditic and male flowers reflectance in both the UV and visible regions. Mango flower produce more sucrose that glucose and fructose but different cultivars presented different sugar profiles based on the relative percentage of sugar contents. Pollen viability was high for all cultivars, but pollen germination exhibited an optimal temperature that varied depending on the cultivar. Likewise, for all cultivars, pollen from male flowers exhibited higher germination percentages compared to pollen from hermaphroditic flowers at temperatures above optimal values but at temperatures below the optimal, they had a tendency for lower germination percentages relative to hermaphroditic flowers but this pattern was only significant for Kent. Open natural pollinations tended to produce more fruit and yielded seeds with faster development times than artificial pollinations in three of the four cultivars (Julie, Tommy Atkins and Keitt). They also resulted in faster seed germination in two cultivars (Julie and Tommy Atkins). The combined results suggest that not only animal-mediated pollination is needed for these cultivars but that this mode of pollination also results in improved fruit yields, fruit, and seed traits. Over the three years, plants were visited by a combined total of 50 insect species with Diptera being also the most abundant followed by Hymenoptera, Coleoptera and Lepidoptera. The relative abundances of insect communities changed after the passage of Hurricane Maria over the island of Puerto Rico but only one field (hosting Kent) experienced significant species richness declines in 2018 following the hurricane events. Two of the most dominant insects, *Palpada vinetorum* F. (Diptera: Syrphidae) and *Apis mellifera* L. (Hymenoptera: Apidae), showed a "reduction-recovery" pattern for in the period of 2018-2019 but not so for *Cochliomyia minina* (Diptera: Calliphoridae) which was very abundant in 2018 in three out of four cultivars but then returned to pre-hurricane levels in 2019. In 2017, the trees exposed to higher richness and insect abundances experienced higher yields regardless of cultivars but these relationships when present were often weaker in 2018 (post-hurricane) and 2019. Also, not all fields were equally successful at attracting the same levels of diversity and abundance of insects. The visitation rates were studied and estimated with video cameras for each cultivar in 2018 and 2019. When visitation rates were pooled, I found no significant differences in the average total visitation rate (pooled values of all insects) between 2018 and 2019 in any of the cultivars, but the visitation rates of individual species showed different across years. In 2019, a pollen deposition experiment showed no significant differences among insect species in the mean pollen load deposited on stigmas on virgin flowers in any of the cultivars. When both components of pollination effectiveness (pollen deposition and visitation rate in 2019) were combined, there were significant differences in pollinator effectiveness only for Julie where *P. vinetorum* was expected to deposit more pollen grains than the other species. However, all other pollinators were equally effective in the remaining cultivars. All pollinators showed significant correlations between their visitation rates and fruit yield in at least one cultivar but most significant correlations appeared in 2019 and only one in 2018. Insect diversity and pooled visitation rates estimates from video feeds were not correlated with fruit yields. However, the diversity estimates from video feeds when compared to estimates using net sweeping are significantly lower which suggests that this method as implemented greatly underestimates diversity estimates and that may explain the lack of association between global diversity and fruit yields.

As expected, mango reproductive traits related to flower and inflorescence production is highly variable within and between cultivars. During the time period of this study the role of this variability on fruit production in mango seemed secondary to the role of pollinator diversity and abundance in is their relation to agricultural yields. Pollinators are equally effective at depositing pollen and any spatial-temporal fluctuations in the abundance of individual pollinator species is likely to be compensated by the action of other insect species in the pollinator community. Results from pollination experiments suggest that open pollinations may result from cross-pollinations across cultivars which needs further exploration as these result in desirable outcomes in some cultivars (e.g. increased fruit production and faster germination traits) but added variation from open pollinations may also result in added variation in other fruit qualities (e.g. taste, color) not explored in this work. From a management perspective maintaining adequate levels of pollinator diversity would be an important requirement for the stability of fruit yields in this crop system especially in the face of future hurricane events. With expected increases in temperature for the Caribbean basin, a concern would be the potential negative effects of reduced pollen germinability on fruit production.

CHAPTER I

GENERAL INTRODUCTION

Food security means providing the population with sufficient and nutritious food with physical and economic access throughout the time (Pinstrup-Andersen, 2009). Food security may be jeopardized with global climate changes and the availability of pollinators that could change the production of agricultural crops (Wheeler and Von Braun, 2013; Di Falco et al., 2011; Ladányi and Horváth, 2010). Many studies agree that Earth temperatures will continue to increase as will the frequency and intensity of extreme atmospheric events and changes in global precipitation patterns (Trenberth, 2011; Alexander et al., 2006) and this changes in climate variability will impact terrestrial, ecological, social and economic systems globally, but above all insular systems (Gould et al., 2015; Khalyani et al., 2016; Jennings et al.,2014). Temperature increases are expected to from 1.5 ºC to 2.5 ºC by the end of the century with an increase in the frequency and intensity of extreme atmospheric events such as hurricanes, droughts, heat waves and floods (Campbell et al., 2011; Scatena, 1998). In addition to climatic challenges, agricultural crop yields are also vulnerable to the decline of pollinators anthropogenic activities such as pesticide use, habitat transformation and the effects of invasive species among others (Kevan, 1975; Allen et al.,1998). Understanding, how crop production may be influenced by climate and pollinator variability is crucial to develop appropriate mitigation strategies.

Mangifera indica (mangó) grows widely in tropical and subtropical areas around the world. This is a crop of great economic importance worldwide with the Asian continent is the leading producer with 75% production followed by Latin America and the Caribbean with the 14th% and Africa with a 10% (FAOSTAT, 2000). In the Caribbean area, Puerto Rico is among the main producers of this crop (Central América Data, 2016) and in 2014 was positioned as the fourth most produced and the second largest economically supplied fruit in Puerto Rico (Departamento Agricultura, 2014). In Puerto Rico, flower pollinators are unknown but elsewhere *M. indica* floral visitors (pollinators or nectar stealers) have been reported to be extremely variable between geographical areas in the Neotropics. For example, in Taiwan and Australia, Hymenoptera are reported as frequent visitors and potential pollinators (Hsin Sung et.al, 2006; Anderson et al., 1982). In Israel, Diptera species in addition Hymenoptera species are the reported visitors and potential pollinators of mango flowers (Dag and Gazit 2000) but only Diptera are reported as main pollinators of mango flowers in India (Ramírez and Davenport ,2016; Singh, 1988). Overall, the geographical variability of flower visitors and potential pollinators of mango crop appears to be considerable, but we know little about their true functionality as pollinators, about the temporal variability of pollinator communities and the role of this variability in mango production.

Mango fruit yields are often below the number of flowers that trees produce (Ramirez and Daveport, 2012; Popenoe,1917). Low pollination service could be one factor explaining low fruit yields in animal-pollinated species (Singh, 1954; Singh, 1997) but is not necessarily the only factor (Iyer et al., 1989; Chaikiattiyos et al., 1996, Shü, 2006).The sampling of flower-visiting insects in any crop is an essential component in determining the efficiency and effectiveness of pollinators and their effects on reproductive systems (Howlett et al., 2017), and this knowledge can help clarify how availability of different pollinators relates to fruit productivity in mango. Temporal fluctuations of pollinating species may also lead to temporal fluctuations in pollinator services. Hurricanes which are endemic to the Caribbean region will affect the populations of individual organisms, but also their plant-animal interactions (Khaliq et al., 2014; Spiller and Agrawal, 2003; Spiller and Schoener, 2007). These phenomena can lead to drastic changes in the abundance of pollinators and are believed to have been an important evolutionary factor in the reproductive systems of native plants (Rivera & Ackerman, 2006). Mango is non-native to the Caribbean but it has naturalized successfully in the region (Warschefsky, E. J., and von Wettberg, E. J. ,2019). One possibility is that mango may be resilient to these events given the diversity the diversity of pollinators that reportedly visit this crop elsewhere. Data on the effects of hurricanes on pollinator communities is scarce but suggests that hurricanes may have different effects on plant-animal interactions in generalists versus specialists. *Ardisia escallonioides* (Primalaceae) is non-native to Florida that has a generalist pollination system and was not affected with the passage of Hurricane Andrew in Florida USA (Pascarella, 2006). However, the population of a specialist pollinator the moth, Periploca sp. (Lepidoptera: Cosmopterigidae), declined after Hurricane Andrew (Pascarella, 2006). Following Hurricane Lili in the Bahamas, the population of two hummingbird pollinators *Coereba flaveola* (Coerebinae) and *Calliphlox evelynae* (Trochilidae) declined affecting the reproduction of both pollinators (Rathcke, 2000). On the other hand, hurricanes can result in massive defoliation, loss of branches and death of vegetation (Brokaw and Grear, 1991; Brokaw and Walker, 1991; Francis, 2000; Walker, 1991 and Whigham et al., 1991). Which may influence reproduction independently of pollinator fluctuations. Given, that Puerto Rico is the largest producer of mangoes in the Caribbean, it is important to evaluate how these phenomena can affect production in this crop.

Last it has been documented that mango crops could exhibit low fruit production that could also be linked to poor stigma receptivity (Young, 1942; Shü, 1983, Singh and Sharma, 1972; Pimentel et al., 1984; Dag and Gazit, 2000). Studies suggests that changes in ambient temperature can affect pollen germination in mango (Shen and Huang, 1979; Tseng and Chang, 1983; Young, 1942). In the Caribbean region and Puerto Rico, temperature increases are expected to from 1.5 ºC to 2.5 ºC by the end of the century (Campbell et al., 2011; Scatena, 1998). Exploring how these increases may influence pollen germination of locally cultivated varieties is a priority. In Puerto Rico, there is a shortage of information about important pollinator communities, their functionality and their potential responses to extreme atmospheric disturbances and the potential effect of temperature changes on pollen germination. This information would enable the development of plans to adapt to changes in pollinators and climate variability based on scientific information. Which in turn would allow us to outline management strategies preventively for this crop.

The study takes advantage of *M. indica*'s living material existing at the Juana a Diaz Agricultural Experimental Station (JDAES) managed by the University of Puerto Rico, Mayagüez Campus. This mango germplasm was brought to Juana Diaz Experimental Station in 1968 after several evaluations were carried out in Mayaguez and Rio Piedras (Kinman, 1918; Mattern and Pennock, 1971). Initially Kinman, began in 1918 to carry out certain evaluations with mango cultivars from India (Amini, Bennett, Bulbulchasm, Cambodiana, Davys, Divine, Itamaracá Mullgoa, Paheri, Sandersha, Sans Parelli, Sufaida, Totafari, Martinique) and local cultivars (Blanco, Mangotina, Redondo, Largo, Piña) making a small collection. These studies were carried out at the Tropical Agriculture Research Station (TARS) in Mayagüez with the purpose of looking for cultivars that would serve for export (Kinman, 1918). In 1948, the University of Puerto Rico Mayagüez through the Tropical Agricultural Station in Mayagüez expanded the collection of mango and began evaluating with 150 cultivars (Mattern and Pennock, 1971). Most of the cultivars were brought from India, some local cultivars were added and evaluated at the San Juan Experimental Station, for approximately 20 years (Mattern and Pennock, 1971).That mango collection was abandoned and a new collection was started in 1968 which included a total of 78 cultivars (25 introduced from Florida (USA), 10 local selections, 13 cultivars introduced mainly from the West Indies, Philippines, Hawaii and 30 from the previous evaluated cultivars (Mattern and Pennock, 1971). All this material was evaluated at three Experimental Stations in the towns of Lajas, Isabela and Juana Diaz (Mattern and Pennock, 1971). Of those, the Juana Diaz Station that was considered the ideal place (Mattern and Pennock, 1971) and it was this research center whichcontinued to bring new cultivars until they had about 105 different cultivars (Mattern and Pennock, 1971). The JDAES lost several cultivars to diseases, pests, mis-management and atmospheric disturbances and only 84 remain. All these cultivars are grown in one site, but there is little information about them other than some assessments about the economic importance of some of the cultivars. This study focused on four cultivars of commercial importance (Keitt, Kent, Tommy Atkins) that were developed in Florida and one of potential urban-residential use (Julie) that is commonly cultivated in the Caribbean islands and was developed independently of the other three cultivars. The main question of this study was: What are the potential vulnerabilities of mango yield at the pollination stage and what are the implications of these influences on agricultural safety in PR? To answer this question, this dissertation address three specific questions developed in four different chapters. In Chapter II, I asked the following questions: 1) Are there differences in reproductive characteristics (inflorescence size and structure, flower production and sex ratios, flower color and nectar, pollen production among the four cultivars? 2) What is the association between temperature variation and pollen performance? A working hypothesis was that reproductive traits of Floridian cultivars would be more similar to each other than to those of the Julie cultivar which originated in the West Indies. I also expected to find that pollen germination would decline with increases in temperatures beyond an optimal value given what we know about the association between pollen performance and temperature. To evaluate these hypotheses, Chapter II had the following objectives: a. To evaluate a variety of flower and inflorescence characteristics with a variety of flower and inflorescence morphometrics, conducting chemical analyses to evaluate general nectar composition and applying uv photography to evaluate potential variation reward advertisement neither of which have been evaluated in mango flowers before. b. To carry out an experiment to evaluate the effect of temperature on pollen germination performance. In Chapter III, I evaluated the following questions: 1) How dependent is the reproductive success of these four cultivars on flower visitors? 2) Is reproductive success related to the mating system (self- vs cross pollination) in these cultivars? To answer these questions, I tested the hypothesis that different cultivars would show different breeding systems (i.e., some will necessarily depend on pollinators and others not) based on what we find in the literature and that different mating systems (self. vs cross) would show differences in reproductive success (e.g., fruit production and seed germination) if genetic variation is important for these cultivars. To evaluate these hypotheses, Chapter III had the following objective of conducting pollination experiments to determine: a. the need for pollinators to set fruits and seeds and, b) the effect on fruit production, seed germination and germination times of different forms of pollen matings (self- vs cross pollination).

In Chapter IV, I address the following questions: 1) Are the insect communities visiting mango flowers consistent between seasons? 2) Are there relationships between mango reproductive success with pollinator diversity and abundance. To answer these questions, I evaluate the following hypotheses: a. When they occur, hurricanes could lead to a reduced diversity and abundance of local *M. indica* pollinators and result in reduced yields; b) Pollinator diversity and not just abundance may help increase pollinator services and plant reproductive success To evaluate these Chapter IV had the following objectives: a. Evaluate the communities of floral visitors in fields of four mango cultivars and evaluating the abundance and richness in three observation seasons, b. Evaluate the possible differences in the functional relationships between reproduction and pollinator richness and abundance.

In Chapter V, I evaluate the pollination functionality of four different insect flower visitors of *M. indica*. Here I aske if are their differences in pollination efficiency, visitation rate and pollination effectiveness between floral visitors, 2) Is there a relationship between pollinator effectiveness, and fruit production? To answer this question, I test the hypothesis that local floral visitors are functionally similar in their pollination efficiency (pollen deposition) or their effectiveness (expected pollen deposition when visit rates are considered) based on the premise that pollinator diversity enhances fruit yields (Chapter VI). To evaluate this hypothesis, the study aimed to conduct field experiments and video obsevations to 1) evaluate the pollination efficiency and visitation rates of individual insect species that dominate insect communities that visit mango flowers based on local studies (Chapter VI) and 2) determine how pollination efficiency and visitation rates relate to fruit production in mango

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CHAPTER II

FLORAL BIOLOGY OF FOUR MANGIFERA INDICA CULTIVARS

ABSTRACT

Understanding the variability of the floral biology of crops s and the impact of abiotic factors that influence this variation can be used to inform conservation and management of their living germplasms. This study evaluated the floral characteristics, pollen germination and flowering phenology of four commercial cultivars of *Mangifera indica* (Julie, Keitt, Kent and Tommy Atkins) at the Juana Diaz Agricultural Experimental Station (JDAES) in Puerto Rico using quantitative and qualitative methods. A main question was whether these traits were significantly different among cultivars and if they exhibited a high degree of temporal variation. A second question was whether pollen germination varied between hermaphroditic and male flowers and whether or not any variation was influenced by temperature. Results showed significant different across cultivars in variables related to inflorescence size (width, length), architecture, flower production and flower sex ratios. However, these differences were not necessarily consistent across years. Flowers of *M. mangifera*, reflect color in the UV region, but these patterns are qualitatively different in Keitt which also shows qualitative differences between hermaphroditic and male flowers both using UV and visible spectrum photos. *M. indica* trees produce more sucrose that glucose and fructose but different cultivars presented different sugar profiles based on the relative percentage of sugar contents. Pollen viability was high for all cultivars, but pollen germination exhibited an optimal temperature that varied depending on the cultivar. Likewise, for all cultivars, pollen from male flowers exhibited higher germination percentages compared to pollen from hermaphroditic flowers at temperatures above optimal values but at temperatures below the optimal, they had a tendency for lower germination percentages relative to hermaphroditic flowers although this pattern was only significant for Kent. The results may have important implications to the management of mango cultivars specially within the context of projected climate changes.

INTRODUCTION

Mangifera indica L. (Anacardiaceae) is a tropical fruit tree native to Asia that is widely cultivated in many tropical locations (Galán, 2009; Jirom and Hedström, 1985). Not only it is considered of great economic importance given its high nutritional value (Mukherjee, 1972) but it is also considered of high cultural value joy for some cultures that considered this crop a symbol of life and have called it the "King of Fruits" (Kostermans and Bompard, 1993; Mehta, 2017; Ciruli, 2018). To date, it is estimated that the domestication of *M. mangifera* has resulted in more than 1000 cultivars worldwide although only a fraction of these are grown commercially (Galán, 2009). In many ways this unusually high variety of cultivars has translated into variable floral and reproductive characteristics among cultivars that will likely influence their capacity to set fruit and seeds. Understanding the variability of reproductive characteristics of *M. indica* cultivars and their interplay with abiotic factors can help support the management of live germplasms specially within the context of ever-changing climate conditions worldwide.

The floral biology (e.g., number of flowers, amount of nectar, production of pollen grains, etc) of a species will influence its reproductive capacity and performance (Mukherjee, 1972; Galen, 1999). The mango tree is an andromonoecious plant, which carries hermaphroditic and male flowers within the same paniculate inflorescence (Galán, 2009; Weberling, 1989). In mango, paniculated inflorescences or "thyrses" can be elongated or short depending on the cultivar (Galán, 2009) and carry a variable number of flowers depending on the with some studies reporting 500 to 10,000 flowers, 200 to 6,000 and even 1000 to 6,000 depending upon the cultivars (Mukherjee, 1953; Kalambe et al., 2007; McGregor, 1976; Free, 1993). The ratio of hermaphroditic to male flowers per inflorescence is also variable. For example, this ratio has been reported as 0.8 in the Haden cultivar, 8.6 in the Irwin cultivar (Shü, 1999), 1.4 to 2.1 in the Ataúlfo cultivar (Gehrke, 2007) and in other studies report 0.6 ratio in Sunderia cultivars (Kalambe et al., 2007). Mango flowers are small but flower size, morphology, color and nectar production can also be quite variable (Galán, 2009 Kosterman and Bompard, 1993). The size and number of pollen grains produced are also variable. Pollen rain size can range from 25 to 45 μm (Ramírez and Davenport

2016; Popenoe, 1917) and the amount of pollen grains per anther which can range from 35 to 700 grains per flower as reported for several cultivars (Mulgoba, Malda, Kala Alonso, Alonso Blanco, Amini, and Sanderscha). Pollen production may also differ between hermaphroditic and male flowers. In the Ataúlfo cultivar hermaphroditic flowers had up to 950 grains per anther and male flowers had 645 grains (Gehrke-Vélez, 2007). Understanding this variability and how it may relate to reproductive variability would be important to mango producers as only hermaphroditic flowers can set fruit.

It has been suggested that the floral variability displayed among mango cultivars is ultimately rooted with multiple factors including genetic differences between cultivars, climatic conditions and tree productivity (Singh, 1997; Galán, 2009). Ambient temperature could also influence the functionality and development of floral structures but even then, its effects can be expressed differently among cultivars. In the cultivars Mulgoba, Malda, Kala Alphonso, Alphonso blanco, Amini, and Sandersha pollen germination is optimal at temperatures between 23.9ºC and 26.7ºC (Popenoe, 1917) whereas in the Kensington, Irwin, Sensation and Nam Dok Mai cultivars pollen germination is optimal between 20ºC a 25ºC (Sukhvibul et al., 2000). In that study pollen germination halted when the temperature reached 30ºC. The combined studies suggest that pollen germination in mango is optimal at warm temperatures and that optimal temperatures may vary among cultivars but this information is not available for all mango cultivars.

This study takes advantage of the existing mango germplasm at the Juana Diaz Agricultural Experimental Station in the island of Puerto Rico to further investigate the nature of the floral variability of four mango varieties (Julie, Kent, Keitt and Tommy Atkins) of local commercial interest and discuss the potential implications of observed variability. Here we asked two guiding questions about the variability of reproductive traits. First, we asked if there were differences among cultivars in characteristics related to the reproductive biology (inflorescence size and structure, flower production and sex ratios, flower color and nectar, pollen production and germination) and if some of these traits were variable across years?, Second, we asked if there was an association between climatic variables pollen performance (temperature)?. The Kent, Keitt and Tommy Atkins cultivars originated in South Florida (Warschefsky and Wettberg, 2019) while the Julie cultivar is cited as having originated in the West Indies (Morton, 1987). Recent work has also indicated that Floridian cultivars are genetically less variable than the Caribbean ones (Warschefsky and Wettberg, 2019). Thus, a working hypothesis is that reproductive traits of Floridian cultivars would be more similar to each other than to those of the Julie cultivar which originated in the West Indies. We also expected to find significant associations between climatic variables and pollen performance given what has been published on the subject for mangos. In 2017 while this study was being conducted, the island of Puerto Rico experienced Hurricane Maria, a category 4 storm with recorded wind speeds of up to 250 km/hr (Feng et al., 2018) and the most intense tropical storm experienced in over 60 years (Feng et al., 2018). Thus, we also expected to observe a reduction in values for reproductive traits evaluated before and after this event. The methods in this study included a variety of flower and inflorescence morphometrics, but also chemical analyses to evaluate general nectar composition and uv photography to evaluate potential variation reward advertisement neither of which have been evaluated in mango flowers before. To determine the role of abiotic factors on pollen performance the work used an experimental approach. Assuming that these cultivars are genetically different, we expected these cultivars to show significant differences in their floral biology and responses to climatic variability. Results can provide important information on the floral biology of four mango cultivars in Puerto Rico that can be used to develop in breeding programs and to develop management and conservation plans for this living germplasm of *Mangifera indica*.

MATERIALS AND METHODS

Study site: This study was conducted at the Juana Diaz Agricultural Experiment Station (JDAES) in southern Puerto Rico (180 01'N, 660 31'W) which has an extension of 111.23 ha. The station is located in an area classified as a subtropical dry forest (Ewel and Whitmore, 1973) and exhibits average monthly temperatures that fluctuate between 22°C and 33°C and receives an annual rainfall of 977.1 mm (Fig. 2.1). This research center was established in 1950 by the University of Puerto Rico, Mayagüez Campus for the evaluation of sugarcane varieties with other tropical crops added over the years. The station maintains several germplasms of tropical fruit trees one of them being the germplasm of *Mangifera indica* which currently has 84 cultivars occupying approximately 13% of the station’s total area (Fig. 2.1A).

Study system: *Mangifera indica* L. (mango) is a perennial tree in the family Anacardiaceae. Mango produces highly subdivided panicles (Coetzer et al., 1995; Goguey, 1997) with minute flowers that range from 5 to 10 mm in diameter with five green sepals and five petals that may take different colors depending on the cultivar (white, yellow, pink and red; Galán, 2009). Flowers bear yellow nectar discs and both hermaphrodite and male flowers have fertile stamens. Hermaphrodite flowers have globose ovaries with a single stigma (Ramirez and Davenport, 2016) (Fig. 2.1C, D). Pollen grains have been reported to vary in size and shape depending on the state of hydration. For example, pollen may exhibit long oval shapes (between 25 to 45 μm) long when dry and exhibit sphere-shapes when hydrated (Ramirez and Davenport, 2016; Popenoe, 1917). Some cultivars may even have trilobed pollen shapes (i.e. Chausa, Dasheri and Krishanbhoges). In vitro pollen fertility rates have been reported to be generally high and between 93 to 99%, although at 10-15 minutes of the dehiscence of the anthers the pollen grains are dried (Randhawa and Damodaran, 1961). The four mango cultivars studied at JDSE were Keitt, Kent, Tommy Atkins and Julie all of which are commercial cultivars that differ in tree size. Keitt is a moderately vigorous tree with open canopy tops and the tree heights have a range 9.1 to 40 m. tall (Campbell, 1992) and Kent had open canopy and producing trees can reach 40 m tall, while in Tommy Atkins are a dense and rounded canopy tree top and tree height range 8 to 30 m tall (Campbell, 1992). Julie produces some of the smallest trees among mango cultivars in general (~ 3.3 m tall; Willis and Marler, 1992). At JDAES, these cultivars often flower only once a year for a period of six weeks sometime between December and April but occasionally cultivars may manifest an additional flowering bout (Cabrera-Asencio and Melendez-Ackerman 2021) cultivars undergo the same management which consists of weekly irrigation, fertilizer application twice per year and monthly mechanical weed removal.

Characterization of inflorescences, floral traits and pollen performance - Inflorescences and flowers (Fig. 1B, C, D, E, F) were collected for each cultivar between the years 2017 and 2019 to evaluate different floral traits with the intensity and method of collection of different reproductive structures varying depending on the trait.

Inflorescences and Flowers - To characterize flower and inflorescence traits, two inflorescences per tree were collected for 10 trees in each of four different cultivars (4 cultivars x 10 trees x 2 inflorescences / tree = 80 inflorescences/year). Inflorescences were collected during the flowering periods of 2017 and 2018 at a height of 3 meters from the ground on the sides facing the adjacent roads. Fixing data collection on a particular position within the tree was meant to reduce the variation that may result from sampling different parts of the plant (Herrera, 2009) while allowing for accessible sampling within trees. The following variables were recorded for each inflorescence: the length of the inflorescence, the number of hermaphrodite flowers, the number of male flowers per inflorescence, the number of subdivisions, the length of each subdivision and the total number of flowers per and the flower sex ratio (number of male flowers / numbers of hermaphroditic flowers). Each variable was expressed as the average value per inflorescence per tree. We analyzed the variation in reproductive traits related to flower, inflorescence size and architecture with two-way ANOVA with cultivar and year (time) as main effects.

Color UV - Hermaphrodite and male flowers were collected from each cultivar at 10:00 AM once they had completed the anthesis. They were taken to the lab for photography using a Nikon SMZ800 stereoscope with a Nikon camera that used a 395 nanometer "blacklight" light and a UV filter (Baader U) placed to the camera lens to be able to observe possible flower differences in the reflection of flowers in ultraviolet region of the light spectrum. A qualitative evaluation of the visible differences for each flower pair (hermaphrodite vs male flower) was made for each cultivar with a total of five flowers per gender observed per cultivar. We looked for evidence of UV reflectance of flowers and petals and the potential presence of UV patterns.

Sugar Content - Nectar standing crops in flowers in the four varieties was minute and not possible to extract directly from the nectaries with microcapillary and Hamilton® calibrated needles as it is traditionally done (Kearns and Inouye, 1993). Therefore, an alternative option to the analysis of nectar rewards in mango was to carry out an analysis of the relative proportion of different sugars (e.g., glucose, sucrose, fructose) as a proxy for a nectar analysis of flowers following a modified version of a protocol by Isaac R.A. (1990). For this purpose, two samples of 50 flowers per cultivar (representing five flowers per tree) were collected at 9:00 AM on different dates during flowering in 2019. Once collected, flowers were taken to the laboratory where the nectarium was extracted from the calyx of each flower. The 50 extracted nectaries were weighed and macerated and placed in an 80% alcohol container, adding 0.01g of $CaCO3$ and shaking the solution to combine all the material. The container with the macerated material was then heated in a water bath at 130°C for 30 minutes and then sealed and placed in the refrigerator. The next day it was processed on a high-resolution liquid chromatograph, HPLC Water ® with refractive index, which detects the different types of sugars and their proportion in a known volume. The percent content of glucose, sucrose and fructose between the four cultivars, the amount of each type of sugar was standardized by the combined weight of the nectaries of each cultivar. For each cultivar, the concentration and the percentage of glucose, fructose and sucrose were expressed as the mean and standard error of the two sampling dates.

Number of pollen grains - For each cultivar, the amount of pollen from the anthers of hermaphrodite flowers and male flowers was also determined by collecting 10 flowers per tree (5♀♂, 5♂) per cultivar for a total of 100 flowers (50 hermaphrodite y 50 male) per cultivar. In the laboratory these were observed under a stereoscope (marca Nikon SMZ-8000) where the anther was extracted from each flower and the pollen was placed on a slide where it was preserved in a glycerin gel drop prepared using methods described by Atwater and Lott (2011). Pollen grains in the slides were counted under the microscope using a gridded grid to quantify pollen grains in one anther per flower for a total of 100 anthers/cultivar. Two-way ANOVA was used to test for differences in the amount of pollen grains per flower by different cultivars and different genders.

In vitro pollen germination experiment- Anthers from 10 hermaphrodite flowers and 10 male flowers were collected per cultivar to determine the rate of pollen germination at different set temperatures. The procedure used a nutrient medium based on the Brubaker- Kwack medium (1963) containing 10mg of boric acid (H_3BO_3), 20 mg magnesium sulfate heptahydrate ($Mg_2SO_4 7H_2O$), 30 mg calcium nitrate($Ca(NO_3)_2 4H_2O$), 10mg de potassium nitrate (KNO_3) and 40ml distilled water together with an 80 % of sucrose solution. A drop of the nutrient solution and a drop of the sucrose solution were extracted with a dropper and placed on a slide to mount the pollen from collected anthers using the hanging drop technique described by Stanley and Linskens (1994). The experiment consisted on placing prepared pollen slides from 10 anthers per cultivar under five controlled temperature treatments (20ºC, 25ºC, 30ºC, 35ºC y 40ºC). The experiment was performed using a Panasonic MODEL growth chamber where independent samples were left at a given temperature for about 24 hours after which slides were evaluated under a microscope and germinated and non-germinated pollen grains were counted and calculated the percent germination of pollen grains as the number of germinated grains divided by the total number of pollen grains on the slides. A Three-way ANOVA was used to test for differences in pollen germination with temperature, cultivar and flower gender as main effects.

Pollen viability - To determine the viability of pollen grains, four male and four hermaphrodite flowers were collected per cultivar in the morning following flower anthesis. The anthers were placed in a drop of acetocarmin at 1% (Radford et al., 1974) on a slide where pollen was extracted from the anther. The next day, slides were evaluated under the microscope to count the number of pollen grains with dye (viable) and those that were not to calculate the percentage of viable pollen grains per cultivar, where any pollen that was dyed was considered viable. The percentage viability of pollen of each cultivar were calculated as the total number of pollen grains with dye over the total number of pollen grains counted. Two-way anova was used to evaluate the pollen viability as a function of cultivar and flower gender.

RESULTS

Characterization of inflorescences, floral traits and pollen performance:

Inflorescences - ANOVA analyses showed that a large portion of the variation in inflorescence traits is explained by differences among cultivars but also by significant year*cultivar effects. Analyses detected significant differences among cultivars in inflorescence length but also found a significant cultivar*year interaction (Table 2.1, Fig. 2.2A). On average Keitt produced significantly longer inflorescences relative to Julie with other cultivars (Kent and Tommy Atkins) producing inflorescences of intermediate length relative to Keitt and Julie but significantly different from either cultivar (Table 2.1). However, these relative differences in inflorescence length among cultivars were only apparent in 2017 and not in 2018. In that year, Keitt experienced an average reduction of 8.5 cm in inflorescence length (Fig. 2.2A) while the remaining cultivars presented similar averages for inflorescence length across years (Fig. 2.2A). The number of subdivisions within an inflorescence was significantly different among cultivars but also between years (Table 2.1, Fig. 2.2B). Inflorescences of Tommy Atkins had significantly more subdivisions than the inflorescences of Keitt, Kent and Julie but all cultivars presented less subdivisions in 2018 relative to 2017 (Table 2.1, Fig. 2.2B). However, for Tommy Atkins reductions in the number of inflorescence subdivisions were not significant across years and that was reflected in a significant year*cultivar interaction effect (Table 2.1). The width of the inflorescences was significantly different among cultivars and across years, but this difference was not expressed equally across years (Table 2.1). In 2017, Tommy Atkins and Kent produced significantly wider inflorescences relative to Keitt and Julie but in 2018, Tommy Atkins produced significantly wider inflorescences relative to all the remaining cultivars and the average inflorescence width of the remaining varieties was not statistically different (Table 2.1, Fig. 2.2C).

Flower production –Cultivars differed in the total amount of flowers per inflorescence as well as the total amount of male and hermaphroditic flowers per inflorescence, but cultivar*year interactions were only detected for the total number of hermaphroditic flowers (Table 2.1). In general, Tommy Atkins inflorescences had more flowers and when flower gender was considered, this cultivar produced more male and hermaphroditic flowers throughout the duration of the study (Table 2.1, Fig 2.1D, Fig. 2.3A, B). On average, inflorescences of all cultivars tended to produce fewer total flowers and hermaphroditic flowers in 2018 than in 2017 but in the case of hermaphroditic flowers these reductions were less dramatic in the Julie cultivar than in Kent, Keitt and Tommy Atkins (Table 2.1, Fig. 2.3B). The production of male flowers within an inflorescence was consistent across years regardless of cultivar (Table 2.1, Fig. 2.3A), but more male flowers were produced by Tommy Atkins than by the other cultivars (Table 2.1, Fig. 2.3A). Values for sex ratios of ♀♂ hermaphroditic: ♂ male flowers within inflorescences were lower than one for all cultivars but these exhibited significant differences and that were not manifested consistently across years for all cultivars (Table 2.1, Fig. 2.3C). In 2017, values were lower than 0.5 in for Julie and Tommy Atkins and higher than 0.5 for Keitt and Kent (Table 2.1, Fig 2.3 C). In 2018, inflorescence flower sex ratio was reduced for Kent and Keitt (less hermaphroditic flowers) which changed the relative differences in the inflorescences' flower sex ratios among cultivars in that year almost canceling the relative differences in sex ratios among cultivars that was observed in 2017 (Table 2.1; Fig. 2.3C).

Color UV- Images show that all four cultivars evaluated have white and/or pink petals. Flowers from all cultivars show a prominent line that runs from the nectarium (bottom of the petal) towards the middle of the petal which can be yellow or more reddish. Without the UV filter, these lines are more contrasting in the Keitt and Kent cultivars and less so in the Tommy Atkins and Julie cultivars but they seem to be very prominent under the UV filter regardless of the gender (Fig. 2.4). Under the UV filter, the contrast between the nectar guides and the petal background is also more apparent. Closeup images of the flowers also show morphological differences of the petals between hermaphroditic and male flowers that seem to be pronounced in the Kent and Keitt cultivars which show the tip of the petals folded underneath in hermaphroditic flowers and not in males flowers (Fig. 2.4). When you take the UV photos of the flower, one notices that the nectar guides in petals are less contrasted in Julie relative to the other cultivars. (Fig. 2.4A to P). Keitt flowers present different color patterns relative to the other cultivars in the visible and the UV spectrum (Fig. 2.4A to P). Slight differences in color patterns between males and hermaphroditic flowers are also apparent in Keitt but not so in the other cultivars.

Sugar Content- Results on the sugar profiles showed several patterns in terms of the variation in sugar concentration and relative percentages of sugar compositions (Table 2.2). First, for all cultivars, nectars exhibited higher concentrations of sucrose relative to glucose and fructose with glucose concentrations showing intermediate values between sucrose and fructose with the exception of Julie which had equivalent concentration values of glucose and fructose (Table 2.2). When cultivars were compared, concentration values for sucrose and glucose were 2-3 times higher in Kent and Keitt relative to Julie and Tommy Atkins and concentration values for fructose were twice higher for Keitt relative to the other cultivars (Table 2.2). When evaluating the relative percentage of sugars within a cultivar, the values for glucose and fructose were 4-5 times lower than those of sucrose in Julie, Kent and Keitt, but only twice as low in Tommy Atkins (Table 2.2).

Number of pollen grains- ANOVA tests found significant differences in the number of pollen grains per anther among cultivars but no significant differences between gender nor significant cultivar*gender interactions for both flower genders (Fig. 2.5). Tommy Atkins anthers produced the highest amounts of pollen per anther and Julie anthers the lowest with Kent and Keitt producing intermediate amounts and statistically similar to each other (Figure 2.5).

Temperature effects on pollen performance

Pollen germination experiment - Results showed that in vitro pollen germination occurred in all tested temperatures but Three-way ANOVA analyses indicated significant cultivar, gender and temperature effects as well as significant interaction effects (Figure 2.5). As general rule, percent pollen germination for all cultivars exhibited an optimal value around a given temperature or range of temperature which varied across cultivars (i.e. Julie: 25°C-30°C, Kent: 20°C-30°C, Keitt: 25°C-

35°C, Tommy Atkins: 25°C- 30°C, Fig. 2.5). Also, for all cultivar, pollen from male flowers had higher germination percentages than pollen from hermaphroditic flowers at temperatures above the optimal value but at temperatures below the optimal, they had a tendency for lower germination percentages relative to hermaphroditic flowers although this pattern was only significant for Kent and Keitt (Fig. 2.5). Gender differences in average pollen germination percentage were largest at the temperature extremes above the optimal values for percent pollen germination but even more so for the high temperature extreme (20°C vs 40°C: Julie 9% vs 32%, Keitt 8% vs 37%, Kent 18% vs 32%, Tommy Atkins 13% vs 22%) and these trends were consistent for all cultivars (Fig 2.5). There were no differences in the percentage of germinated pollen grains at the optimal temperature values for any of the cultivars (Fig 2.5).

Pollen viability – Pollen viability tests using pollen dye indicated high viability for all cultivars (87% - 99%) irrespective of gender (Table 2.3). There no significant differences between cultivar or gender (Table 2.3).

DISCUSSION

As expected, the four cultivars studied showed high variability in reproductive traits related to inflorescence size and architecture, flower advertisement (color and nectar sugar content), pollen production and performance and flowering. Below we discuss the implications of this variability in lieu of our original hypotheses and how these traits may have been influenced by environmental change given our results and the broad literature available on mango reproduction. We also discuss the implications of our results to fruit yields in the Caribbean under current projections for climate change in the region.

Cultivar origin vs tree size - Traits related to reproductive effort such as inflorescence size, flower and pollen production are often positively correlated with plant size (e.g. Lauri et al., 1996; Otarola et al., 2013; Chaudhari and Singh, 2019). Indeed, life history theory on the evolution of reproductive organs is based on the premise of trade-offs between plant growth and reproductive effort (Andersson, 2003). However, variability in reproductive organs may also originate from genetic variation (Barrett and Harder, 1996). The literature suggests that mango cultivars vary widely in reproductive traits presenting a wide range of inflorescence sizes, architecture (i.e, number of subdivisions and number of flowers per inflorescence (Singh, 1997; Mukherjee and Litz, 1997; Gehrke-Vélez, 2007; Gehrke-Vélez et al., 2011). In this study we examined three cultivars of Floridian origin (Keitt, Kent, Tommy Atkins) and one cultivar of Caribbean origin. The maximum tree heights reported for these cultivars are notably different and Keitt, Kent and Tommy Atkins reported as the tallest variety and Julie the smallest (Campbell,1992; Willis and Marler, 1992). Thus, our two complementary hypotheses were that reproductive traits in Floridian cultivars would be more similar to each other than to the Caribbean cultivar and that for continuous reproductive traits (inflorescence length, width, and branching, number of flowers per inflorescence, and pollen production), differences among cultivars would be consistent with their reported height differences where larger cultivars would exhibit larger values for these traits. The combined results of this study do not support the hypothesis of morphological convergence based on geographic origin of the cultivars. On the other hand, the support for the size hypothesis is weak but present for some traits. One of the tallest cultivars Tommy Atkins, presented higher values relative to the other varieties that were temporally consistent for flower production and inflorescence subdivision. Meanwhile the Julie cultivar, with the shortest tree form, presented smaller values relative to the other cultivars only for inflorescence length and only for one year. Indeed, the combined results suggest that many of the values for reproductive traits measured in this study changed dramatically across years. One hypothesis is that these changes may result from environmental variation (see below) which is consistent with *M. indica* studies (Asif et al. 2002; Litz 2009; Ramírez and Davenport, 2012). While, mean inflorescence size for Keitt, Kent and Tommy Atkins are certainly within the ranges reported for these cultivars in Egypt (Abourayya et al., 2011), reported inflorescence length for Tommy Atkins is much larger in this study compared to that of a separate study in Colombia (Corredor and García, 2011) (28.7 5cm vs 21.13 cm respectively) which further supports the idea that these inflorescence traits may be highly plastic and most likely influenced by environmental factors. Observations from other flower characteristics (color and nectar) were qualitative and do not allow us to test how variable these traits are within or between cultivars. However, at least qualitatively, flower color and nectar composition do not help distinguish between Caribbean and Floridian cultivars. The tallest cultivars (Keitt and Kent) had higher values for sugar contents relative to Tommy Atkins and Julie. One interesting finding was the presence of UV reflection in *M. indica* flowers with one cultivar showing UV patterns (via contrasts) not present in the other cultivars where the UV reflection is more evenly distributed. The presence of UV reflection has been studied extensively and is deemed important in flower detection by animal pollinators and specially insects (Bargini and

Medeiros, 2012). Results on the influence of UV patterns on the behaviors of different types of insects is insects has been equivocal with some experimental studies arguing that these patterns may be more important for the attraction of hymenopterans than dipterans (Klomberg et al., 2019) and others finding that their presence may be equally important for these groups (Koski and Ashman, 2014). To the extent that cultivar differences in flower color or nectar were consistent, one question is whether or not observed differences in flower color or nectar may lead to differences in the types of pollinators that visit the different cultivars. In at least one study, the Keitt cultivar was reported to have low attractiveness relative to Kent and Tommy Atkins (Carvalheiro et al., 2012). However, prior studies evaluating the pollinator communities of these cultivars at JDAES did not see consistent differences among these cultivars in the abundance of the three dominant flower visitors (*Palpada vinetorum, Apis mellifera and Cochliomyia minima*) visiting these different the different areas where these cultivars are located (Cabrera-Asencio and Melendez-Ackerman 2021*)* suggesting that at least for the most dominant flower species these qualitative differences in color-nectar features may not be important although one cannot discard potential effects of other less common flower visitors.

Environmental change and reproductive traits - Results showed a tendency for reduced production of total flowers and hermaphroditic flowers per inflorescence in 2018 relative to 2017. Reduced inflorescence size was also observed for Keitt in 2018 and in that year reduced inflorescence subdivision was observed for all varieties except for Tommy Atkins. These reductions could have been associated with the passage of hurricanes Irma and María in September of 2017 recorded on Puerto Rico landfall as Category 1 and Category 4 storms respectively. These events especially María, led to considerable changes island-wide tree damage and mortality (Zimmerman et al., 2021; Lugo, 2020). One hypothesis is that reduced photosynthetic capacity (via defoliation) following events can lead to reduced reproductive effort following Hurricane events (Rathcke, 2000) but hurricane-driven effects on plant reproduction in perennial systems are not common and results do not always support this conclusion. For example, in South Florida, the introduced understory shrub, *Ardisia escallionoides* showed increased flowering following the passage of hurricane Andrew (Pascarella, 1998). Individual trees in this study exhibited little mortality but did experienced damage and defoliation and increased reproductive effort was attributed to reproductive release following canopy opening and increased light environments. Mango fruit yields were severely reduced following the passage of Hurricane Andrew in Florida and many years after this event but these reductions were coupled with high levels of tree hurricane-induced tree mortality and severe structural tree damage in addition to defoliation (Crane et al., 1993; Crane and Balerdi, 1996). At JDAES, none of the mango trees suffer mortality from the 2017 hurricane events although most experienced considerable defoliation (Cabrera-Asencio and Melendez-Ackerman 2021). While defoliation in perennials can lead to reduced capacity for growth and reproduction, these effects are not necessarily universal and can manifest in the form of tradeoffs (e.g., increased growth and reduced reproduction or vice versa, Koptur et al., 1996 and references therein) or reproductive responses that may compensate loss of vegetative resources. Flower production in *M. indica* growing at JDAES was reduced following the hurricane events in all cultivars following the 2017 events (this study), but reduced fruit production did not necessarily follow (Cabrera-Asencio and Melendez-Ackerman 2021). This was unexpected given that some cultivars experienced a reduction in hermaphroditic flowers and their proportion is an important determinant of influence fruit set in *M. indica* (Iyer et al., 1989; Chaikiattiyos et al., 1996). It is known that inflorescences of *M. indica* often produce many more flowers than fruits and that fruit sets are often low (< 12%, Shü, 2006). Thus, it is possible that the capacity to produce numerous flowers can help *M. indica* (at least for some cultivars) to compensate for the loss of photosynthetic resources following the hurricane events in terms of fruit production even when the number of flowers is reduced at least if tree structural damage following these events is minimal. Compensatory reproductive responses following hurricane defoliation may also be influencing resource allocation decisions at the inflorescence level in terms of the allocation of resources to produce male vs female flowers at least in some cultivars. In *M. indica*, sex ratios have been found to respond to environmental factors and their variation can influence fruit set (Geetha et al., 2016, Ramirez and Davenport, 2016). Sex allocation theory predicts that when resources are limited, the production of male flowers should be favored over females (or hermaphrodites which have both female and male functions) as a way to optimize the use of resources for reproduction (Primack and Lloyd, 1980; Elle, 1999, 2000; Obeso, 2002; Moore and Pannell, 2011). In this study, two out of four cultivars modified their sex ratios such that the production of hermaphroditic flowers (presumably more costly than male flowers) was reduced in the 2018 flowering season following the hurricane events which is consistent with this hypothesis. Results from a variety of studies also suggest that there are potential for trade-offs related to the allocation of resources between reproductive structures or between growth and reproduction is possible in *M. indica* (Geetha et al., 2016). In *M. indica,* sex ratios have been found to respond to genetic and environmental factors and their variation can influence fruit set (Geetha et al., 2016; Iyer et al., 1989; Chaikikiattiyos et al.,1996; Ramirez and Davenport, 2016). However, it seems reducing fruit yield with reduction in hermaphroditic flowers tends to occur around a threshold sex ratio value of 0.04 (or 4% of the total flowers, (Davenport and Nunez-Elisea, 1997). Differences in sex ratios may also respond to genetic signatures among cultivar as shown by different recorded sex ratios among different cultivars (some cultivars showing low ratios of hermaphrofitic:male flowers and other showing high ratios) even when grown under similar conditions (Geetha et al., 2016; Iyer et al., 1989; Chaikiattiyos et al., 1996). Nevertheless, it should be pointed out that even within a cultivar, sex ratios may show considerable geographic variation in their degree of maleness. All mango cultivars reported here produced inflorescences with very low hermaphroditic: male flower ratios (<<1) but three of those varieties, Keitt and Tommy Atkins have been known to produced inflorescences with very high hermaphroditic to male flower ratios elsewhere (Sex ratios: Keitt = 1.44 in Shu 2006; Keitt = 1.76 and Tommy Atkins = 1.34 in Abourayya et al. 2011, Keitt = 1.45, Kent = 4.0 and Tommy Atkins = 27.0 in Coetzer et al.,1995).

Pollen traits – Temperature variation had significant influences in pollen germination in *M. indica* but these effects manifested differently across cultivars and across genders. Grains showed optimal germination rates at some intermediate temperature with pollen from hermaphroditic flowers having higher germination rates than pollen from male flowers at low temperature but lower germination rates at high temperatures. Pollen germination is influenced by a myriad of enzymatic reactions and enzymatic reactions are bound to be influenced by temperature influences in non-linear ways. Pollen germination is known to be sensitive to elevated temperatures (Halterlein et al., 1980; Bajaj et al., 1992). The presence of flower gender differences in pollen germination in *M. indica* is an interesting observation that suggests physiological differences between the pollen grains that come from hermaphroditic and male flowers.

Pollen germination and viability in *M. indica* are traits considered to be cultivar and temperature (Abourayya et al., 2011; Singh, 1954; Ramirez and Davenport, 2016; Perez et al., 2019). Pollen viability (using staining techniques) in this study was comparable or else higher (>90% viability all cultivars), with what has been reported in other studies that also evaluated Keitt, Kent, Tommy Atkins (Dag et al., 2000; Abourayya et al., 2011, Jayaraman et al., 2016) and other mango cultivars (Sukhvibul et al., 1999, Ramirez and Davenport, 2012). However, this study did not evaluate pollen viability under different temperature environments and that is something that should be done in future studies as it has been shown that pollen viability in mango may vary as a function of temperature at least in some varieties (Issarakraisila and Considine, 1994). On the other hand, similar pollen viabilities among cultivars and flower genders in this study at least suggests that observed differences among cultivars and flower genders in pollen germination across different temperature regimes are unlikely to be related to initial differences in pollen viabilities among cultivars or flower genders. Whenever examined, pollen germination of different plant species or crops often germinate best at some optimal temperature value where germination is often decreased below or above that temperature value (Hedley et al., 2004; Irenaeus and Mitra, 2014). Consistent with this pattern, our results do show that pollen from each cultivar seems to have an optimal value for pollen germination, but that value is not necessarily the same for all cultivars. This is also consistent with experimental studies evaluating the thermal responses of pollen germination from different cultivars of *M. indica* (Dag et al., 2000; Sukhvibul et al., 2000; Perez et al., 2019). However, most studies in *M indica* have focused on evaluating temperature responses to values below the optimum (i.e., cold stress) rather than heat stress (but see Dag et al., 2000) and only one has addressed the potential for differences in pollen germination performance between pollen grains from male and hermaphroditic flowers (Pérez et al., 2019). Pérez and collaborators (2019) evaluated germination between mango pollen grains from male and hermaphroditic flowers over different temperature treatments in four mango cultivars that included the Keitt and Kent cultivar but did not differ in pollen germination rates. Only one variety (Kent) showed significant differences in pollen germination between pollen grains from different flower genders (hermaphroditic > male) under cold stress and only at the lowest temperature regime. However, our study did show clear differences in pollen germination between pollen from male and hermaphroditic flowers under heat stress with male pollen performing better at high temperatures and female pollen performing better at low temperatures in two out of four cultivars. Therefore, pollen grains from the same plant, even when genetically equally not only are showing different phenotypic responses to temperature-stress but also its relative expression (in terms of pollen germination) changes depending on whether temperature extremes relate to cold stress or heat stress. This suggests that mechanisms influencing tolerance to temperature extremes may be different at low vs high temperatures at least for some cultivars. Hypotheses about what mechanisms may drive these differences may come from the literature on the physiology of pollen germination and literature about the evolutionary drivers of sex expression. We know that heat stress can reduce pollen germination in crops like cotton (Kakani et al., 2005), soybean (Djanaguiraman et al., 2013) and others (Paupiére et al., 2014). Although the physiological mechanisms of heat tolerance have yet to be addressed in mango, there is growing evidence from other systems as to the potential mechanisms by which pollen grains may deal with heat stress even when there are still many unknowns. Pollen responses to heat stress may rely on the rapid activation of genes that lead to rapid accumulation of heat shock proteins (HPs) (Muller and Rieu, 2016; Raja et al., 2019), which are high molecular weight proteins in charge of slowing down the process of enzyme degradation to high temperatures (Raja et al.,2019). Their production is most likely influenced by nutrient availability. Pollen germination requires energy which in its initial phase relies on nutrients from stored reserves (autotrophic phase) and in later stages on nutrients from the stigma (heterotrophic phase) to provide nutrients for pollen tube growth (Garcia et al., 2017). The ‘male vs female function’ hypothesis in angiosperms states that in co-sexual plant species, differential allocation between the male and female functions of a flower may be favored by selection when resources are limited (Charlesworth and Charlesworth, 1981; Lloyd,1984; Brunet, 1992). Experimental studies with *Nigella sativa* (Ranunculaceae) have shown that physiological trade-offs between the pistil and anthers are possible (Andersson, 2003). If resources provided to male and hermaphroditic flowers in *M. indica* are also finite during flower development, then one possibility is that there are stored resources need for the production or expression of proteins involved in heat stress responses may be more available in pollen grains from male flowers than in hermaphroditic flowers. While negative correlations between pistil and pollen functions are not necessarily universal (Brock et al., 2017; Yang and Guo, 2004; Campbell, 2000), the potential for differential resource allocations between the male and female functions in mango has been invoked to explain variability in other floral traits such as sex ratios between male and female flowers (Asif et al., 2002). While a decline in pollen germination under high heat stress may come about a variety of physiological mechanism (i.e. decrease cell membrane stability, enzyme degradation, reduced metabolic response; Rieu et al., 2017, Raja et al., 2019), these alone cannot explain differences in pollen germination success between male and female flowers.

In agreement with other studies (Abourayya et al. 2011; Asif et al. 2002; Corredor 2011) the results show high variability in characters related to inflorescence size, flower production and sex ratio. Future studies should address how this variability will be influenced by changes in climatic variables as predicted by climate models vs. other factors (resource availability). Of particular importance is how thermal stresses will affect the expression of floral traits that are known to influence fruit production. Results showed that pollen viability decrease considerable in these four cultivars with temperature increases. Climate models in the Caribbean predict an increase of 2 to 5 °C (Campbell et al. 2011), and world statistical models project increases of 2.7 °C by 2050 and 4.6 °C by 2090 (Meehl et al., 2007; Girvetz et al., 2009). Considering the ranges in average temperatures at JDAES (22°C and 33°C) and that thermal tolerances for mango pollen most likely lie between 10°C to 33°C (Issarakraisila and Considine, 1994), these increases if they were to materialize would create added pressures in this crop. Evaluating other mango cultivars and germplasms to determine which genetic strains are more tolerant and susceptible on different temperature environments would be important. Establishing alternate sites in cooler areas for this mango germplasm may also be important to preserve living mango germplasm as well.

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Table 2.1. Anova analyses for the floral characterization per cultivar per two-year. SE values represent standard errors around the means.

Anova	Length of Inflorescences (cm)		# Subdivisions		Inflorescences width	
Model	F	P	F	P	F	P
Year	4.31	0.0395	8.49	0.0041	7.65	0.0001
Cultivar	6.59	0.0003	22.77	<0.0001	0.79	0.3756
Cultivar*Year	4.59	0.0042	2.37	0.0725	5.00	0.0027

Anova	# Flowers		# Male flowers		# Hermaphrodite Flowers		Sex ratio	
Model	F	P	F	P	F	P	F	P
Year	4.50	0.0356	0.39	0.5317	2.98	0.0865	2.17	0.1432
Cultivar	17.07	<0.0001	32.92	<0.0001	19.89	<0.0001	5.98	0.0007
Cultivar*Year	1.72	0.1648	0.36	0.7852	6.47	0.0004	4.70	0.0036

Table 2.2. Mean sugar content in the nectar of flowers of each mango cultivars: Julie, Keitt, Kent and Tommy Atkins. The mean (+S.E.) were obtained from the concentration and the percentage of glucose, fructose and sucrose, (n=2).

Cultivar	Sucrose Concentration µg/mL	% Sucrose*	Glucose Concentration µg/mL	% Glucose	Fructose Concentration µg/mL	% Fructose
Julie	235.45 ± 14.84	1.29 ± 0.19	69.51 ± 5.57	0.38 ± 0.06	64.70 ± 7.19	0.36 ± 0.07
Keitt	729.21 ± 167.18	4.92 ± 0.82	211.45 ± 43.12	1.43 ± 0.20	114.75 ± 22.59	0.78 ± 0.10
Kent	550.93 ± 124.98	5.17 ± 0.23	133.90 ± 80.93	1.12 ± 0.45	80.65 ± 5.88	0.79 ± 0.16
Tommy Atkins	335.95 ± 87.80	1.92 ± 0.76	110.73 ± 34.86	0.64 ± 0.28	74.03 ± 8.74	0.41 ± 0.11

* The percentage is the concentration per volumen over the weight (µg) of the nectarium.

Table 2.3. Anova analyses for viability per gender. Mean (±SE) of percent of pollen viability of male and hermaphrodite flowers in four cultivars of *Mangifera indica* (n=4). SE values represent standard errors around the means.

Cultivar	% Viability of male flowers	% Viability of hermaphrodite flowers
Julie	87.00 ± 10.50 a	93.00 ± 4.73 a
Tommy Atkins	94.00 ± 6.00 a	99.00 ± 5.81 a
Keitt	95.50 ± 3.86 a	99.00 ± 1.00 a
Kent	87.00 ± 7.55 a	92.00 ± 5.23 a
Model	F	P
Cultivar	1.01	0.4050
Gender	1.41	0.2469
Cultivar*Gender	0.20	0.9979

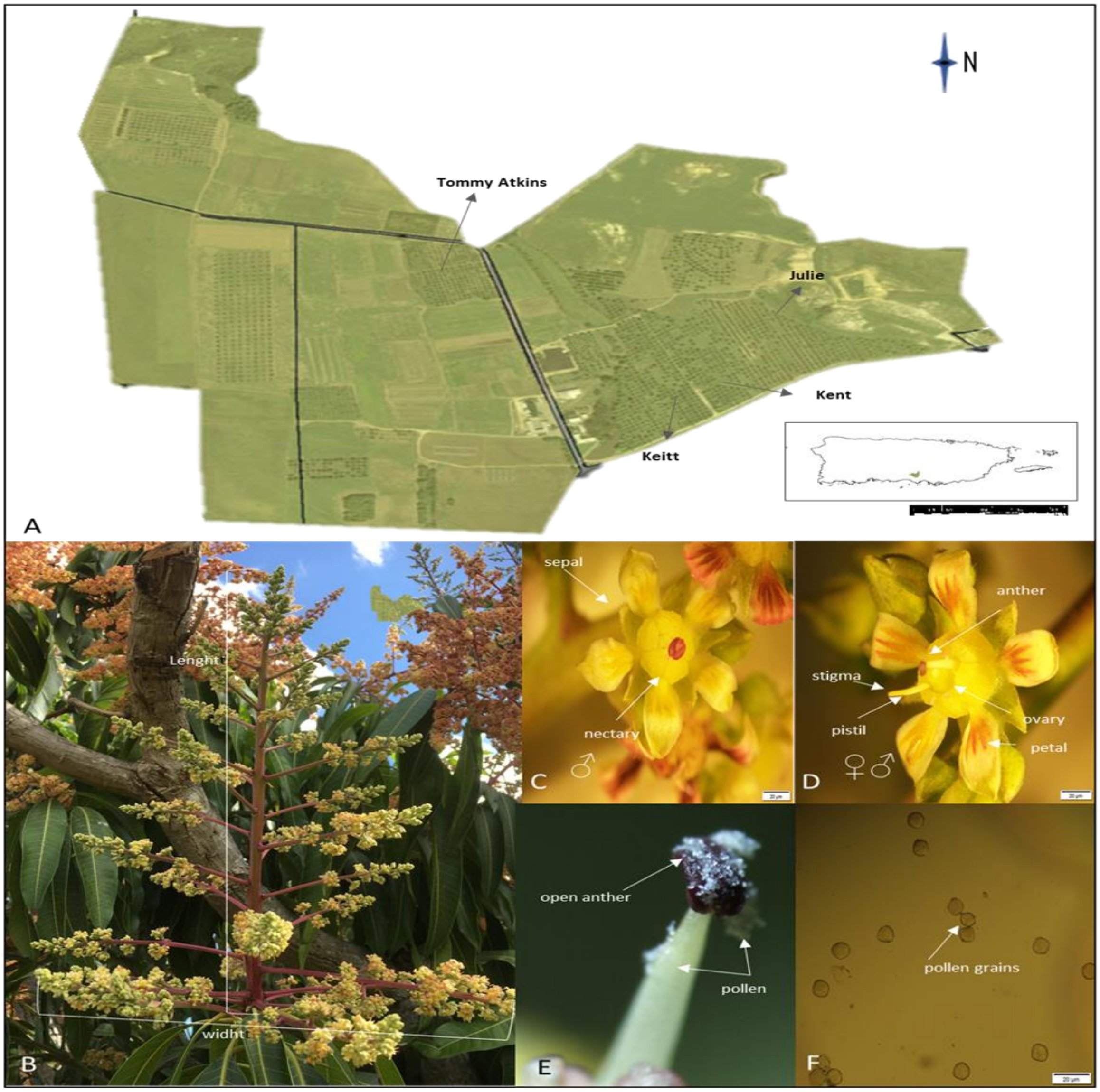

Figure 2.1. (A) Location of Juana Diaz Agricultural Experimental Station indicating the location of the mango germplasm of the four cultivars Julie, Keitt, Kent and Tommy Atkins used in this study. (B) Inflorescences of the Tommy Atkins cultivar, length and width take in the inflorescences. (C) Keitt male flower, parts of flower sepal and nectary. (D) Keitt hermaphrodite flower, parts of flower pistil, anther, ovary, petal. (E) Open anther and pollen of Kent. (F) Pollen grains of Julie.

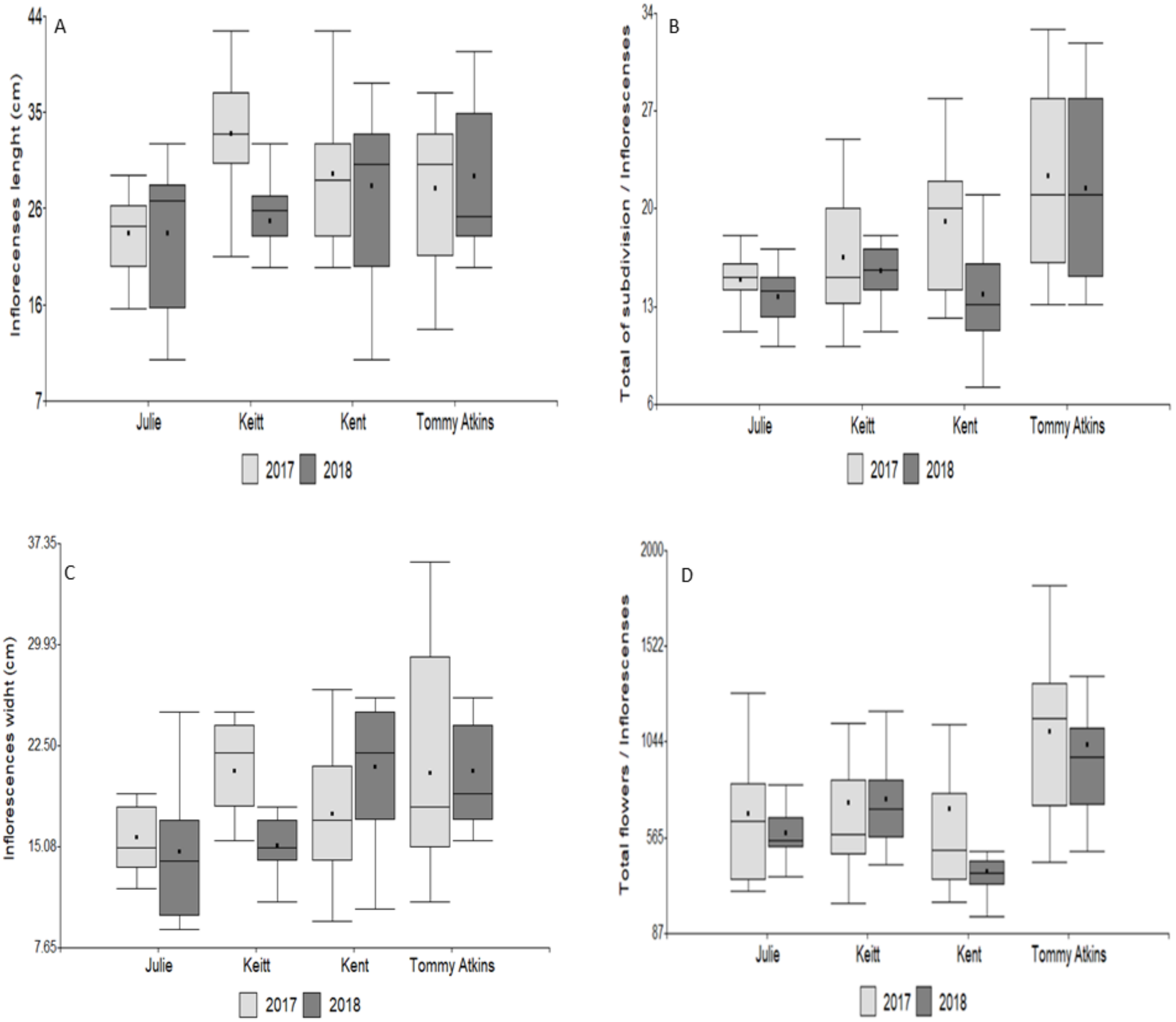

Figure 2.2. Floral Characterization of four mango cultivars Julie, Keitt, Kent and Tommy Atkins. A) Inflorescences length, B) Total of subdivisions in the inflorescences, C) Inflorescences width, D) Total flowers per Inflorescences.

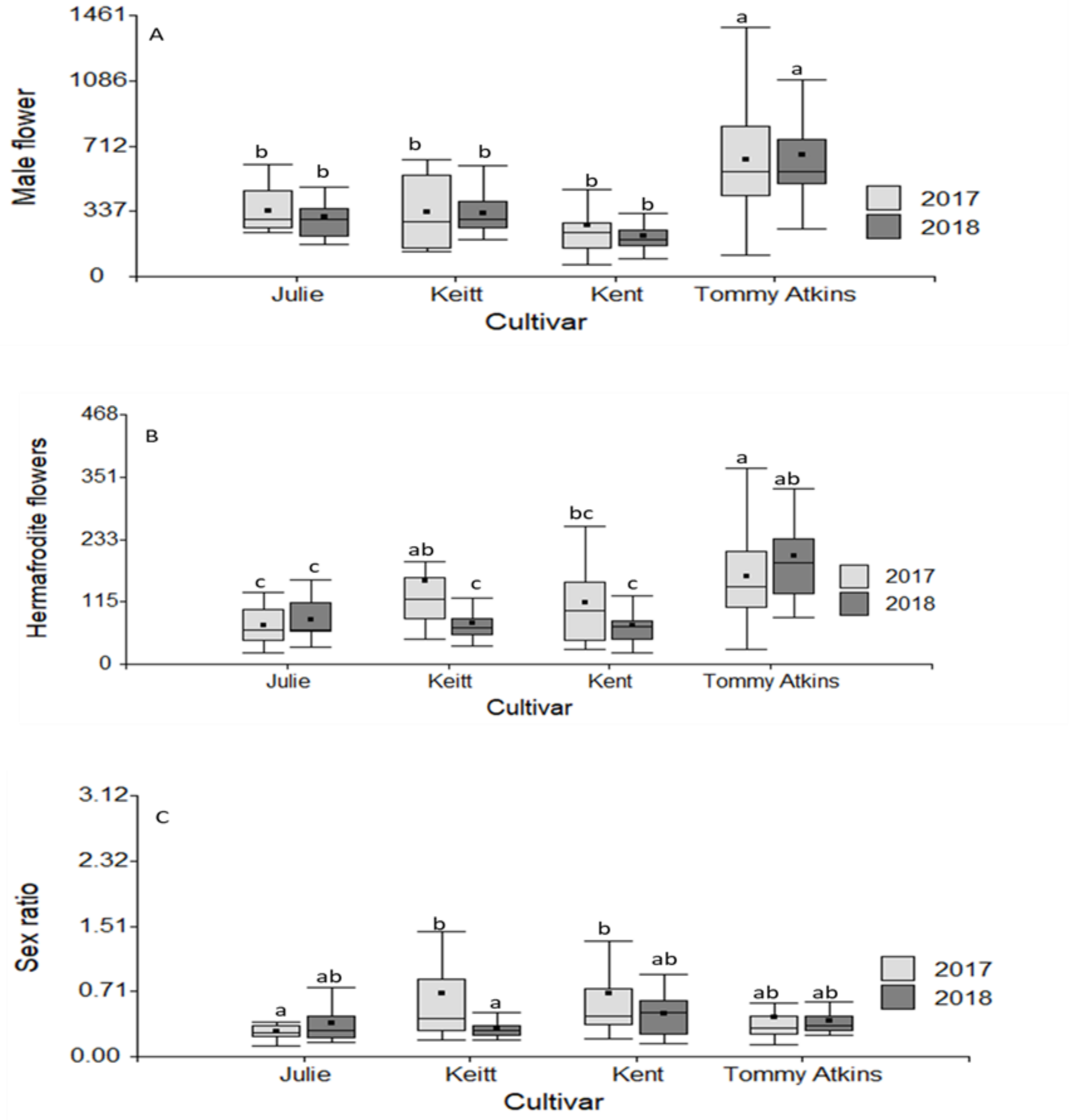

Figure 2.3. A) Number of males, B) hermaphrodite and C) sex ratio flowers per Inflorescences per cultivar (Julie, Keitt, Kent, Tommy Atkins) per year 2017 and 2018.

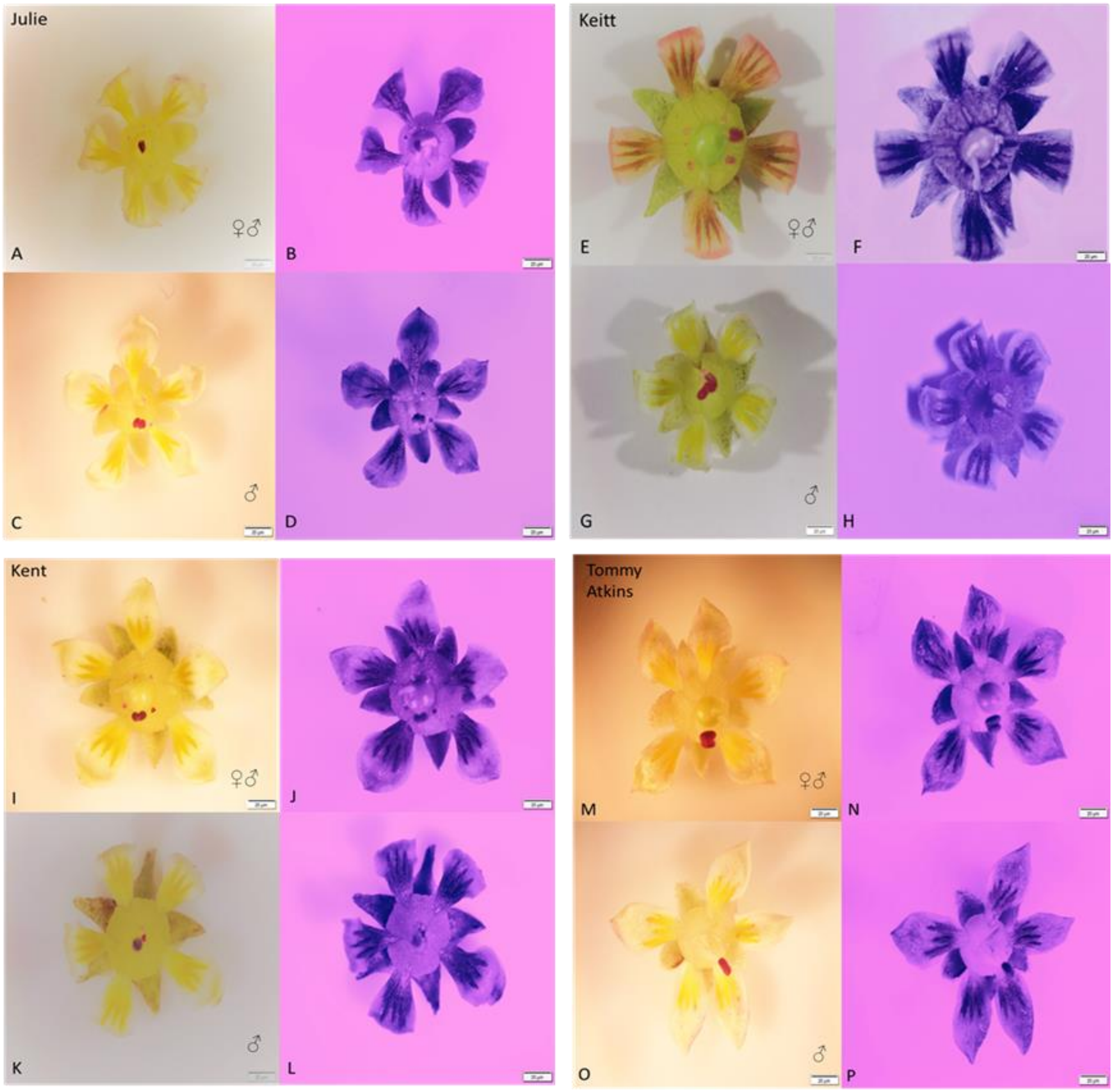

Figure 2.4: Flower color displays of hermaphroditic (left) and male (right) flowers with (white background) and without (pink background) a UV filter. **Julie** - A) hermaphrodite flower without UV, B) hermaphrodite flower with UV, C) male flower without UV, D) male flower with UV; **Keitt** E) hermaphrodite flower without UV, F) hermaphrodite flower with UV, G) male flower without UV, H) male flower with UV; **Kent-** I) hermaphrodite flower without UV, J) hermaphrodite flower with UV, K) male flower without UV, L) male flower with UV: **Tommy Atkins-** M) hermaphrodite flower without UV, N) hermaphrodite flower with UV, O) male flower without UV, P) male flower with UV.

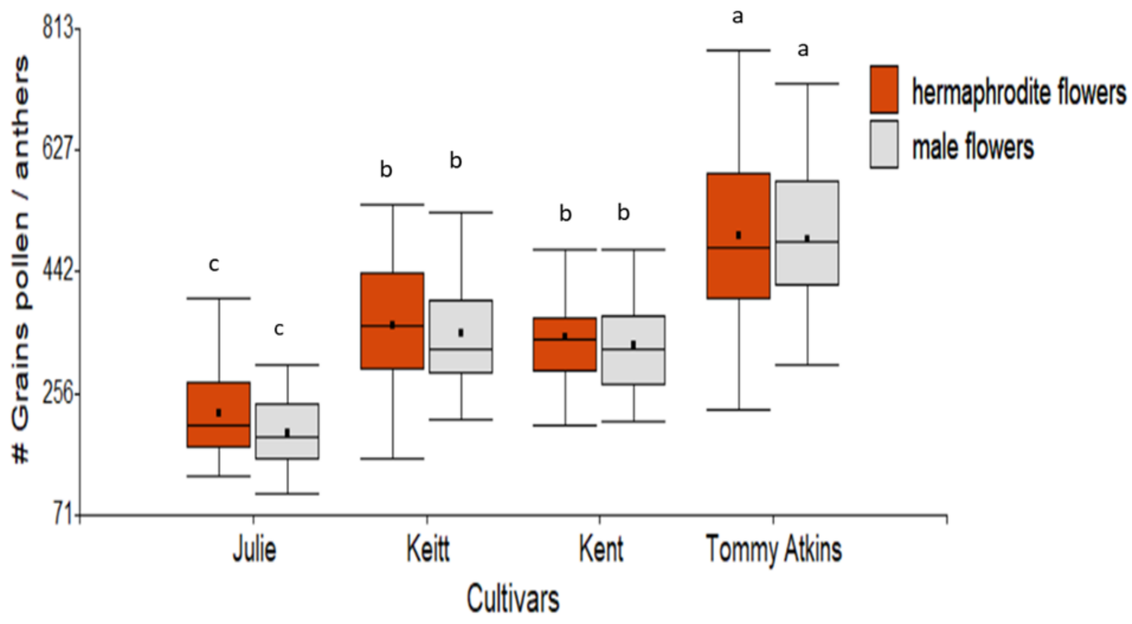

Figure 2.5. Numbers of Grains polen per hermaphrodite and male flowers per cultivar. Results of the Anova for: Cultivars F= 161.31, P <0.0001; Gender F=2.91, P = 0.0890; Cultivar*Gender F=3.98, P=0.8074. Bars represent means ± SD, bars with different letters indicate significantly differences at, p<.05, (Di Rienzo et al.2018).

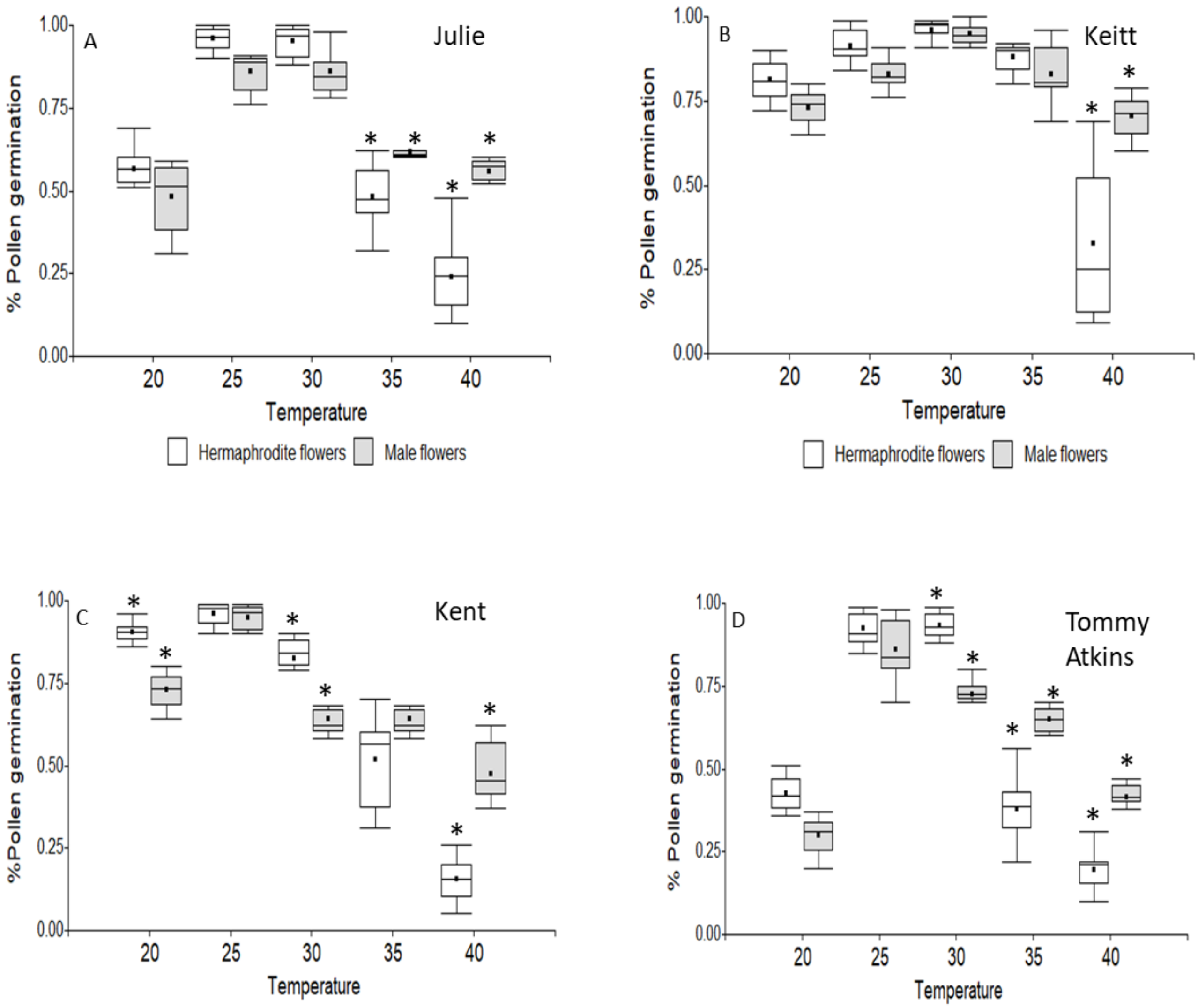

Figure 2.6. Results on the percent in vitro pollen germination under five controlled temperatures (20°C, 25°C, 30°C, 35°C, 40 °C) for four cultivars of M. indica. A) Julie, B) Keitt, C) Kent, D) Tommy. Results of the Anova for: Cultivars F= 143.49, P <0.0001; Gender 11.07, P = 0.0010; Temperature F=656.07, P=0.0001; Cultivar*Gender F= 0.44, P=0.7259; Cultivar*Temperature F=47.79, P=0.0001; Gender*Temperature F=128.92, P=0.0001; Cultivar*Gender*Temperature F=7.45, P=0.0001. Bars represent means ± SD, bars with different letters indicate significantly differences at, p<.05, (Di Rienzo et al.2018).

CHAPTER III

BREEDDING SYSTEM EVALUATION OF FOUR MANGIFERA INDICA L. CULTIVARS

ABSTRACT

Germplasms in the form of living collections also can make important contributions to the understanding of how processes that occur at the pollination stage influence reproduction and plant yields. This study evaluated the breeding system of four commercial cultivars (Julie, Keitt, Kent and Tommy Atkins) at the Juana Diaz Agricultutal Experimental Station (JDAES) in Puerto Rico to evaluate their degree of self-compatibility, the dependence of their reproductive systems on flower visitors to set fruit and the potential effect of pollen type (self *vs* cross *vs* open pollination) on their seed and fruit performance using artificial pollination experiments. Open natural pollinations tended to produce more fruit and yielded seeds with faster development times than artificial pollinations in three of the four cultivars (Julie, Tommy Atkins and Keitt). They also resulted in faster seed germination in two cultivars (Julie and Tommy Atkins). The combined results may suggest that not only animal-mediated pollination is needed for these cultivars but that this mode of pollination also results in improved yields, fruit, and seed traits. They highlight the importance of studying the breeding system of all living germplasm and for *M. indica*, a fruit crop with variable breeding systems throughout its distribution results reiterate the importance of local pollinators and the need to evaluate the circumstances by which pollen donors may influence fruit characteristics in this crop.

INTRODUCTION

The protection and conservation of germplasm material from any agricultural crop is essential to global food security and has become a priority given the loss of biological diversity worldwide (Thrupp, 2000; FAO, 2018). A crop’s germplasm constitutes the collective catalogued genetic pool available (wild and domesticated) for crop development and improvement (Orton, 2019). Germplasms provide the raw material to develop new crops and as such it is the foundation for current and future agricultural development (Peefers and Calwey, 1988; Nwachukwu et.al. 2016). Many ex-situ germplasms collections of agricultural crops are maintained as seeds and tissue but also as field living collections of old, current and wild varieties of crops with the purpose to study, manage, use and conserve their genetic diversity (National Research Council, 1991). While seeds are the primary collection for herbaceous plants whether annual or perennial, woody perennial crops rely heavily on living collections because their longer life history cycles and different seed characteristics (e.g., larger, heavier, fleshier) preclude many of these cultivars from relying on seed-based genetic banks (Migicovsky et al., 2019). This is particularly true for tropical fruits whose fleshy fruits with high moisture content prevent seed storage and transport through conventional cold dry storage methods (Bonner 1990, Tweddle et al., 2003; Li and Pritchard, 2009; Walters et al., 2013). Living collections of woody perennial crops while expensive to maintain, have the added value of offering important opportunities to conduct biological research to support conservation and management of agricultural diversity (Migicovsky et al 2019).

Basic biological information of agricultural plant species such as its flowering biology, mating systems, seed production and viability are important aspects of the long-term effective management and future use of agricultural germplasms (Engels and Visser, 2003). For example, determining seed vigor, in addition to germination percentage, could provide the germplasm's curator with early indications of viability decline in the genetic stock (Bewley and Black, 1994). Knowledge of the mode of reproduction of a crop and how it reproduces naturally would be essential to evaluate which possible methods of reproduction can be employed for artificial breeding, for crop improvement and to predict a cultivars' behavior under field conditions (Fryxeli, 1957). A crop's breeding system will also influence the level of genetic variability present in the crop population (Camarena et al., 2012). The role of animal pollinators in agriculture is often cited as an important and endangered ecosystem service, yet for many crops that data is absent, incomplete or in need of updating (Klein et al., 2007). For tropical crop species, an estimated 70% of them, have cultivars where production is improved by animal pollination (Roubik, 1995). Even when during the process of domestication, self-pollination has often been a favored trait, many crop species are predominantly cross-pollinated (Halloran and Luckett, 1994). Woody perennial crop species tend to be predominantly outcrossing (Migicovsky et al., 2019), and would need to rely on animal pollination for reproduction. When crop plants may exhibit self-compatibility seed quality may be enhanced by outcrossing (Richards, 2001). However, without reliable information on the breeding system and the mechanics of natural pollination, identifying the factors that lead to failure or success in crops of interest will be difficult.

Mangifera indica (mango) is considered the second most important tropical fruit and in terms of production this food plant is the fifth largest fruit crop in the world behind bananas, grapes, apples and oranges and the second most important tropical fruit crop (Bally et al., 2009). The species, originally from Asia, has had a long and complex history of domestication with over 1000 cultivars developed and established throughout the worlds' tropics (Litz, 2009; Warschefsky and Westberg, 2019). It has been claimed that almost all known cultivars have originated from the selection of chance seedlings from natural cross-pollinations (Iyer and Schenell, 2009). Yet, the literature suggests that the breeding and mating systems of this tropical fruit crop is highly diverse among cultivars. For example, some mango cultivars such as Dasheri and Ataúlfo are self-incompatible (Singh et al., 1962; Davenport, 2009) and it is assumed that insects play an essential role in their pollination even if the role of particular pollinator species is not understood (Anderson et al., 1982). In contrast, cultivars such as Sensation, Osteen, Keitt and Kent reportedly show capacity for self-pollination (Dijkman and Soule, 1951; Perez et al., 2016). However, in the case of the Osteen, Keitt and Kent cultivars, molecular tests show a higher percentage of progeny resulting from outcrossing mating relative to selfing (Perez et al., 2016). It has even been suggested that wind and the falling of grains by gravity could facilitate pollination in some mango cultivars but experimental data has shown that even if possible, pollination success is very low with this mechanism relative to cross-pollination (Popenoe, 1917; Maheshwari, 1934; Mallik ,1957; Singh, 1997; Singh and Sharma, 1972). One study suggests that cross-pollination may lead to higher fruits sets than self-pollinations but that this may vary among cultivars (Paull and Duarte, 2011). The combined data available for *M. indica* cultivars from the literature suggest that their mode of reproductions is understudied and incomplete given the large number of cultivars available.

This study aimed to characterize the breeding system of four commercial cultivars of *M. indica* (Julie, Keitt, Kent and Tommy Atkins) grown at germplasm had the Juana Diaz Agricultural Experimental Station (JDAES) in Puerto Rico to evaluate their degree of self-compatibility, the dependence of their reproductive systems on flower visitors to set fruit and the potential effect of pollen type (self vs cross vs open pollination) on their seed and fruit performance. Of these four cultivars three of them come from Florida material (Keitt, Kent, Tommy Atkins) being part of a germplasm of 84 mango cultivars planted in 1962. In this study, I will investigate the following question: 1) How dependent is the breeding system of these cultivars on animal pollination? 2) What is the effect of mating type (self vs cross-pollination) on reproductive success, fruit and seed traits of these cultivars? To answer these questions, the study evaluated two hypotheses. First, I expected different cultivars to show different breeding systems, where some cultivars will necessarily depend on pollinators and others will not. Second, I expected that the different mating types would show differences in fruit set, fruit and seed traits under the assumption that cross-pollination may influence the genetic variation and phenotypic expression of the developing embryo and therefore the quality of fruits and seeds. There are many crops of local economic importance whose yield or quality may be enhanced by good pollinator activity (Richards, 2001). Klein et al. (2007) found inadequate information is available on the pollination biology and pollinator requirements of many crops. In the case of mango, depending on the cultivar and geographical location, data reflects a potentially large variability among cultivars in their pollination biology, natural pollinators and breeding systems (Perez et al., 2016; Sharma and Singh, 1970; Dag et al. ,2000). To evaluate these hypotheses, I conducted pollination experiments to elucidate the reproductive success (fruit production, fruit development and seed germination) of different mating (self vs cross vs open pollination). This work builds of plant reproductive ecology theory to provide important information to support germplasm conservation in *M. indica*, an economically important crop in the world's tropics.

MATERIALS AND METHODS

Site of study: This study was conducted on the grounds of the Agricultural Experimental Station (JDAES)located in Juana Diaz in the South of Puerto Rico (180 01'N, 66 031'W, 22.25 msl). This research center was established in 1950 by the University of Puerto Rico, Mayagüez Campus has a combined area of 111.23 ha. Recorded average monthly temperature range between 22°C to 33°C and monthly average rainfall of 977.1 mm (Harmsen et al. 2014). The site is located in a subtropical dry forest life zone which makes it an ideal area to maintain different fruit germplasms

(Ewel and Whitmore, 1973). One of the main germplasmic materials brought to JDAES belongs to the *Mangifera indica* orchards. Today's collection maintains 84 of the 105 mangos cultivars that were initially planted in 1968. The total mango germplasm occupies two sectors of JDAES covering approximately 14.16 ha (Fig. 3.1).

Study system: *Mangifera indica* L. is a perennial tree belonging to the family Anacardiaceae. The flowers of the mangó are found in a panicle that is composed of a main axis with several secondary axes branched into tertiary and sometimes quaternary axes (Coetzer et al., 1995; Goguey, 1997). Plants produce minute flowers (5-10 mm diameter), with yellow nectar discs and can be hermaphrodite and male both of which have stamens (Kostermans, 2012). Hermaphrodite flowers have a globose ovary with a single stigma (Ramirez and Davenport, 2016). The mango pollen is variable size (25 to 45 $\boldsymbol{\mu}$m long), and has an oval shape when dry and is sphere-shaped when hydrated (Ramirez and Davenport, 2016; Popenoe, 1917). The four mango cultivars studied are Keitt, Kent, Tommy Atkins and Julie. There are some noticeable differences among the four varieties selected for the study. Keitt which is the most sown cultivar in Puerto Rico has a medium to moderate tree height (9.1 m to 40 m), an open canopy and a fruit size that ranges from 13 to 15 cm long (Campbell,1992). The Kent cultivar is the preferred cultivar in Latin America and considered a tall tree can reach 40m that produces fruits with sizes ranging from 11 to 13 cm long (Campbell, 1992). The Tommy Atkins cultivar, the most preferred for the color of its fruit (skin orange-yellow, crimson or dark red blush color), has trees with a dense and round canopy and the fruit can measure 12-14.5cm. long (Campbell, 1992). Julie is the cultivar with the smallest tree height reaching up to 3m. This cultivar is believed to have come from Jamaica and is one of the favorites throughout the Caribbean (Morton 1987). For all varieties, flowering occurs mostly once a year starting in January (or end of December) with the flowering season lasting five to six weeks (I. Cabrera-Asencio, unpublished data). In Juana Diaz Agricultural Experimental Station, all cultivars are managed equally, the germplasm have been given the same management to all trees. Providing weekly irrigation, twice per year fertilizer and monthly weed management.

Pollination experiments: From January to April 2019, 10 trees per cultivar were selected and five different pollination treatments commonly used to evaluate a species breeding system (control, apomixis, autogamy, self and cross pollination, Eckert, 2010) were conducted on each tree. The control treatment consisted of non-manipulated flowers sitting on open inflorescences (natural pollination). In the remaining treatments, flowers were manipulated and their respective inflorescences were covered with an organza bag. The apomixis treatment consisted of emasculated flowers (asexual fruit production). The autogamy treatment consisted of unmanipulated flowers covered with an organza bag (autonomous pollination). The last two treatments consisted of manual pollinations (self-pollination and cross pollination) which were performed when the pistil and anther of selected mature flowers were put in contact with each other: In the self-pollination treatment flowers were pollinated using pollen from the same tree and the same inflorescences; In the cross-pollination treatment flowers were pollinated with pollen from another tree (at least nine meters away) and inflorescences. For all treatments with the exception of the control, inflorescences were covered with organza bags before the opening of tagged flowers to avoid the presence of flower visitors before the experiment began. The organza bags were kept until the flowers either senesced or else formed fruits. Each treatment contained 10 replicates for a total of 500 pollinated flowers distributed across all treatments. Pollinations were performed weekly during the first four weeks of flowering and then checked weekly to record fruit development time, % fruit initiation (number of fruits initiated/flowers observed x 100) and final fruit production or % fruit set (number of fruits matured/flowers observed x 100). Matured fruits from all treatments were collected to record their weight and determine their seed germination success (see below).

Seed germination: To determine the germinability of seeds for all treatments, the seeds were extracted from the fruit and the endocarp (or "bone") removed in order to examine the embryo. Once the embryo was removed they were placed in trays where they were covered with wet paper towels to keep them moist and they were kept in darkness at a constant temperature of 30°C in an enviromental test chamber (Panasonic MODEL MLR-352). For each tree, the percent seed germination was determined as the number of seeds germinated / the number of seeds available for that treatment.

Statistical analyses for breeding system and germination: The apomixis and autogamy treatments did not produce fruits and were excluded from the statistical analyses which were used to evaluate differences among the other sets of treatments (self-pollination vs cross-pollination treatments, control vs average of self and cross-pollination treatments, control vs cross-pollination) in the following dependent variables for each cultivar: fruit set (%), fruit development time (days), fruit weight, seed germination (%) and seed germination (days). For the dependent variables fruit set (%), seed germination (%) and seed germination (days), we used paired t-tests to evaluate differences between paired treatments. For the non-parametric data there use the two-sample inference with Wilcoxon test. To analyze fruit development time (days) and fruit weight, we used the values for the differences between paired treatments (i.e., self- vs cross-pollination and control vs. average of self- and cross-pollination) and then used Two-way ANOVA to evaluate if there were statistical differences among cultivars in the mean deviation values between treatments. When the data was non-parametric we used Kruskal Wallis Test.

We constructed a reproductive index (IR) using the following formula for each treatment: IR = (% fruit set/100) x (cultivar's average development time of all treatments/minimum development time of treatment) x (average weight fruit) x (% seed germination/100) x (cultivar's average days to first germination/ minimum number of days to first seed germination). We used a Two-way ANOVA to evaluate if there were statistical differences in IR between cultivars and pollination treatments.

RESULTS

Breeding systems in four cultivars:

Fruit Traits:

Fruit set: Out of the five pollination treatments two treatments, autogamy and apomixis failed to produce fruits. For these treatments, flowers senesced 14 days after the treatment was applied and this was equivalent for all cultivars. I found no significant differences in the proportion of flowers that set fruit between the self and the cross-pollination treatments regardless of cultivar (Fig. 3.2 A, C, E, G). However, for three cultivars (i.e. Julie, Keitt, Tommy Atkins), flowers in the control treatment (i.e. open pollinations) produced proportionally more fruit on average than the combined average for the self- and the cross-pollination treatments (Fig 2 B, D, H). Julie and Tommy Atkins had the highest average percent of fruit set with 78% (±14.92) and 78% (±14.90) respectively compared to an average fruit set for the combination of self and cross pollination treatments (Fig. 3.2 B, H). Following the cultivar Keitt with had and a moderate average fruit set with 55% (±19.44) (Fig. 3.2 D).

Fruit development time: ANOVA analyses yielded significant differences among cultivars and pollination treatments in fruit development time but also a significant cultivar*treatment interaction in this variable (Table 3.1 A). On average, fruits for the Keitt and Kent cultivars had longer fruit development times than fruits from the Julie and Tommy Atkin cultivars regardless of pollination treatment (Fig. 3.3). Fruits from the Julie and Tommy Atkin cultivars developed on average 16 days faster than fruits from the Keitt and Kent cultivars (average number of days(d) ± SE: Julie = 117d ± 1.52, Tommy Atkins = 117d±1.44, Keitt=136d±1.58, Kent=131d±1.52). Significant differences in fruit development time among pollination treatments were only evident for the Julie and Tommy Atkins cultivar but they did not express equally. In the Julie cultivar, control pollinations resulted in fruits that developed on average 9d (111.81±1.32) faster than those from self and cross pollinations with no significant differences in fruit development time between self- and cross-pollinations (Fig 3.3). In contrast, in the Tommy Atkins cultivar, fruits from the self and control treatments developed 1-2 days faster than those from the cross-pollination treatments with no significant differences between those two (averages ± SE: Control: 117.2d ± 1.29, Self: 118.0d ± 1.58, Cross: 116.8d ± 1.46).

Fruit weight: Results from two-way ANOVA detected significant effects of cultivar, pollination treatment and their interaction on fruit weight (Table 3.1-B). Overall, Julie produced lighter fruits than the other cultivars regardless of treatment with fruits from the other cultivars (Kent, Keitt, Tommy Atkins) weighing 140g more on average. Meanwhile, cross pollinations tended to yield slightly heavier fruits than self and control pollinations but only in the Keitt and Tommy Atkins cultivars (Fig. 3.4). Fruits from cross pollinations were on average 58g heavier than controls in the Tommy Atkins cultivar and 51g heavier than controls in the Keitt. In both of these cultivars, cross pollinations tended to be heavier than self-pollinations but that tendency was not significant (Fig. 3.4).

Seed Traits:

Seed germination: The average percentage of seed germination ranged from 32% to 80% (Figure 3.5). None of the cultivars exhibited significant differences between self and cross-pollination treatments in the percentage of germinated seeds (Fig. 3.5 A, C, E, G). However, for three of the cultivars (Julie, Keitt and Tommy Atkins), the control (open) pollination treatment had significantly higher germination percentages than the combined average for the self and cross pollination treatments (Fig. 3.5 B, D, H). In the Julie cultivar there was a 23% difference between the average percentage for seed germination of the control treatment and the combined hand pollination treatments while for the Keitt and Tommy Atkins cultivar that difference was 15% and 23% respectively.

Seed germination days: On average seeds took 3.80 to 4.07 days under a control environment (Fig. 3.6). There were no differences between pollination treatments in the days it took seeds to germinate except for the Tommy Atkins cultivar where seeds from control (open) pollinations developed slightly faster than seeds from hand pollinations (open: 4 ±0.12d SD, self + cross: 3.86 (±0.12d SD) (Fig 3.6. H).

The reproduction index (IR) which combined all reproductive characteristics was significantly different among cultivars with Tommy Atkin cultivars showing significantly higher reproductive indexes relative to the Julie and Keitt cultivars (Table 3.2). The effects of pollination control treatment in Tommy Atkins were significant in the indexes compared with the others cultivars (Table 3.2).

DISCUSSION

Knowledge of the breeding system of crops is an important aspect of crop management. Breeding systems in *M. indica* appear to be highly variable and understudied given the large number of cutivars available (>1000; NHB, 2003, USDA, 2013-2015) and the number of cultivars studied approximately N=173 (Bally et al., 2009, USDA, 2013-2015). Pollination experiments in this study set out to test whether the four mango cultivars evaluated within a germplasm maintained at the JDAES, depended on animal pollinators for successful fruit production. The work also addressed the hypothesis that the mating type (self vs cross) during pollination would affect fruit production, fruit and seed traits. This was based on the untested premise that different pollen donors may generate differences in the genetic makeup of resulting embryos that in turn could influence the likelihood of setting fruit and traits observed in fruits and seeds. In studies with other some mango varieties, the probability of fruit set in *M. indica* has been shown to increase with self-pollination (Huda et al. 2015; Gehrke-Vélez et al., 2012; Ramirez and Davenport 2016) while in others the same has been true with cross pollination (Sharma and Singh, 1972; Dag et al., 1998; Dag et al., 2000). In others, open pollinations have been more effective than artificial pollinations (Roemer, 2011; Gehrke-Velez et al., 2012; Saeed et al.,2016). The findings of this study on the breeding system of *M. indica* indicated that all four cultivars evaluated at JDAES (Julie, Keitt, Kent and Tommy Atkins) while self-compatible are highly dependent on animal pollinators. For most traits evaluated, the mating type through controlled artificial pollinations (self vs cross-pollination within a cultivar) failed to have a significant effect on reproductive success. Yet, the effect of pollen donor cannot be discarded. Indeed, open natural pollinations tended to produce more fruit and yielded seeds with faster development times than artificial pollinations in three out of the four cultivars (Julie, Tommy Atkins and Keitt). They also resulted in faster seed germination in two cultivars (Julie and Tommy Atkins). The combined results may suggest that not only animal-mediated pollination is needed for these cultivars but that this mode of pollination also results in improved yields, fruit, and seed traits. Below, we discuss several hypotheses that may explain these results based on what we know from other studies as well as the implications of the different results presented to the management of this mango germplasm.

One study examined 429 open flowers of various cultivars of *M. indica* exposed to natural pollinators and found that the number of pollen grains in pistils is relatively low ($\bar{X}$: 1.2 grains/pistil; Popenoe 1917) which suggest that levels of pollen deposition are low under natural conditions (Popenoe,1917). In this study, artificial pollination as performed by saturating stigmas with pollen to ensure pollination. Even then, open pollinations as more effective at promoting fruit production. Such result suggests that pollen amounts per se do not explain differences between open and artificial pollinations. That result also suggests that the observed reproductive advantage of open vs artificial pollinations may occur through various mechanism that may or may not be genetic in nature and that may involve other factors not considered by this study. For example, the cultivars studied here are embedded in a living germplasm collection that consists of 84 cultivars. While controlled pollinations were done with pollen from the same cultivars, open pollinations may carry pollen from other cultivars and influence the genetic composition and phenotypic expression of resulting embryos through hybridization in positive ways. Exogenous pollen could also come from *M. indica* trees available outside the germplasm, as mango is a naturalized species in the Puerto Rican landscape and one of the most common in secondary forests (Marcano, 2017). Another possibility for the differences between open and artificial pollinations is that artificial pollinations may, in some cases, result in reduced fruit set if flowers are fragile or susceptible to mechanical manipulation (Sturrock, 1944,1961). Young and Leding (1954) indicate that it is not very easy to perform manual pollinations in mango. In this study, manipulated flowers did not fall right away following the experiments but had to be covered with mesh which was not the case for flowers with open pollinations. While this is a typical set up for these kinds of experiments (Eckert 2010, Huda et al. 2015) it may have created an unfavorable microenvironment for pollinated flowers. Inadequate timing of receptivity (pollinating immature stigmas with mature pollen and vice versa) may influence the fate of artificial pollinations in *M. indica* (Dag et al., 2000), we are confident that this was not a factor affecting results in our study. I ensured that our study included only mature anthers and pollen to prevent this. In some mango cultivars (i.e. Irwin, Beni-Keitt and Amrapali) open pollinations have resulted in a higher probability of fruit set (Honsho et al. 2012, Srivastav et al. 2014, Amin et al. 2015). Meanwhile in a study by Saeed and collaborators (2016) in a mango cultivar without name they indicated the open pollinations led to larger fruit sizes and weights relative to the treatments with and without pollinators. Thus, one possibility is that the observed differences between open and artificial pollinations in our study are explained by large differences in pollen pools (i.e., pools more variable in open pollination and less variable in artificial pollination).

On the surface, the lack of differences between the reproductive success of self vs. cross pollination treatments in the four cultivars of mango, may appear as a paradox given that in obligate animal-pollinated systems genetic loads (i.e. high frequencies of recessive deleterious alleles) may be high (Armbruster and Rogers, 2004). Yet, the lack of differences between the reproductive success of self vs. cross pollination treatments could also be a byproduct of the process of domestication and generation of these cultivars. The loss of genetic diversity (i.e. genetic erosion) in agricultural crops is a known phenomenon that has occurred during the process of a species domestication but was exacerbated with modern agricultural practices (Smýkal et al. 2018). Artificial selection of particular crop phenotypes based on the desirability of particular traits (fruit color, size, shape, weight of fruit) leads to the propagation of individuals that will have a reduced gene pool, relative to their wild counterparts (Warschefsky and Wettberg, 2019). This process not only reduces genetic variation but may also purge artificial populations of recessive deleterious alleles which in turn could explain the lack of differences between self- and cross-pollination treatments in reproductive success and in the expression of some traits in this study (fruit set, fruit development, fruit weight, seed germination, days of seed germination). It should be noted, however, that differences between self-and cross-pollination treatments do occur in many other mango varieties and that levels of self-compatibility are highly variable among cultivars (Singh et al., 1962; Davenport, 2009; Popenoe, 1917; Maheshwari, 1934; Mallik, 1957; Singh, 1997; Singh and Sharma, 1972). This suggest that at least for *M. indica*, genetic erosion does not occur equally across cultivars within the species and that may relate to the context of domestication and the genetic origins of different cultivars among other factors.

The original hypothesis in this study considered the possibility of differences among cultivars in the different reproductive responses evaluated on the premise that artificial selection leads to genetic differentiation among cultivars. It also considered the possibility that cultivars generated in Florida (Keitt, Kent, Tommy Atkins) would be different from the one generated in the Caribbean (Julie) on the premise that Floridian cultivars may have common genetic origins. Indeed, a recent genetic analyses of mango cultivars worldwide, showed that Floridian cultivars were genetically distinct (i.e., lower diversity values) and different from Caribbean cultivars (Warschefsky and Westberg 2019). In addition, the JDAES germplasm of mango material in the primary collection originated from cuttings that were grafted and planted in 1948 from Indian material but cuttings from Florida USA were added in 1962 (Mattern and Pennock, 1971). The cultivars studied here showed significant differences among them in fruit production, fruit and seed traits. However, the expected similarities and differences among cultivars based on domestication and historical origin (Floridians vs Caribbean cultivars) of the cultivars did not always hold. In addition, even when the reproduction of Floridian cultivar behaved similarly in some aspects, these responses did not necessarily match those observed for the same cultivars kept in the Florida germplasm. For example, our results showed that for Tommy Atkins and Keitt at JDAES, the mating system had no bearing on fruit set (equal success for cross- and self-pollinations) and that behavior is different from those same cultivars in Florida where self-pollinations improved the likelihood of setting fruit over cross pollinations (Ramirez and Davenport 2016). The occurrence of somatic mutations and intra plant genetic variations in trees that make up the germplasm cannot be discarded either as a possible driver of these differences. All trees that make up the JDAES germplasm originated from vegetative material (i.e., scion) that was grafted into local rootstock (i.e, original tree base planted from seed) from the Caribbean mango material present on the island (Eugenio Toro, pers. comm). Agricultural studies acknowledge the possibility of interactions between the scion (graft) and the rootstock that may influence plant characteristics (Harie et al., 2018). Such interaction may have developed in the germplasms studied here given their age (60 years). Breeding objectives through grafting often include achieving: a) early generation cycle, b) dwarfing growth habit ability, c) high yielding ability, d) good fruit quality, e) resistance to biotic and abiotic stresses nature, f) tissue compatibility and, g) resistant to biotic and abiotic stresses (Harie et al., 2018). In mango, such studies are scarce but at least one studied the relationship between different rootstock type and grafting success of the Apple mango cultivar (Beshir et al., 2019) and the relationship between rootstock on scion on yield in the Kensington Pride cultivar (Bally, 2011). Both studies evaluated the effect on the scion. In the Apple cultivar, results showed that the time of grafting increase the diameters, length and numbers of leaves of the scion, while in Kensington Pride some rootstocks increased the yield. No study has evaluated the rootstock effect in fruit traits in *M. indica* but at least in one study with the crop *Citrus reticulata* results suggest that at least for some crops there can be reciprocal interactions between the rootstock and the scion that can affect fruit traits (yield, weight, quality) three years after the grafting (Tietel et al., 2020). In that study they had to wait three years for C. *reticulata's* grafting developed and found Therefore, the potential for rootstock-scion interaction effects on fruit set in *M. indica* cannot be discarded and needs to be considered in future studies attempting to describe the role of pollen type and stock origin on crop reproductive success in perennial crop germplasms such as mango.

Another limitation of the study was the inability to explore the role of individual trees within a cultivar as pollen donors in the artificial pollination experiment given how time-consuming hand pollinations as. Also, the design was not set up to detect the pollen donors in open pollination. The effect of pollen donor on fruit traits (i.e., xenia) is an important concern of crop management (Bulant et al., 2000). In this work, pollen donor effects were seen in subtle ways when comparing artificial and open pollination (within cultivar). As stated before, the origin of pollen in the open pollinations can be diverse and include multiple parents within a cultivar and parents from multiple cultivars depending on the pollinator behaviors. From a commercial perspective, the ideal mango fruit weights between 350 and 400 (g) and has short development time (Bally et al., 2009). Mango cultivars with seeds that have short germination times will also be preferred because of the effect of dehydration (Corbineau et al. 1986). It should be noted that for Keitt and Tommy Atkins, cross pollinations did produce heavier fruits than the other pollination types and heavier than the ideal mango fruit weight ($\bar{X}$: Keitt= 403 g.; Tommy Atkins= 409 g), which also suggests that important levels of within cultivar genetic variation even if small is still present and may influence the desired fruit weight.

Improvements in seed germination from open pollination over other pollination treatments were mostly absent except in the Tommy cultivar. However, the observed mean differences amounted to a little over three hours which from a biological context may or may not make a difference in the subsequent establishment of mango propagules and would need to be evaluated. Ultimately, open pollination which are carried out by natural pollinators were found to be an important factor in fruit production. Therefore, the management of fruit characteristics of these four cultivars should consider relying on information on local pollinator activity.

This study highlights the importance of studying the breeding system of all living germplasms. For *M. indica*, results reiterate the importance of local pollinators and the need to evaluate the circumstances by which pollen donors may influence fruit characteristics in this crop. Detailed breeding experiments that help identify which are the best parents in terms of fruit and seed traits would help advance management strategies of M. *indica* cultivars. These experiments should be complemented with molecular studies that help clarify the origin of pollen donors from open pollination. Studies that evaluate the potential effects rootstock-scion interactions of fruit production would be a necessity to evaluate and improve the gene banks represented in *M. indica* living germplasm.

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Table 3.1. Model two way- ANOVA to evaluate differences in days of fruit development and fruit weight in different breeding systems in four cultivars.

A. Anova	Days of fruit development	
Model	F	p
Cultivar	323.25	0.0001
Treatment	15.73	0.0001
Cultivar * Treatment	7.79	0.0001
B. Anova	Fruit weight	
Model	F	p
Cultivar	167.47	0.0001
Treatment	10	0.0001
Cultivar * Treatment	2.16	0.0442

Table 3.2. Two way- ANOVA to evaluate differences in the reproduction index (IR) as a fecution on pollination treatment (self, cross, control) and cultivar (Julie, Keitt, Kent and Tommy Atkins) in *M. indica*.

Cultivar	mating system	Index IR mean± S.E.
Julie	self	11.37 ± 13.62 b
	cross	21.76 ± 13.62 b
	control	56.96 ± 13.62 b
Keitt	self	16.20 ± 13.62 b
	cross	15.75 ± 13.62 b
	control	54.56 ± 13.62 b
Kent	self	40.26 ± 16.28 b
	cross	54.94 ± 16.28 b
	control	81.01 ± 15.23 b
Tommy Atkins	self	29.11 ± 13.62 b
	cross	50.00 ± 13.62 b
	control	169.75 ± 13.62 a
Model	**F**	**P**
cultivar	10.71	0.0001
treatment	25.75	0.0001
cultivar*treatment	3.87	0.0016

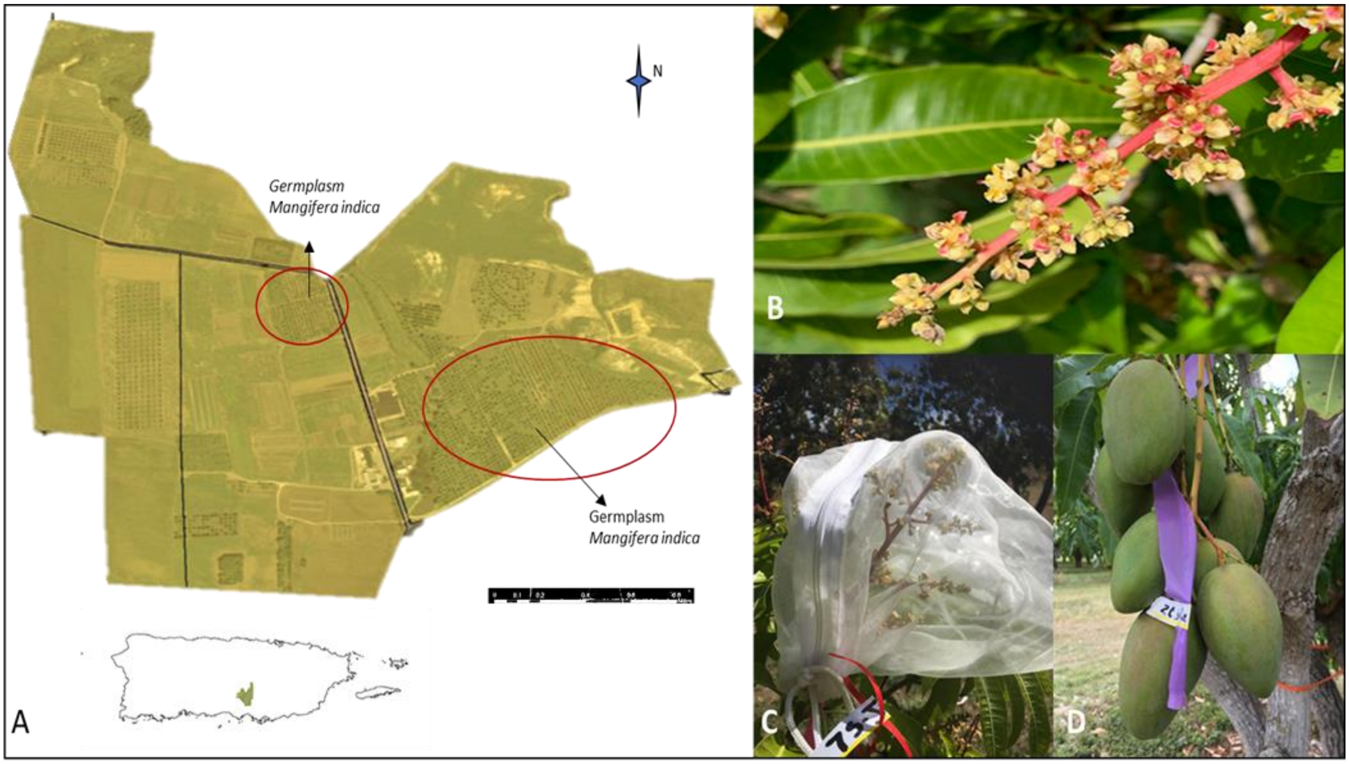

Figure 3.1. A) Location of the germplasm of *Mangifera indica* in the Juana Diaz Agricultural Experiment Station. B) Flower of Julie, C) Self -treatment of Julie, D) Fruits of control treatment in Julie.

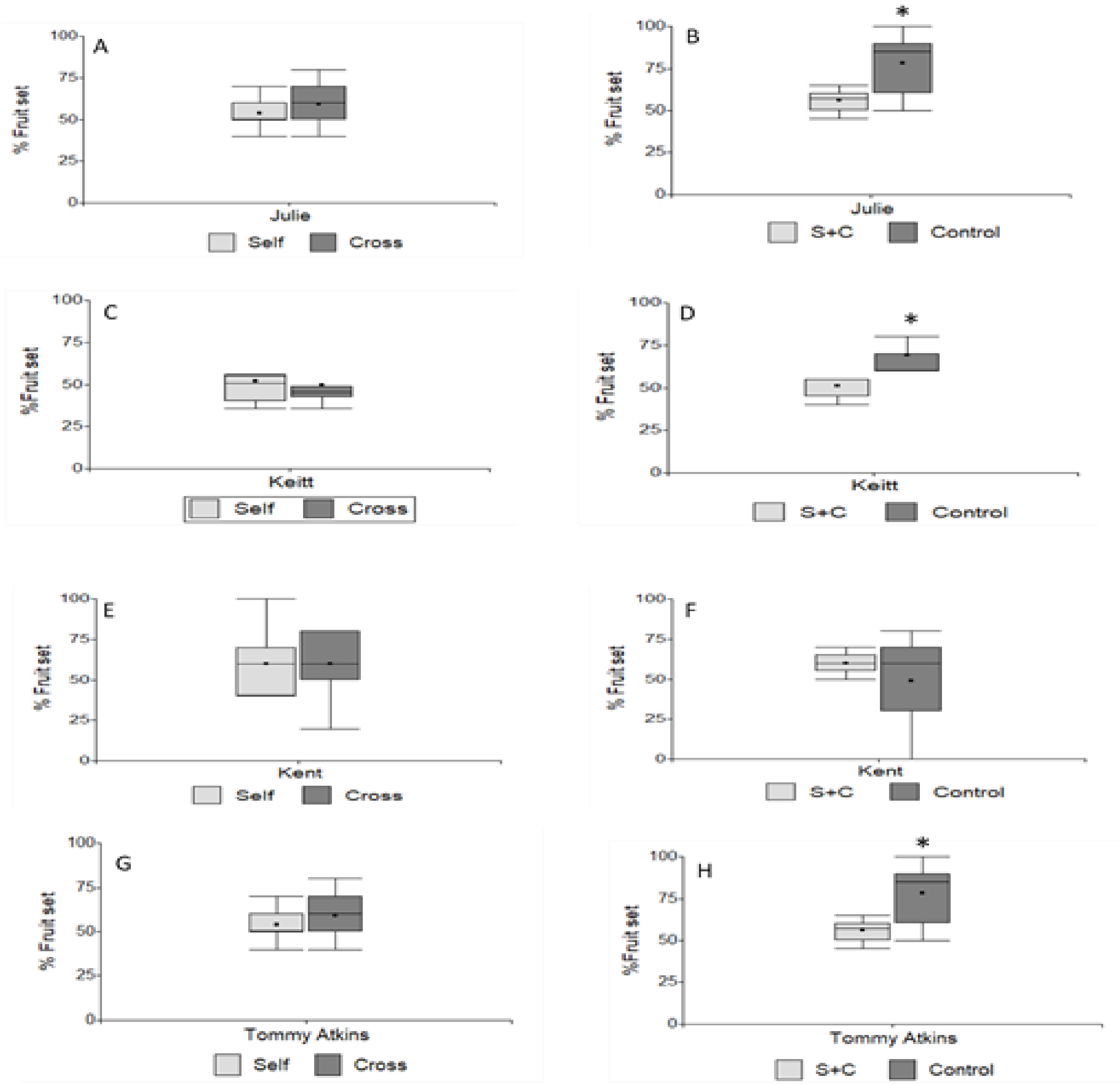

Figure 3.2. Comparisons of percent fruit set between pairs of pollination treatments: self- vs cross-pollination and average of self and cross pollination (S+C) vs pollination of open flowers (control) in four different cultivars of *M. indica* (Julie, Keitt, Kent, Tommy Atkins). Analyses represent paired-t tests for all cultivars except for comparisons for the Keitt cultivar which were with Wilcoxon tests as data did not meet all assumptions for parametric tests. The significant values were indicated by "*" and represent $\alpha = 0.05$.

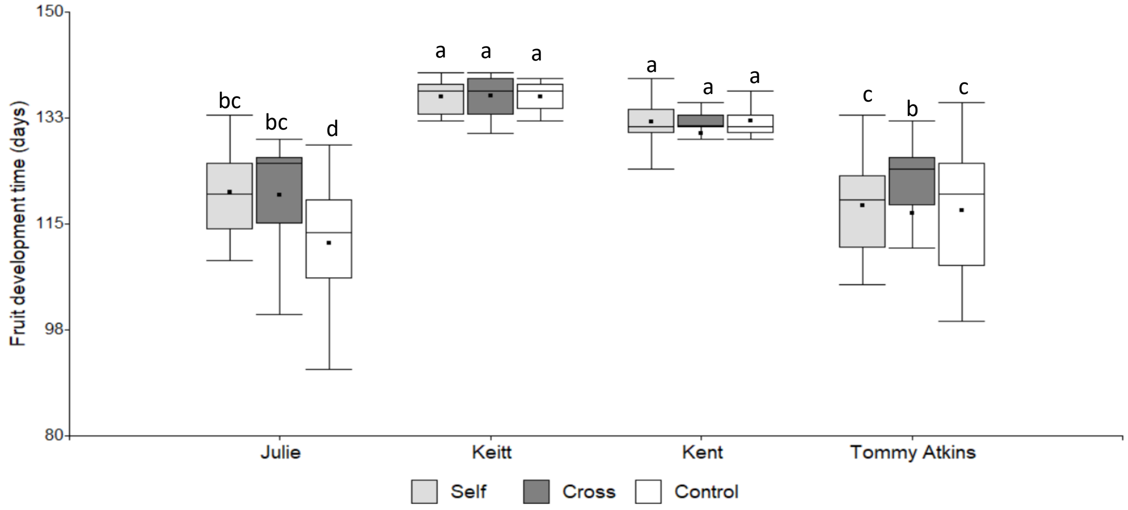

Figure 3.3. Variation in the duration of fruit development as a function of *M. indica* cultivar (Keitt, Kent and Tommy Atkins) and pollination treatment (self, cross, control). Different letters indicate significant differences (at α =0.05) following post hoc tests following Two-way ANOVA analyses (Table 1-A).

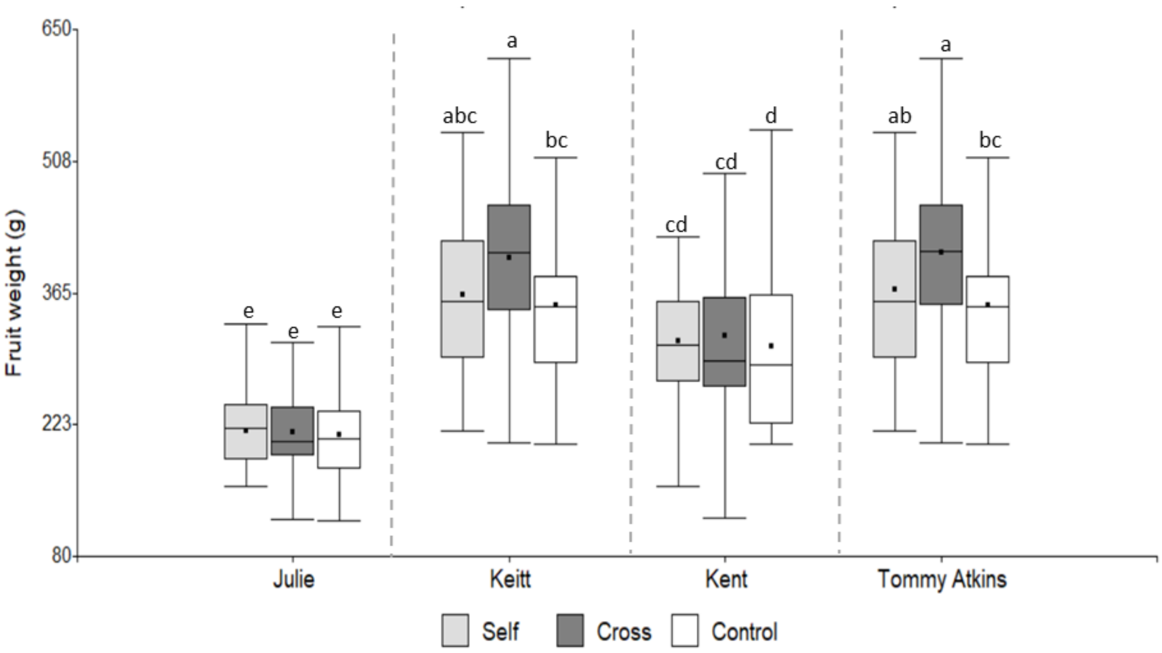

Figure 3.4. Differences in average fruit weight among cultivars (Julie, Keitt, Kent, Tommy Atkins) and pollination treatments (self, cross, control) in *M. indica*. Different letters indicate significant differences at $\boldsymbol{\alpha} = 0.05$ as determined by post hoc analyses following Two-way ANOVA (Table 1-B).

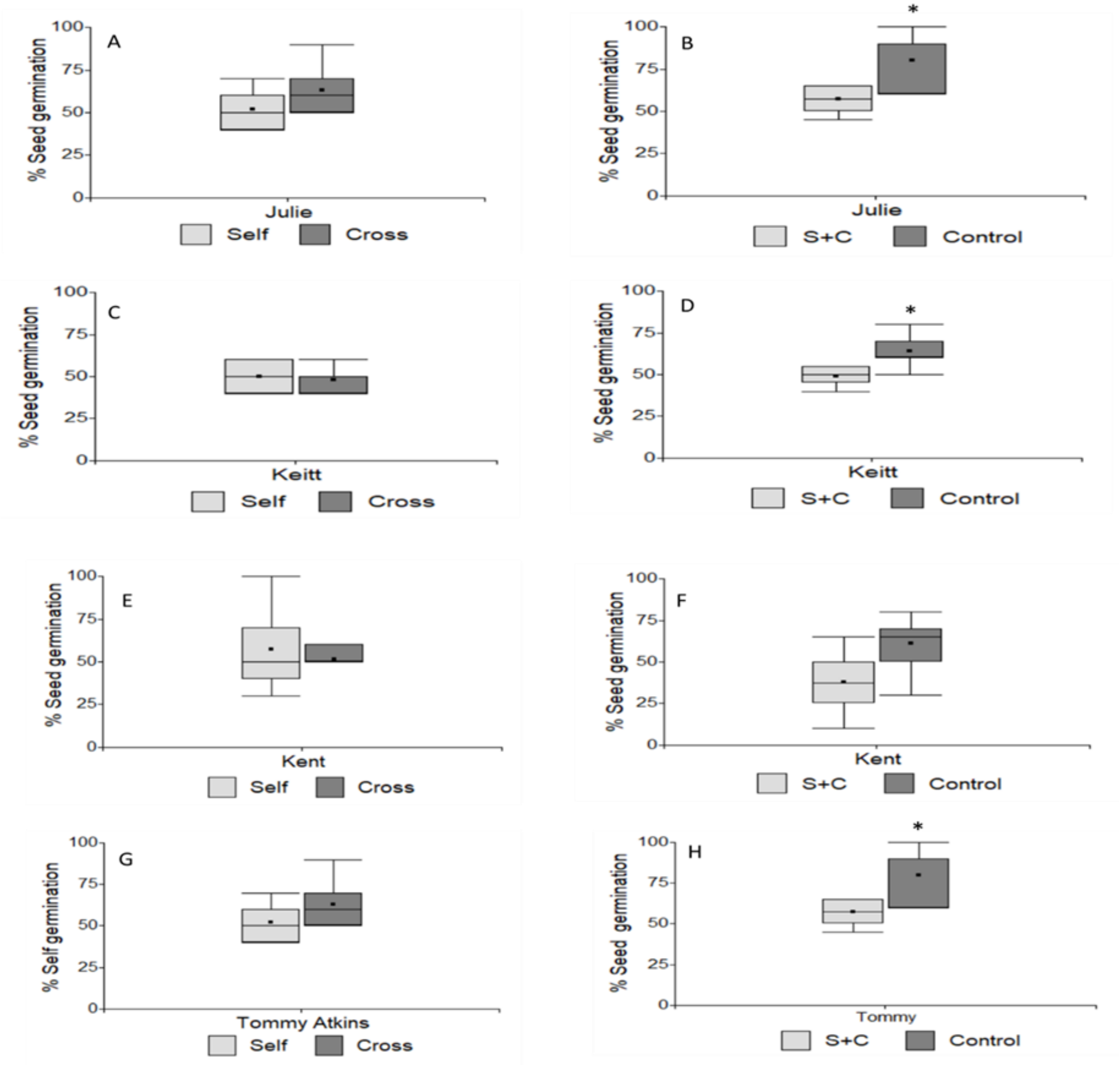

Figure 3.5. Differences in percent seed germination between pollination treatment pairs: self- vs cross-pollination and average of self and cross pollination (S+C) vs pollination of open flowers (control) in four different cultivars of *M. indica* (Julie, Keitt, Kent, Tommy Atkins). Analyses represent paired-t tests for all cultivars except for comparisons for the Keitt cultivar which were with Wilcoxon tests as data did not meet all assumptions for parametric tests. The significant values were indicated by "*" and represent $\alpha = 0.05$.

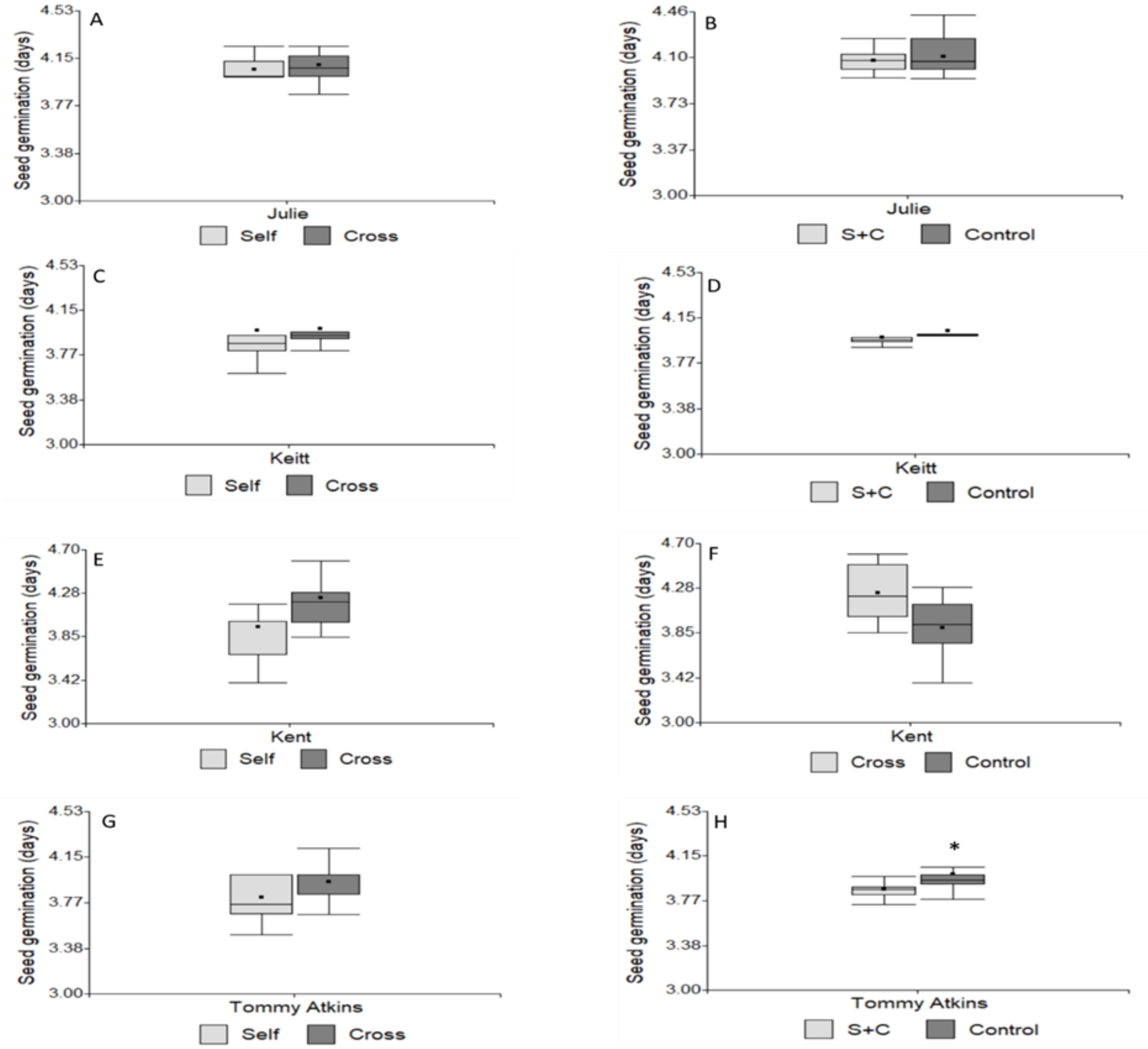

Figure 3.6. Differences in seed germination (i.e. number of days to seed germination) between pollination pairs in four different cultivars of *M. indica* (Julie, Keitt, Kent, Tommy Atkins). Data was analyzed with paired t-tests with the exception of the Keitt cultivar which was analyzed with a Wilcoxon test. Significant differences at $\boldsymbol{\alpha} =$0.05. were indicated with “as a fecution on pollination treatment *”.

CHAPTER IV

COMMUNITY AND SPECIES-LEVEL CHANGES OF INSECT SPECIES VISITING MANGIFERA INDICA FLOWERS FOLLOWING HURRICANE MARIA: "THE DEVIL IS IN DETAILS"

ABSTRACT

Mangifera indica is a widespread economically important tropical fruit. An ongoing study at the Juana Diaz Agricultural Experimental Station in Puerto Rico aims at understanding the factors that influence local pollination success and fruit yields in four fields each hosting a different mango cultivar (Keitt, Kent, Tommy Atkins and Julie) at different temporal scales. Here we describe the results of insect collection campaigns that evaluated flower visitor communities of these fields (from January to April) in the seasons of 2017 (before Hurricane Maria), 2018 (after Hurricane Maria) and 2019 (two years after Hurricane Maria). We expected a reduction in diversity, abundance and yields and even changes in composition following the hurricane events of 2017. Over the three years, plants were visited by a combined total of 50 insect species, mostly Diptera (also the most abundant), Hymenoptera, Coleoptera and Lepidoptera. The relative abundances of insect communities changed but overall species richness of insect communities appeared to be recovering by 2019. A clear decline in overall crop yields for two of the four fields (hosting Kent and Tommy Atkins) was seen in 2018 but then recovered in one and surpassed pre-hurricane levels in another in 2019. Mango trees experienced an increase in the abundance for all insect groups in 2019 following the 2018 decline and only one field (hosting Kent) experienced significant species richness declines in 2018. Two of the most dominant insects, *Palpada vinetorum* (Diptera) and *Apis mellifera* (Hymenoptera), showed a "reduction-recovery" pattern for in the period of 2018-2019 but not so for *Cochliomyia minina* which was very abundant in 2018 in three out of four cultivars but then returned to pre-hurricane levels in 2019. In 2017, the trees exposed to higher richness and abundance of species experienced higher yields regardless of cultivars but these relationships when present were often weaker in 2018 (post-hurricane) and 2019 and not all cultivars were equally successful at attracting the same levels of diversity and abundance of insects. Our results do support the importance of pollinator diversity and abundance to improve agricultural yields. They also emphasize that within the context of future extreme atmospheric events, that there needs to be an understanding of not only how these pollinator communities may

recover from these events but also of how individual pollinators (vs. other factors) may influence plant yields to develop informed management strategies following such events.

INTRODUCTION

Observed declining trends in the diversity and abundance of pollinators, may threaten global economies and our capacity to meet increasing global food demands (Potts et al., 2010a,b; Cameron et al., 2011; Bartomeus et al., 2013; Jevanandam et al., 2013; Aizen et al., 2019). A significant portion of the world's crop production (35%) depends on animal pollination (Klein et al., 2007) and many of those many are dependent on insect pollinator (Rader et al., 2015). In the United States alone, the value of this 'pollination service' to the agriculture has been estimated at $43B (Losey and Vaughan, 2006). While managed pollinators like honey bees are main contributors of worldwide agricultural production (Potts et al., 2010b), studies suggest that wild pollinators are also important and as they can increase agricultural yield and quality of fruit crops (e.g. Garibaldi et al., 2013; Garratt et al., 2014, Rader et al., 2016). Given the importance that pollination services represent to food and economic security, calls for strategies to conserve, manage and monitor pollinator abundance and biodiversity have been on the rise to reduce potential risks (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, 2016). A core element of the International Pollinator Initiative's 2013-2018 action plan led by Food Agriculture Organization (FAO) and the Convention for Biological Diversity (CBD) centers around activities related to monitoring research and assessment on the status and trends of pollinators and pollination (Convention on Biological Diversity, 2018). Information on the diversity and abundance of pollinator species is an important step towards understanding the stability and resilience of crop pollination services (Rader et al., 2013; Rogers et al., 2013). Developing monitoring strategies will be critical when evaluating pollinator management strategies especially in the face of climate variability and the prospect of increases in extreme atmospheric events world-wide.

Predicting the effects of extreme atmospheric events such as hurricanes on the abundance and diversity of insect pollinators is complex. Indeed, the literature offers no agreement about the effects of hurricanes on insect communities with some species increasing while others decreased in abundances following these events (Torres, 1992; McGinley and Willing, 1999; Showalter and Ganio, 1999; Koptur et al., 2002; Gandhi et al., 2007; Spiller et al., 2016). Some responses are modulated by spatial differences in microhabitat (e.g Showalter et al., 2017) and others by species interactions within and between trophic levels (e.g. Showalter, et al., 2017; Novais et al., 2018). From the perspective of diversity changes, it has been argued that while extreme weather events (hurricanes and frosts) often result in immediate reductions of alpha diversity, insect assemblages tend to be fairly resilient returning to pre-disturbance levels within 1-5 years (Marquis et al., 2019; Chen et al., 2020). It should be noted that most studies evaluating the effects of these events on insect communities do not often include pollinators.

Studies addressing the dynamics of insect pollinators following hurricane events have a narrow geographic scope and most are focused on non-agricultural systems. One study explicitly evaluated changes in insect pollinator assemblages in Florida (United States) after hurricanes Frances and Jeanne, showed a decline in species diversity accompanied by a reduction in the reproductive success of *Laguncularia racemosa* (Landry, 2013). In a second study, the insect pollinator community visiting *Ardisia escallonioides* following Hurricane Andrew in 1998, changed little in composition and abundance of their generalist pollinators (Pascarella, 1998). A third study, also in Florida, showed that after hurricane Andrew populations of *Pegoscapus jimenizeii* (fig wasps), a specialized pollinator of *Ficus aurea*, dropped but were able to recover in only 5 months (Bronstein and Hossaert-McKey, 1995). On the other hand, hurricanes across Yucatan, México, are one of several factors contributing to the decline of feral and managed colonies of stingless bees with generalist species persisting even when some of their food species disappear (Villanueva-Gutierrez et al., 2005; Roubik and Villanueva-Gutierrez, 2009). Clearly more studies are needed to evaluate the potential role of these events on pollinator communities and how these may affect plant reproduction. This would be particularly important for agricultural systems when their production is dependent on animal pollination.

Mangifera indica (mango), is native to Asia but has been widely dispersed in tropical and subtropical areas around the world (Jiron and Hedström, 1985; Galán, 2009). It is an economically important crop worldwide with over 1,000 cultivars (Galán, 2009). The Asian continent is the principal producer (75%) for this crop plant followed by Latin America and the Caribbean region

(14%) and Africa (10%) (FAOSTAT, 2000). The scientific literature suggests that flower visitors of *M. indica* are quite diverse taxonomically and that there is considerable variation among geographic regions. For example, studies in Taiwan and Australia reported that Hymenoptera were the only flower visitors and potential pollinators of *M. indica* (Anderson et al., 1982; Hsin Sung et.al, 2006) whereas in India, Diptera were named as dominant flower visitors (Ramírez & Davenport, 2016, Singh, 1988). Meanwhile, studies in Israel and Africa, showed that the main visitors of mango plants were a mixture of both Hymenoptera and Diptera species (Dag and Gazit, 2000) or Hymenoptera, Diptera and Coleoptera (Carvalheiro et al., 2010, 2012) while in some areas, *Apis* bees were important pollinators (Wongsiri and Chen, 1955). The combined data suggest that *M. indica* flowers can attract a large diversity of floral visitors and therefore has a pollination system that may be generalized (Kumar et al., 2012, Fajardo et al., 2008, Anderson et al., 1982, De Siqueira et al, 2008, Corredor and García, 2011). While generalized pollination systems may offer resilience capacity within the context of environmental change (Waser et al., 1996, Gonzalez-Varo et al., 2013), this may not always be the case (Memmott et al., 2007) or may not occur at a speed that is necessary in economically important species.

In the Caribbean region, the island of Puerto Rico is among the principal producers of *M. indica* (Central América Data, 2016) and Puerto Rico’s climate is changing. Since 1950, air temperatures have increased by 2°C as well as the frequency and intensity of extreme climatic events (e.g. heat waves, droughts and tropical storms) (Gould et al., 2015). Indeed, tropical storms and hurricanes have become more common and more intense during the past two decades (Enviromental Protection Agency [EPA], 2016) and this trend is expected to continue according to most climate models (see reviews by Gould et al., 2015; Fain et al., 2018). Following hurricane events, flower resources available to pollinators may be reduced through the direct impact to plants (e.g. via mortality or defoliation; Rathcke, 2000) for pollinating insects. Hurricane-driven ecosystem modification may also reduce the availability of suitable habitat (e.g. flooding of ground nesting sites for insects, Savage et al., 2018) and result in declines in pollinating fauna (Landry, 2013). Therefore, both mango trees and their pollinator communities can be affected by the current trends in atmospheric events and these could affect local yields in Puerto Rico. Detecting declines in pollinator abundances and reproductive success of *M. indica* as a result of these events has yet to be documented in hurricane prone areas. On the other hand, predictions about the effects of hurricanes effects on plant-pollinator interactions are not necessarily straightforward as the impacts on pollinator abundances by these phenomena have not been documented frequently and only for a limited number of taxa.

In 2017, hurricanes Irma (Category 1 on land) and Maria (Category 4 on land) hit the island of Puerto Rico leading to considerable changes in the island's green landscape and changes in ambient temperature. It was estimated that when Hurricane Maria hit Puerto Rico on 20 September 2017, 23 to 31 million trees island-wide were severely damaged or killed (Feng et al., 2018) with some areas in Puerto Rico reporting ambient temperature increases of up to 4°C for a full year (U.S. Forest Service, 2018). Such stresses could reduce the ability of plants to produce fruits through a reduction of photosynthetic resources available to produce flowers and fruits and through a reduction of their pollinator communities (Rathcke, 2000). Following the 2017 hurricane season, we took advantage of an ongoing monitoring study of the flower visitors of four cultivars of *M. indica* on the island of Puerto Rico to address the potential influence of these hurricane events on the flower visitor communities of *M. indica* cultivars and how hurricane-influenced changes in these communities related to plant reproductive success. We specifically asked if insect communities visiting mango flowers varied significantly across years and how these changes related to plant reproductive success. One hypothesis is that when they occur, hurricanes could lead to a reduced diversity and abundance of local *M. indica* pollinators and result in reduced yields. At the same time, a current paradigm is that pollinator diversity and not just abundance may help increase pollinator services and plant reproductive success (Gomez et al., 2007; Albrecht et al., 2012). Thus, a minor goal was to evaluate the functional relationships between reproduction and pollinator diversity and abundance. We also sought to determine how these relationships may change following hurricane events and whether they differed among four fields each of which hosted a different mango cultivar. Lastly, we examined the relative importance of *Apis mellifera* to mango reproduction. *Apis mellifera* is often seen as a replacement for local species but managed *A. mellifera* failed to improve yields for *M. indica* in Africa (Carvalheiro et al. 2010). Furthermore, we know little on how *A. mellifera* responds to climatic events in the Caribbean Region. Results of this study provide insights into the dynamics of insect communities visiting flowers of *M. indicia* within the context of extreme weather events (i.e. hurricanes). From a management perspective, our results may help inform proposed strategies for managed pollination following such events.

MATERIALS AND METHODS

Study Site- We conducted our study was conducted at the Juana Diaz Agricultural Experiment Station (JDAES) in the island of Puerto Rico (18° 01'N, 66° 31'W) (Fig. 1). The Station covers 111.23 ha and was established in 1950 by the University of Puerto Rico, Mayagüez Campus. It is located in the Southern side of the island which lies within a subtropical dry forest life zone (Ewel and Whitmore, 1973). The site has an average monthly temperature that ranges from 22 to 33 °C and an annual rainfall of 977.1 mm (Harmsen et al 2014). According to the USDA Survey, JDAES soils are classified as "Prime" with irrigation systems that are primarily driven by extracting groundwater (Beinroth et al., 2003). Since 1968, the Station has maintained a mango germplasm which today has 84 cultivars that occupy approximately 14.16 ha (Fig. 4.1). Data was collected in four fields within the germplasm area and each field hosted a different cultivar (see below). For simplicity, each field is named after the cultivar it hosted.

Study system- *Mangifera indica* L. (Anacardiaceae) is a perennial tree that produces highly branched inflorescences (Coetzer et al., 1995; Goguey, 1997) with flowers that range from 5 to 10 mm in diameter that carry 5 green sepals and 5 petals with yellow nectary discs and colors ranging from white to yellow, pink or red (Galán, 2009) (Fig. 4.1). Inflorescences carry both hermaphroditic and male flowers. In hermaphroditic flowers the ovary is globose with a single stigma (Ramirez and Davenport, 2010). The Keitt cultivar evaluated in this study is derived from the Mulgoba cultivar, which has a moderate to tall tree height (9.1 to 40 m), an open canopy, and fruit sizes ranging from 13 to 15 cm (Campbell, 1992). This is the most planted cultivar on the southside of the island. The Kent cultivar evaluated here was derived from the Brooks cultivar and can reach heights of 40 m, has a foliage that looks more compact, and produces fruit sizes ranging from 11 to 13 cm that are a favorite in Latin America (Campbell, 1992). The Tommy Atkins cultivar was derived from the Haden cultivar and produces a dense, rounded top tree with a fruit that can measure between 12 to 14.5 cm and is often preferred for its fruit color (skin orange-yellow, crimson or dark red blush color) for export (Campbell,1992). Julie is the smallest of the four cultivars studied. It grows only up to 3.3 m tall and produces smaller fruits relative to the remaining three cultivars. This is the main mango exported from the West Indies to Europe, believed to have been imported from Jamaica to other countries in the Caribbean and a favorite throughout the Caribbean region (Morton, 1987). For any of these cultivars, flowering has historically occurred within the course of five to six week during the dry months, which in Puerto Rico occurs between the months of January and April although some variation of the onset of flowering may occur depending on the year.

Data collection - We collected insects visiting the flowers of 10 plants of each of the four *M. indica* fields (N = 40 plants) three days a week during their annual flowering cycles. In the 2017, and 2018 seasons, flowering occurred from January to April whereas for the 2019 season, flowering started early and ran from the last week of December and continued until April. Trees in the general mango germplasm were planted in rows with a 7 m minimum distance from one planted tree to another and a total area surveyed per cultivar of 0.70 ha. Likewise, cultivars varied in their distance from each other: 64.7 m between the Keitt and Kent cultivars, 477.0 m between Keitt and Tommy Atkins and 150.4 m between Kent and Julie (Fig. 4.1). For each tree in a cultivar, we selected five inflorescences to be the focus of the insect collections (50 inflorescences/cultivar). Inflorescences on a tree were observed for insect visitors that were seen performing legitimate visitations (i.e. when the corolla was open and the visitor was collecting resources (nectar or pollen from it). Once detected, insects were collected by sweeping an entomological net over the inflorescence. Observations and collections were done three days every week: Monday, Wednesday and Friday. Each day, collection was carried out between 09:00 h and 14:00 h following a systematic scheme. The day was divided into 1-hour periods with four 15-min intervals each assigned to a given cultivar. Within each 15-min interval, inflorescences for all trees were observed (1.5 min/tree) and insects visiting flowers in legitimate ways were collected in separate bags recording the time and the tree ID on each bag. At each hour period, the order of cultivars was rotated, and the order of trees within a cultivar as well to make sure that cultivars and their respective trees were observed at different daytime periods within the flowering season. All collected insects were taken to the Laboratory to be mounted or preserved in 75% alcohol. All specimens were identified to species except for one which was identified to genus. For each observed tree we also recorded the number of inflorescences per tree during the flowering peak and collected and weighed fruits to generate a value for the number of fruits per tree and fruit yield (kg).

Data analyses - For each field (Julie, Keitt, Kent, Tommy Atkins), an insect data matrix was constructed with the variable's year, field, collection week (1, 2, 3, etc.), pooled insect abundance for each *M. indica* field. This abundance matrix was then analyzed with non-metric multidimensional scaling (NMDS) analysis and time vector overlays (using year as the time variable) (Clarke, 1993; McCune and Grace, 2002) to evaluate potential differences in flower visitor communities among fields and across time periods. This analysis was carried out in PC-ORD version 5.0 (McCune and Mefford, 2006) using the Bray–Curtis similarity index and performing 250 iterations with randomized data to select a dimensionality, and then performing one iteration with the chosen dimensionality to find a stable solution with minimal stress (McCune and Grace, 2002). To explore which species best explained the variance among insect communities in multivariate space, we used Kendall correlation analyses that specifically tested associations between species abundance and the first and second NMDS axes (McCune and Grace, 2002). For these analyses we considered species abundances with a Kendall correlation coefficient of (r) ≥ 0.4 to be significantly correlated with the NMDS multivariate axes and later used the three most dominant ones (and the most consistent in activity across cultivars) to evaluate their association with plant reproductive success (see below). For the purpose of this work, we provide results on total insect species diversity (hereafter richness) and abundance as well as abundances for the three most dominant species as these were the most consistent visitors for all four fields.

Our intent was to evaluate differences in total insect abundance, species richness as well as the abundance of dominant species between years and fields and how these variables related to mango reproduction. We first tested for the presence of systematic spatial variation of variables which could influence statistical results when using ordinary least square methods (Dormann et al., 2007). To that effect, we did exploratory analyses of Moran's I values for all numeric variables in GeoDa (Anselin et al., 2006) first pooling data from all cultivars to check for spatial autocorrelation issues at a landscape level. We then used values for each individual cultivar to test for autocorrelation at a local scale. Moran's I analyses did reflect spatial autocorrelation issues at the landscape scale but not at local scale (i.e within a cultivar). Therefore, first we performed 'permutational multivariate analysis of variance' (PERMAVOVA) using the Bray-Curtis dissimilarity index to test for multivariate dissimilarities integrating variables related to pollinator species richness and abundance (insect species richness, abundance, and individual abundances of dominant insect species) with year, field and their interaction as main effects. Unlike a regular MANOVA, PERMANOVA is not dependent on the correlation structure among groups (Marti, 2014). To visualize relationships based on dissimilarity, we generated a dendrogram from a cluster analysis based on the unweighted pair group method with arithmetic mean (UPGMA) and the Bray Curtis dissimilarity index using MSVP v3.22 (Kovach, 2007). We followed PERMANOVA with a series of complementary analyses to evaluate changes in insect community variables and plant reproduction and the relationship among these at the field scale to circumvent spatial autocorrelation issues and using ln transformed variable values to meet the requirement of parametric tests. To evaluate the influence of insect abundance and diversity and the number of inflorescences per tree on the number of fruits/tree and total yield (kg/tree) of each mango field each year, we used generalized linear models to evaluate how the data fitted the models under different distributions (poisson, normal, exponential and negative binomial) and using the lowest AICc value (an adjusted AIC to account for small sample sizes, Cavanaugh, 1996) as our selection criteria for the best model and using a 2 unit minimum criterion for model selection (Buhrmann and Anderson, 2002). For models under a normal distribution (all but two), ordinary least square models were also run to generate R^2 estimates. For each field, we ran repeated measures ANOVAs to test for univariate differences across years in total insect species richness and abundance, fruit yield and the number of inflorescences as well as pairwise Pearson correlation analyses to test for associations between fruit yield with the number of fruits and also between the number inflorescences with fruit yield. We also ran multiple correlation analyses to test for associations between the abundance of each of the three dominant insect species observed for each tree and fruits yield per tree for each field and each year. To account for a potential increase in type II errors from multiple analyses (N = 28 correlations), p values were corrected using Bonferroni corrections (Hammer et al., 2001). GLM Analyses were run in JMP v. 13.1 (SAS Cary Institute Inc., 2019) while PERMANOVA, Repeated measures ANOVAs and correlations were run in in InfoStat (Di Rienzo et al., 2018).

RESULTS

Insect community composition - A grand total of 3,795 individuals distributed across 50 insect species were collected in four mango cultivars (distributed across four fields) across the three years of the study (2017: N = 920; 2018: N = 1,318; 2019: N = 1,557) (Appendice A). NMS analyses showed that 94.3% of the temporal variation in the insect community composition was explained by a two-dimensional solution with most of the variation (70.4%) explained by Axis 1 (Fig. 4.2). The NMS analysis showed that observations for different the different fields (Julie, Keitt, Kent and Tommy) based on species similarity tended to form clusters around time periods and not around the fields themselves. That is, within a given year observations for the species composition of flower visitors for the different fields were more similar to each other than what the observations for a given field were across the different years (Fig. 4.2a). Also, insect communities in 2017 (before the hurricane events) and 2019 (2 years later) were more similar to each other than what they were to insect communities for all four fields in 2018, 4-6 months after Hurricane Maria hit the island. Kendall correlation analyses showed that the species that most related to the observed variation in the community composition of flower visitors were *Palpada albifrons* (Diptera: Syrphidae), *Palpada vinetorum* (Diptera: Syrphidae), *Apis mellifera* (Hymenoptera: Apidae), *Cochliomyia minima* (Diptera: Calliphoridae), *Psedodorus clavatus* (Diptera: Syrphidae), *Gonia crassicornis* (Diptera: Tachinidae) *and Peckia sp.* (Diptera: Tachinidae) (Fig. 4.2 b, Table 4.1). Of those, the three most abundant were *Palpada vinetorum, Apis mellifera, and Cochliomyia minima* (mean observations / field ≥ 7.4 individuals/field/tree). Overall, the abundances for *P. vinetorum* and *A. mellifera* were considerably lower in 2018 but that was not the case for *Cochliomyia minima* which became the dominant species with observed abundances that were six times higher than those observed in 2017 (Fig. 4.3). In 2019, abundance values for *Cochliomyia minima* had reduced to pre-hurricane levels but those of *A. mellifera* were significantly lower than abundance values in 2017 (Fig.4.3). In contrast, following the decline in 2018, *P. vinetorum* experienced an overall increase in abundance in 2019 and became, once again, the most dominant species by that year. When considering the abundance of dominant species within individual fields, *P. vinetorum* and *A. mellifera* exhibited significant declines in 2018 in all fields but returned to pre-hurricane levels in all fields with the exception of Kent field (Fig. 4.3, Table 4.2). In that field, the abundance for *P. vinetorum* increased in 2019 but went above pre-hurricane levels and the abundance for *A. mellifera* did not recover in 2019 (Fig. 4.3, Table 4.2). In contrast, the abundance of *C. minima* increased for Keitt, Kent and Tommy Atkins fields in 2018 returning to pre-hurricane levels in 2019 with the Julie field experiencing a decline-recovery pattern between 2018 and 2019 (Fig. 4.3, Table 4.2).

Species richness and abundance – PERMANOVA detected significant multivariate dissimilarities defined by the total insect diversity and abundance and the abundance of *P. vinetorum, A. mellifera* and *C. minima* explained by differences across years, fields, and their interaction. Fluctuations in species richness and abundance were not consistent across fields. A dendrogram following a cluster analysis using the same variables as in PERMANOVA showed fields within a year indeed clustered together but that relationships among fields within a year were not consistent based on their similarity in species richness, total insect abundance and the abundance of dominant species (Fig. 4.4). Repeated measures ANOVA results showed significant differences across years in insect species diversity (i.e., richness) and abundance in only two out of four fields (Kent and Tommy Atkins) but the direction of changes was different for each (Fig. 4.5a,b; Table 4.3). On average, values for species richness were lower in 2018 for the Kent area and these returned to pre-hurricane levels by 2019 while in the Tommy Atkins area, trees experienced annual incremental increases in species richness and an increase in insect abundance in 2018 that remained in 2019. The Julie and Keitt fields did not experience significant changes in neither richness nor abundance across years (Fig. 4.5a,b; Table 4.3). The variance in species richness also seemed to increase for all fields in 2018 and also remained elevated in 2019 for the Julie, Keitt and Tommy fields but not for the Kent (Fig. 4.5b).

Fruit production and crop yields – With the exception of the Julie field in 2018, crop yield was strongly and positively correlated with fruit yields for all fields and all years (Pearson correlations for fruit production (the number of fruits produced per tree) vs crop yield; Julie 2018: r 0.39, P = 0.26; all other fields: r = 0.83 – 0.99, p <<0.01). Results involving crop yield and the number of fruits were always similar in magnitude and direction, thus or the sake of simplicity, we only present results for correlates of plant reproductive output as they relate to crop yield. All fields exhibited declines in the number of inflorescences per tree in 2018 that were followed by significant recoveries in 2019 (Fig. 4.4c, Table 4.4). Nevertheless, at the field level, the number of inflorescences per tree during peak flowering was never a good predictor of plant reproductive output (Table 4.4). Instead, plant reproductive output (i.e. crop yield) was positively associated with insect species richness and abundance (Fig. 4.7) but the significance and magnitude of these relationships were also influenced by field and sampling year (Table 4.4). In 2017, positive relationships between yield, species richness and insect abundance were strong for all fields (Fig. 4.7, Table 4.4). In 2018, following the hurricane events, these associations disappeared for the Julie and Tommy Atkins fields and were only present for the Keitt (only for abundance) and Kent (both for species richness and abundance) although they were weaker than they were in 2017 (Table 4.4). In 2019, relationships between crop yield and species richness and abundance returned for the Julie field and were present in the Keitt field but were absent in the areas with Kent and Tommy Atkins trees. While the Julie and Kent fields had consistent fruit yield values across years, the Kent and Tommy Atkins both experienced an overall reduction in crop yield in 2018 but then recovered to pre-hurricane values in the Kent field and went above pre-hurricane levels in the Tommy Atkins' field in 2019. (Fig. 4.5d, Table 4.3). In the Kent field, individuals showed the highest crop yield values of all fields (ave. 407 ± 58.13 kg), followed by the Keitt (ave: 183.25 ± 31.19kg), Julie (ave: 60.77 ± 7.95 kg) and Tommy Atkins (ave: 77.55 ± 10.63 kg) fields (Figure 4.4d). The abundance of two of the dominant insect species (*P. vinetorum* and *C. minima*) showed positive correlation with reproductive output (crop yields) of mango trees but these relationships were not consistent for all years or fields, nor they were consistent within a single season using conservative alpha values (Table 4.5). With more relaxed alpha values, significant associations between insect abundances and crop yield were encountered in three instances for *P. vinetorum,* two for *C. minima* and none for *A. mellifera*. Using less conservative alpha values, the number of significant correlations increases to eight for *P. vinetorum* (distributed across all years), six for *C. minima* (in 2018 and 2019) and only two for *A. mellifera* (in 2017 and 2019 only in Julie) (Table 4.5).

DISCUSSION

Hurricanes are large-scale weather events with the potential to change insect communities and influence their subsequent dynamics (e.g. McGinley and Willing, 1999; Showalter and Ganio, 1999; Showalter et al., 2017). It has been argued that these events may disrupt plant-pollinator interactions with important consequences to food crops (National Research Council, 2007; Natalia Escobedo-Kenefic, 2018). While insects carry out most of the pollination function in plants, most of what we know about insect responses to hurricane events comes from other functional insect groups (herbivores, predators, omnivores, detritivores; e.g. McGinley and Willing, 1991; Torres, 1992; Showalter and Ganio, 1999; Koptur et al., 2002; Spiller et al., 2016; Showalter et. al. 2017; Novais et al., 2018). Indeed, studies evaluating the response of insect pollinators to hurricane events have been few and limited in geographic and taxonomic scope which impairs making generalizations on the short- and long-term effects of these interactions (Bronstein and Hossaert-McKey, 1995; Pascarella, 1998, Roubik and Villanueva-Gutierrez, 2009; Landry, 2013). Here we discuss our results on hurricane-induced changes of pollinator communities of *M. indica* on the island of Puerto Rico following the passage of Hurricane Maria to: 1) build knowledge of post-hurricane community dynamics of insect pollinators, and contribute to the extensive literature available for hurricane responses of insect communities; 2) understand the role of diversity and abundance of insect communities vs the role of individual insect species on the reproduction of a worldwide economically important species, and how these relationships may be influenced by extreme weather events; 3) discuss how this information may inform pollinator management in crop systems in areas where catastrophic weather events such as hurricanes are frequent events.

The literature related to post-hurricane dynamics in arthropod communities suggests that while arthropod populations can take different pathways (increase, decrease or else exhibit complex responses (e.g., Gandhi et al., 2007), under some circumstances these assemblages can be highly resilient and return to pre-hurricane levels in a short period of time (Chen et al., 2020). However, this does not always occur because species responses to hurricanes can depend on the taxon, guild and the environmental context (biotic and abiotic) in which they occur (Showalter et al., 2017; Novais et al., 2018; Savage et al., 2018). In our system, insect pollinator communities did change after the hurricanes of 2017 but as a whole they were on their way to pre-hurricane levels based on how similar they appeared in multivariate space by 2019. Post-hurricane dynamics of the individual insect pollinator species suggest that these temporal changes in community similarities across years were at least in part driven by changes in their relative abundance and not through the substitution of dominant insect species. At the individual species level, hurricanes clearly reduced the populations of *P. vinetorum* and *A. mellifera* (the two most-dominant species before the hurricanes) but *A. mellifera* abundances was on its way to pre-hurricane levels by 2019 (although not completely) while populations of *P. vinetorum* increased in numbers that year. In contrast, *C. minima* abundances spiked dramatically after the hurricane but decreased to pre-hurricane levels by 2019. A probable explanation for the observed differences in species' post-hurricane abundance dynamics is that hurricane-driven changes in the biotic or abiotic environment influenced the life cycle of different taxa in different ways. For example, many syrphid flies such as *P. vinetorum* have aquatic larvae that are saprophagous and feed on organic matter and microorganisms (Pérez-Bañon et al., 2013; Sánchez-Galván, et al., 2017). We know that the 2017 hurricane season resulted in a large accumulation of debris across the island of which 60% was organic (Lugo, 2018). In managed spaces, disposal of this debris was slow and that material might have created ideal habitats for the growth of syrphid larvae during the wet season. Meanwhile, larvae of *Cochliomyia minima* (Calliphoridae) most likely feed on dead carcasses (Yusseff-Venegas, 2014), which were likely abundant after the hurricane events. Following Hurricane Maria and as a result of canopy cover loss, the island of Puerto Rico exhibited increases in temperatures of up to 4°C in some areas (Lugo, 2018).

Differential taxon responses to hurricane-induced abiotic changes may also explain the observed post-hurricane changes in the dominant taxa. It has been shown that exposure to higher than optimal temperatures in *Apis mellifera* bees may lead to reduced reproductive health in colonies through impaired drone development and reproductive quality, especially if changes are sudden (Rangel and Fisher, 2018 and references therein). High temperature exposures in *Apis mellifera* may also reduce survival and increase oxidative stress in bees (Li et al., 2019). On the other hand, higher temperatures shorten developmental time in Calliphoridae (Bansode et al., 2016), and some species (including some *Cochliomyia*) have high thermal tolerances (42°C-53°C; Richards et al., 2009). If local *Cochliomyia* have these traits, then it may explain their numerical increases following the hurricanes. Syrphid species, on the other hand, have variable responses (negative and positive) to changes in their abiotic environment but most of this work has come from temperate regions (Radenković et al.,2017; Milić et al., 2019). Clearly more research is needed on the direct and indirect influences of post-hurricane environmental changes to understand the mechanism driving changes of the dominant pollinators of *M. indica*.

Various studies have emphasized the need to ensure diversity and abundance of wild pollinators and the importance of protecting non-bees as a way to enhance pollination services (Blüthgen and Klein, 2011; Albrecht et al., 2012; Thomson, 2019) and crop production (Rader et al., 2013). The protection of the associated crop biodiversity is seen as an important element to ensure food security and sustainability of agricultural systems (FAO, 2018). Our results do show a clear association between pollinator diversity and abundance with fruit production in *M. indica.* However, an important result of this study is that fruit yields were highly reduced in one of the fields evaluated even when pollinator diversity (as expressed by species richness) and abundance increased locally. Therefore, the combined results not only suggest that the relationship between pollinator diversity, abundance and production in this crop is complex, but they also suggest that mechanisms other than changes in pollinators' richness and total abundance themselves will also influence fruit production after hurricanes.

Here we provide several mechanistic hypotheses to explain our results for *M. indica*, some of which may or may not relate to observed changes in pollinator communities. One possibility is that not all *M. indica* pollinators are equally effective and that localized declines in the relative abundances of less abundant pollinators other than the dominant pollinator species studied here led to declines in yields after the hurricane for the Kent and Tommy Atkins fields. Even when all fields experienced declines in *P. vinetorum* and *A. mellifera* (the dominant pollinators) not all fields experienced declines in fruit yields. Moreover, unexpected significant increases in *C. minima* abundance in 2018 for three fields (Keitt, Kent and Tommy Atkins) did not necessarily translate into concomitant increases in fruit yields for those fields in 2018. Several authors have discussed and tested potential mechanisms in which diversity could stabilize pollination services over time (e.g. Winfree and Kremane, 2009; Mukherjee and Devy, 2019). These include 'density compensation' (i.e. the abundance of one pollinating species increases as a result of reductions in the abundance of another species), 'response diversity (i.e. some pollinating species are increase and others decrease by the same environmental change) and 'cross-scale resilience' (i.e. different species are responding to the same environmental stressor at different spatial and temporal scales). While our study cannot be used to discriminate the applicability of these mechanisms, results presented here can be used to evaluate some of the premises of these mechanisms as to what extent these may or may not apply. Our data suggest that the hurricane event indeed influenced dominant species differently (*P. vinetorum and A. mellifera* decreased, while *C. minima* increased) and that these effects were not observed at the same spatial scales (i.e. abundances of *P. vinetorum* and *A mellifera* were reduced for all fields in 2018 but not those of *C. minima* which instead increased for three of the four fields). Also, fruit yield was reduced for Kent and Tommy even when those fields experienced increases in *C. minima* abundance. Those fruit yield patterns do not completely align with a hypothesis of 'density compensation' effects by *C. minima* for Kent and Tommy Atkins as their fruit yields decreased even when abundances for this pollinating species increased for these fields. The fact that even with increases in species richness and abundances in 2018, fruit yields declined in the Tommy Atkins field also suggests that this flower visitor may not be as effective at compensating for losses or reduction in pollination services from other species that may have experienced local reductions in abundance The fact that the Julie field experienced a reduction in all dominant pollinators but not a reduction in fruit yields also suggests the possibility that other less frequent pollinators relative to the ones studied here may be acting as stabilizing influences of fruit yields and in a localized fashion. A long-term study with the generalist non-agricultural species *Scrophularia californica* showed that the relationship between the abundances of the most effective pollinators and reproductive success was non-linear and influenced by variation in spatial and temporal differences in pollinator diversity and abundance (Thomson, 2018). These complex relationships also apply to agricultural systems such as *M. indica*, whose management for pollinator services and diversity, especially following extreme atmospheric events, would therefore require an understanding of plant-pollinator relationships using both community and species-level approaches.

One important result was that correlations between plant yields and the abundances of individual pollinator species were not consistent across years or fields and that overall *A. mellifera* showed the least number of significant correlations with yield compared to *P. vinetorum* and *C. minima*. Indeed, prior studies have commented on the low attractiveness of mango flowers to honey bees (Free and Williams, 1976) and at least one experimental study that added managed honeybees found no significant increases in fruit production (Carvalheiro, 2010). Our results with honeybees are therefore not surprising considering those studies and raises questions about how effective the addition of managed honeybees is as a strategy to improve local pollinator services following hurricane events for crop species like *M. indica*. Indeed, the study by Carvalheiro et al. (2012) suggest that introducing areas of native vegetation to support pollinator services and improve crop production may be a better management strategy worth pursuing for *M. indica* management. On the other hand, the relationships between diversity and abundance of insect pollinators with plant yield were not equally strong for all fields and that post-hurricane changes in these relationships were not expressed equally among fields (Fig. 7). Each field hosted a different cultivar. Thus, one possibility is that floral characteristics and rewards may differ among cultivars. Mango flowers are minute, and superficially similar among cultivars but could differ in some characteristics which may lead to differential pollinator attraction (Popenoe, 1917). In at least one study, the Keitt cultivar was reported to have low attractiveness relative to Kent and Tommy Atkins (Carvalheiro et al., 2012). We are in the process of analyzing data on floral traits and nectar rewards to address the possibility of differences in floral attractiveness or floral resources among cultivars. Likewise, we have ongoing experiments to evaluate potential differences in pollination effectiveness of dominant pollinators of *M. indica* to different cultivars to better understand the role of individual pollinator species and fruit production in this system. A last possibility, and equally likely, is that mango cultivars are in different spatial locations across the station and that landscape differences in insect requirements unknown to us may operate to influence the visitation to individual cultivars. Studies with *M. mangifera* in South Africa have shown that existing flowering resources available within and outside cultivated areas (i.e natural vegetation) have the potential to influence mango floral visitation by contributing floral resources of shared visitors even outside the mango flowering season (Carvalheiro et at., 2010, 2012; Simba et al., 2018). Carvalheiro et al. (2010), indeed showed that the diversity levels experienced by *M. indica* trees and their fruit yields were dependent on the distance of trees from natural vegetation patches with more diversity and higher yields exhibited by trees that were closer to natural vegetation. There are patches of natural vegetation about 1km away from the Juana Diaz Agricultural Experimental Station and visually the matrix of cultivated trees surrounding the fields with the Julie and Keitt cultivars seems to have a more abundant tree cover than trees from the fields with Kent and Tommy Atkins (Fig.1).

A second non-mutually exclusive alternative for the unexpected declines in fruit production even when pollinator abundances increased following the hurricane may be related to direct hurricane effects on *M. indica* plants. Hurricane disturbances can damage plants to the point of reducing the resources available to produce flowers and fruits (Rathcke, 2000). A rapid assessment of mango trees at the Juana Diaz Agricultural Experimental Station following Hurricane Maria, indicated that trees lost between 20 to 90% of their foliage as a result of this event with no tree mortality observed (Cabrera-Asencio, unpublished data). Thus, resource allocation decisions related to the production of leaves vs. fruits may also explain some of the crop yield dynamics but cannot account for all the variation in fruit production. Even when foliage recovery was slow and branch death was still observed in 2019, observed fruit production increased above pre-hurricane levels in 2019 (this study). The lack of tree mortality following hurricane Maria at our site contrasted with a study on mango tree damage in Florida following Hurricane Andrew in 1992 (Department of Health and Human Services and Department of Agriculture; Crane and Balerdi, 1993; U.S. Department of Agriculture, 1995). The Florida mango trees exhibited considerable damage with 58.4% of the trees showing massive damage or mortality which resulted in a 75% reduction in mango production even after 4 years. Furthermore, most mortality occurred in areas where trees were tall and not managed for size. By contrast, trees at the Juana Diaz Agricultural Experimental Station were managed for size control which may explain different results.

Within season variation in the number of inflorescences did not relate to crop yields and that post hurricane reductions in inflorescence production in mango, while noticeable for all fields, did not necessarily translate into crop yield reductions. Inflorescences of *M. indica* produce numerous and minute unisexual flowers that reach up thousands of flowers like it has been reported for Tommy Atkins (2,238 flowers/inflorescence, Abourayya et al., 2011). It is also widely known that *M. indica* in cultivation produces many more flowers than its sets fruits (fruit sets ~ 10%; Shu, 2006). Thus, one unexplored possibility is that even with the reductions of inflorescences and pollinators, following the hurricanes, the resulting flower: pollinator ratios still allowed to maximize fruit production in some cultivars. Also, the ratio of hermaphroditic to male flowers in andromonoecious plant systems like *M. indica* can vary in response to environmental stressors (Gheeta et al., 2016) in ways that may reflect an optimal use of available resources to reproduction. Thus, another unexplored and not mutually exclusive possibility is that some cultivars of *M. indica* are also able to adjust floral resources in such a way that resources are used more efficiently towards producing fruits. These ideas would need to be explored thoroughly to understand how hurricane changes in floral resources and pollinators may ultimately influence reproduction in *M. indica*.

Fruit yields in this economically important species can bounce back (under some conditions) in less than 2 years. In the case of *M. indica* and assuming low mortality, two conditions may potentiate this recovery. The majority of insects visiting the *M. indica* cultivars are native to the Caribbean region and probably have, through their evolutionary history, developed adaptations that could make them highly resilient to these systems regardless of taxa. Second, the effects of hurricane-related changes in pollinator faunas on plant reproduction (regardless of the plant system) may depend whether or not pollination systems are generalized or specialized (Dalsgaard et al., 2009) or whether or not plant species have alternative mechanisms of plant reproductive assurance (Jones et al., 2001; Rivera & Ackerman, 2006; Pérez et al., 2018). While most cultivars of *M. indica* depend on animal pollination to set fruit (Kumar et al., 2012, Fajardo et al., 2008, Anderson et al., 1982, De Siqueira et al., 2008, Corredor and García, 2011), it is apparent that globally mangos have a highly generalized pollination system, a condition that is likely favorable where extreme weather events are common such as in the Caribbean. Plant reproductive ecology theory states that the capacity to be pollinated by a large diversity of insects may allow for functional redundancy through shifts in dominant pollinator species, allowing for reproduction to occur in highly variable environments (Waser et al., 1996). A generalist pollination system may explain, at least in part, the naturalization and success of this crop in many areas of Latin America and the Caribbean. Whether such success may continue in the face of climatic change and expected increases in extreme weather events depends on long-term monitoring schemes. The use of information on pollinator diversity and abundance in this system for successful agriculture would certainly require more in-depth information on the role of global insect diversity and abundance vs the role of individual species themselves.

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Table 4.1. Kendall correlation coefficients (τ) with ordination axes resulting from Non-metric Multidimensional Scaling (NMDS) analyses for seven species of the communities on four mangos fields each containing a different cultivar (Julie, Keitt, Kent, Tommy Atkins).

Species	r (axes 1)	r^2	r (axes 2)	r^2
Palpada vinetorum (Palvin)	-0.495	0.245	-0.874	0.765
Palpada albifrons (Palalb)	-0.652	0.425	-0.517	0.267
Cochliomyia minima (Chomin)	0.94	0.884	0.421	0.177
Psedodorus clavatus (Psecalv)	-0.67	0.449	0.213	0.045
Api mellifera (Apimel)	-0.853	0.727	-0.579	0.335
Gonia crassicornis (Gon)	0.843	0.711	0.301	0.091
Peckia sp. (DipUnk)	0.711	0.506	0.205	0.042

For each species, r^2 values represent the percentage of variance explained by each axis.

Table 4.2. Repeated measures ANOVA analyses testing for annual differences in the average abundance (number of insects observed/tree/day) of dominant insect species for each four fields cultivars of *M. indica* each containing a different cultivar.

Species	Field	Year	Mean ± SE	F	P
Palpada vinetorum	Julie	2017	19.60 ± 2.90	16.7	0.0001
		2018	3.00 ± 1.83		
		2019	25.10 ± 2.9		
	Keitt	2017	5.70 ± 1.36	27.1	0.0001
		2018	2.90 ±1.36		
		2019	15.00 ± 1.36		
	Kent	2017	3.70 ± 1.31	44.5	0.0001
		2018	0.80 ± 1.31		
		2019	14.90 ± 1.31		
	Tommy Atkins	2017	5.50 ± 1.23	8.64	0.0023
		2018	0.40 ± 1.23		
		2019	7.40 ± 1.23		
Species	Filed	Year	Mean ± SE	F	P
Apis mellifera	Julie	2017	6.50 ± 0.9	13.4	0.0003
		2018	0.40 ± 0.9		
		2019	4.50 ± 0.9		
	Keitt	2017	6.10 ± 0.80	12.5	0.0004
		2018	0.90 ± 0.80		
		2019	4.60 ± 0.80		
	Kent	2017	5.20 ± 0.77	11.96	0.0005
		2018	0.40 ± 0.77		
		2019	2.70 ± 0.77		
	Tommy Atkins	2017	4.60 ± 0.63	26.75	0.0001
		2018	0.40 ± 0.63		
		2019	6.10 ± 0.63		
Species	Field	Year	Mean ± SE	F	P
Cochliomyia minima	Julie	2017	1.3 ± 1.7	38.4	0.0001
		2018	18.6 ± 1.7		
		2019	4.1 ± 1.7		
	Keitt	2017	27.60 ± 2.29	43.57	0.0001
		2018	1.90 ± 2.29		
		2019	1.10 ± 2.29		
	Kent	2017	1.70 ± 0.55	54.17	0.0001
		2018	7.70 ± 0.55		
		2019	0.20 ± 0.55		
	Tommy Atkins	2017	2.00 ± 2.77	35.65	0.0001
		2018	33.90 ± 2.77		
		2019	10.20 ± 2.77		

Significant P- values (<0.05) are indicated in bold.

Table 4.3. Repeated Measures one-way ANOVAs on reproductive traits and insect community traits recorded annually in four fields of *M. indica* between 2017 and 2019.

Trait	Cultivar	F	p
Number of Inflorescences	Julie	21.89	**0.0001**
	Keitt	36.67	**0.0001**
	Kent	74.17	**0.0001**
	Tommy	21.89	**0.0001**
Yield	Julie	2.53	0.11
	Keitt	0.67	0.53
	Kent	14.80	**0.0002**
	Tommy	17.21	**0.0001**
Total Insect Abundance	Julie	0.88	0.43
	Keitt	1.07	0.37
	Kent	14.14	**0.0002**
	Tommy	15.12	**0.0001**
Insect Species Richness	Julie	2.55	0.11
	Keitt	6.52	**0.0070**
	Kent	5.57	**0.0030**
	Tommy	13.61	**0.0003**

Table 4.4. Results for simple regression coefficients from general linear model (GLM) analyses evaluating the variation in reproductive success (plant yield) as a function of insect species richness, insect abundance and the number of inflorescences / trees in four field of *M. indica* in three separate years.

Year	Field	Coefficients	Estimate ± SE	Model:AIC	F	R^2	*p*
2017	Julie	Richness	1.13 ± 0.15***	13.05	41.9	0.84	0.0002
	Julie	Abundance	1.26 ± 0.17***	12.58	44.3	0.85	0.0002
	Julie	Inflorescences	0.46±0.24	26.20	1.34	0.28	0.32
		(Inflorescences)^2	0.43 ± 0.35				
	Keitt	Richness	3.57 ± 0.52***	35.50	36.64	0.82	0.0003
	Keitt	Abundance	3.20 ± 0.40***	32.54	44.3	0.85	0.0002
	Keitt	Inflorescences	0.01 ± 0.003**	29.40	3.13	0.47	0.11
	Kent	Richness	3.86±0.47***	22.23	54.3	0.87	0.0001
	Kent	Abundance	2.42 ± 0.27***	20.70	64.2	0.89	0.0001
	Kent	Inflorescences	0.0008±0.0004	39.52	3.05	0.28	0.12
	Tommy Atkins	Richness	0.97 ± 0.21	17.71	17.65	0.69	0.003
	Tommy Atkins	Abundance	1.53 ± 0.23***	12.18	36.6	0.82	0.0003
	Tommy Atkins	Inflorescences	0.004 ± 0.003	26.17	1.26	0.15	0.27
2018	Julie	Richness	0.45 ± 0.33	21.73	1.49	0.16	0.28
	Julie	Abundance	0.32 ± 0.23	21.59	1.63	0.17	0.24
	Julie	Inflorescences	0.06± 0.17	23.31	2.8	0.26	0.13
	Keitt	Richness	2.13 ± 0.87*	32.50	4.63	0.37	0.06
	Keitt	Abundance	1.77 ± 0.42**	26.89	14.15	0.64	0.0060
	Keitt	Inflorescences	0.71 ± 0.38	34.07	2.8	0.26	0.13
	Kent	Richness	1.47 ± 0.51	26.17	3.6	0.45	0.0300
	Kent	Abundance	1.45 ± 0.28	19.21	21.31	0.73	0.0020
	Kent	Inflorescences	0.06 ± 0.17	23.31	1.19	0.27	0.61
	Tommy Atkins	Richness	0.57 ±0.56	28.55	2.31	0.12	0.26
	Tommy Atkins	Abundance	0.57± 0.35	27.50	2.8	0.27	0.13
	Tommy Atkins	Inflorescences	0.09± 0.42	29.83	0.04	0.004	0.85
2019	Julie	Richness	1.02± 0.31 **	17.7	8.89	0.52	0.0200
	Julie	Abundance	0.91 ± 0.16***	10.26	27.5	0.77	0.0080
	Julie	Inflorescences	0.70 ± 0.39	23.32	1.62	0.17	0.16

Keitt	Richness	1.44± 0.49*	31.7	6.76	0.46	0.03
Keitt	Abundance	2.53± 0.32***	21.4	32.28	0.8	0.005
Keitt	Inflorescences	2.07 ±1.09	34.77	2.86	0.26	0.13
Kent	Richness	0.69± 0.61	22.58	1.01	0.11	0.34
Kent	Abundance	0.85 ± 0.53	21.54	2.11	0.21	0.28
Kent	Inflorescences	-0.70± 0.49	23.32	0.02	0.002	0.90
Tommy Atkins	Richness	0.25± 0.32	13.45	0.48	0.06	0.51
Tommy Atkins	Abundance	0.34± 0.18	10.80	3.05	0.28	0.12
Tommy Atkins	Inflorescences	0.51± 0.26	10.80	3.06	0.28	0.12

Starred values indicate P-values for estimates of regression coefficients: * = 0.05, ** = 0.01, *** = 0.001. Models with significant P-values (<0.05) are indicated in bold.

Table 4.5. Pearson correlation coefficients for the number of fruits per tree and the abundance of dominant insect species per year per field.

Insect Species	Year	Field	r	p
Palpada vinetorum	2017	Julie	0.9707	0.0001
		Keitt	0.9599	0.0001
		Kent	0.3989	0.2534
		Tommy Atkins	0.8236	0.0033
	2018	Julie	0.7684	0.0094
		Keitt	0.337	0.3409
		Kent	N	N
		Tommy Atkins	-0.2129	0.4098
	2019	Julie	0.80547	0.0049
		Keitt	0.9377	0.0001
		Kent	0.6623	0.0519
		Tommy Atkins	0.8585	0.0014
Apis mellifera	2017	Julie	0.6939	0.0259
		Keitt	-0.1113	0.7595
		Kent	-0.1125	0.7568
		Tommy Atkins	0.5287	0.1161
	2018	Julie	-0.0381	0.9166
		Keitt	0.4296	0.2152
		Kent	N	N
		Tommy Atkins	-0.3389	0.338
	2019	Julie	0.8174	0.0038
		Keitt	0.6112	0.0604
		Kent	-0.4153	0.2662
		Tommy Atkins	N	N
Cochliomyia minima	2017	Julie	0.0219	0.9520
		Keitt	0.198	0.5834
		Kent	N	N
		Tommy Atkins	0.9138	0.0002
	2018	Julie	0.6802	0.0304
		Keitt	0.5535	0.0969
		Kent	0.8618	0.0013
		Tommy Atkins	0.7495	0.0126
	2019	Julie	0.6158	0.0579
		Keitt	0.0808	0.8242
		Kent	-0.2806	0.4644
		Tommy Atkins	0.8265	0.0031

Values in bold indicate significant values with a corrected alpha value equal to 0.0002 when corrected for multiple comparisons.

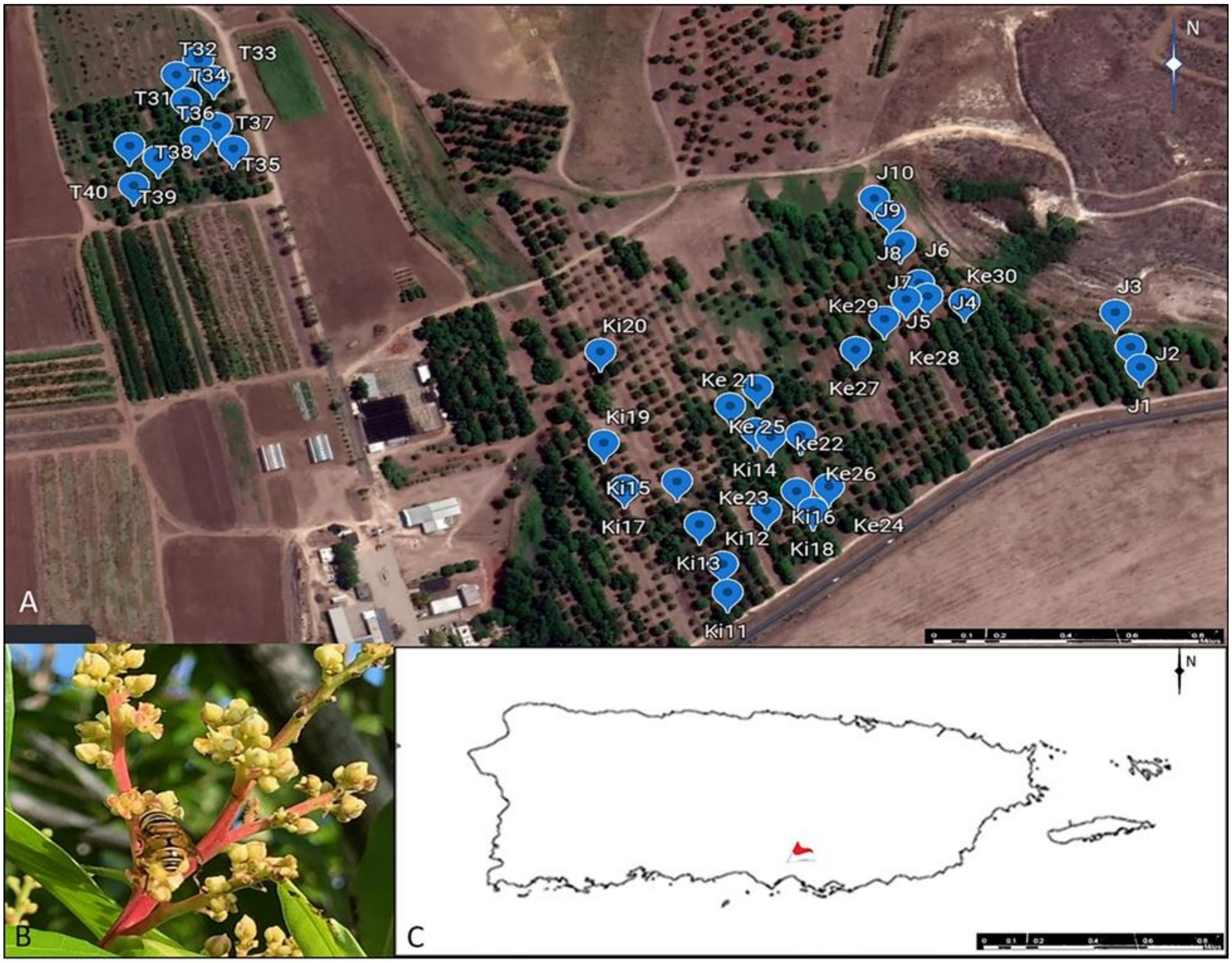

Figure 4.1. (A) Layout of the Juana Diaz Agricultural Experimental Station indicating the location of the mango germplasm and the four field (Julie (J), Keitt (Ki), Kent (Ke), Tommy Atkins (T)) used in this study. (B) *Palpada vinetorum* visiting inflorescences of the Julie field. (C) Location of the Agricultural Experiment Station Juana Diaz, PR. Basemap was obtained from Google Earth Pro, (November 27,2020) Juana Diaz, Puerto Rico. 18°01’37 73, 66° 31’ 23 56’W, Maxar Technologies, https://earth.google.com (February 2, 2021).

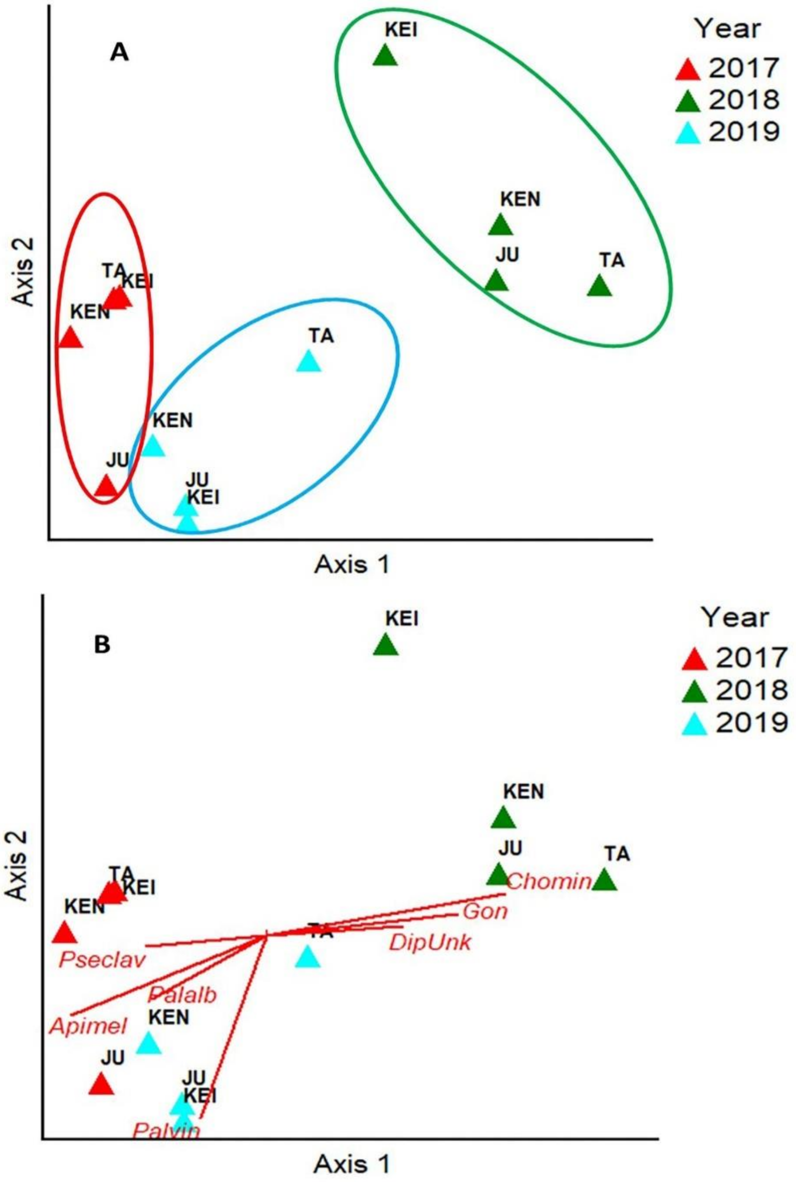

Figure 4.2. Non-parametric multidimensional scaling analysis (NMS) based on Bray-Curtis similarity index evaluating differences in insect species composition among years and fields (Julie, Keitt, Kent, Tommy Atkins). (A) Observation groupings based on within year similarities. (B) Correlations lines showing the strongest associations between insect species and multivariate axes based on insect species abundance. Apimel= *Apis mellifera*; Chomin= *Cochliomyia minima*; Gon= *Gonia* crassicornis.; Pseclav= *Pseudodorus clavatus*; Palalb=*Palpada albifrons*, Palvin=*Palpada vinetorum*, DipUnk= *Peckia* sp.

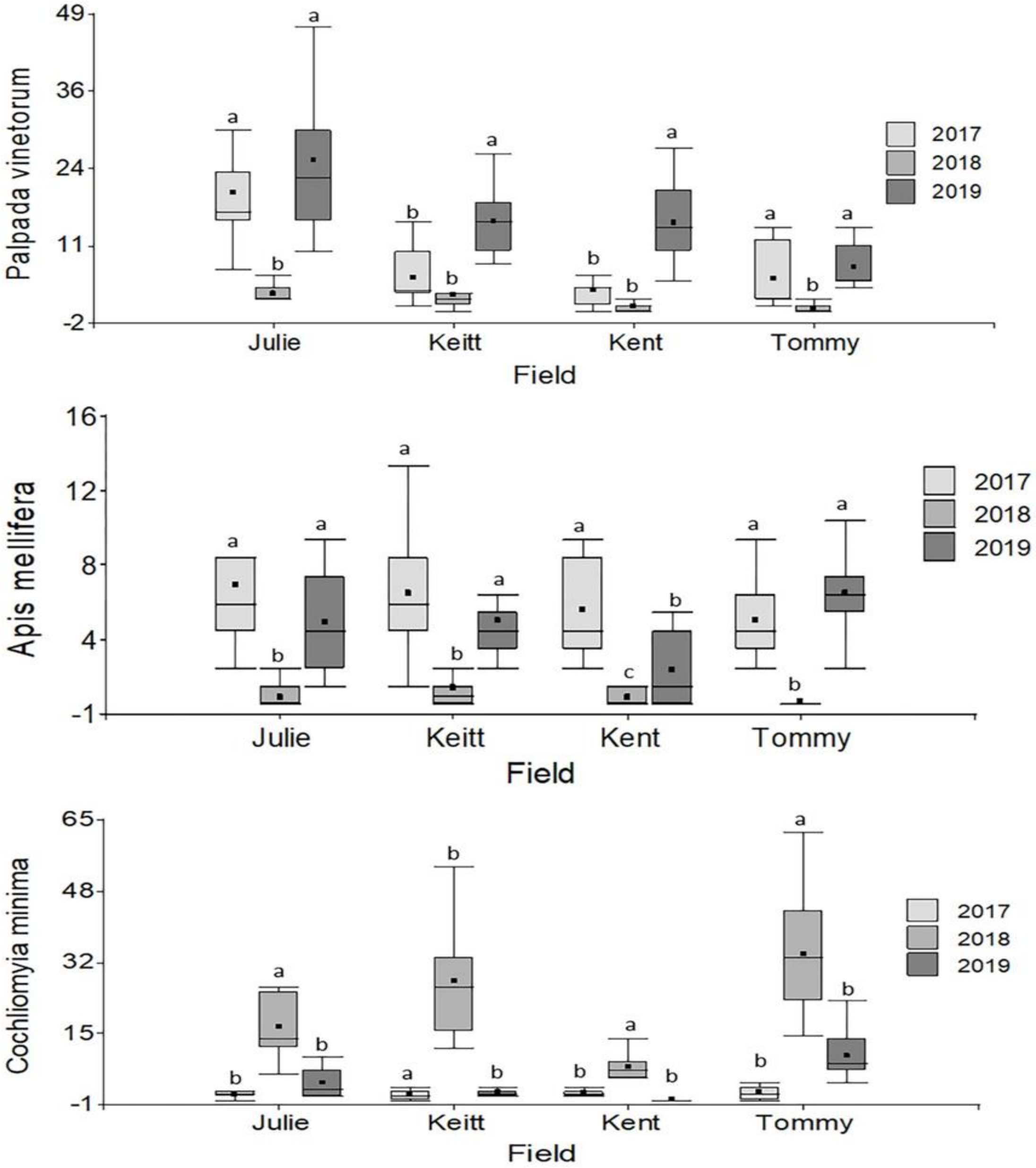

Figure 4.3. Boxplots illustrating differences in insect abundances per field (Julie, Keitt, Kent, Tommy Atkins) per year for the three most dominant flower visitors. (A) *Palpada vinetorum*, (B) *Apis mellifera*, (C) *Cochliomyia minima.* Repeated Measures analyses performed for each year on each field detected significant differences across years all insect species (Table2) of *M. indica* each containing a different cultivar (Julie, Keitt, Kent, Tommy Atkins) detected significant differences across years all insect species (Table 2). Different letters within a field indicate significant differences among years.

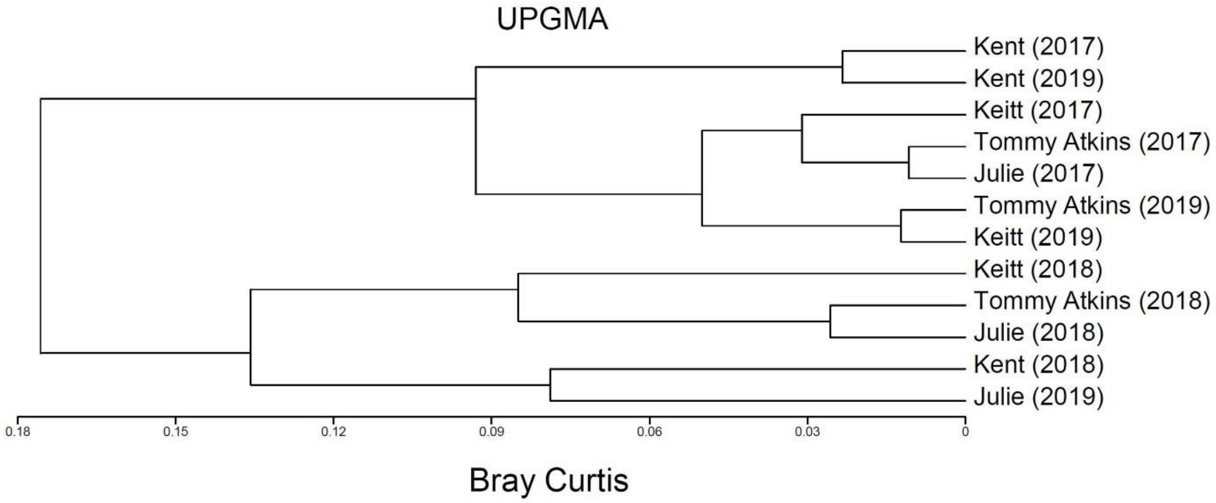

Figure 4.4. Dendogram depicting multivariate clusters using Bray Curtis dissimilarity index based on total insect species richness, total abundance, and abundances of each of the three dominant insect pollinators of *M. indica* (*Palpada vinetorum*, *Apis mellifera*, *Cochliomyia minima*) recorded for four fields (Julie, Keitt, Kent, Tommy Atkins) in 2017, 2018 and 2019 at the Juana Diaz Agricultural Experimental Station in Puerto Rico.

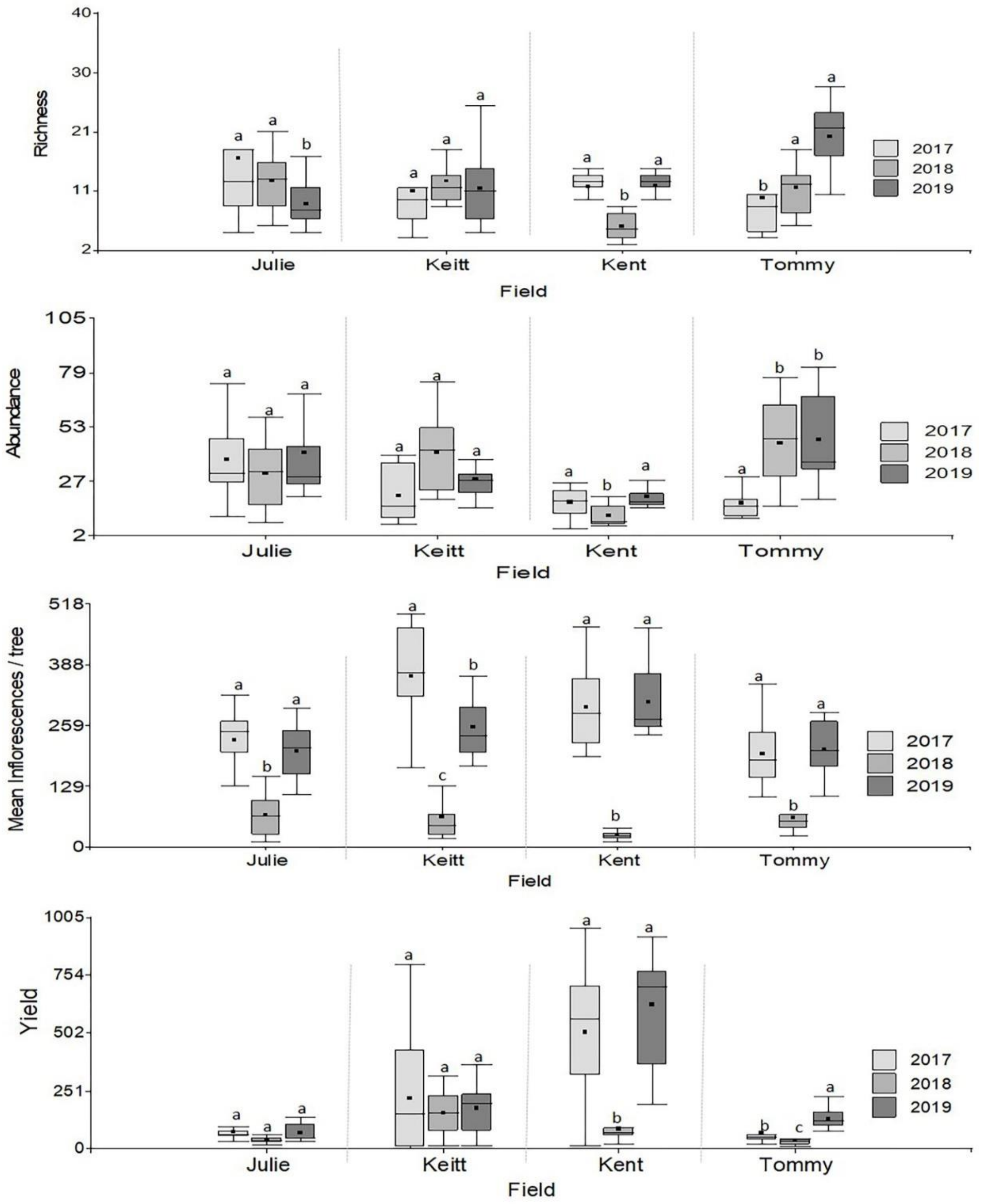

Figure 4.5. Boxplots illustrating differences in variables related to the state of pollinator communities experienced by trees (insect richness and abundance) and variables related to tree reproductive effort (number of inflorescences and fruit yield) in *M. indica* in 2017, 2018 and 2019. (A) Richness, (B) Abundance, (C) Yield (kg), (D) Number of Inflorescences / trees. Different letters within a field indicate significant differences among years following Repeated measures ANOVAs analyses (Table 3).

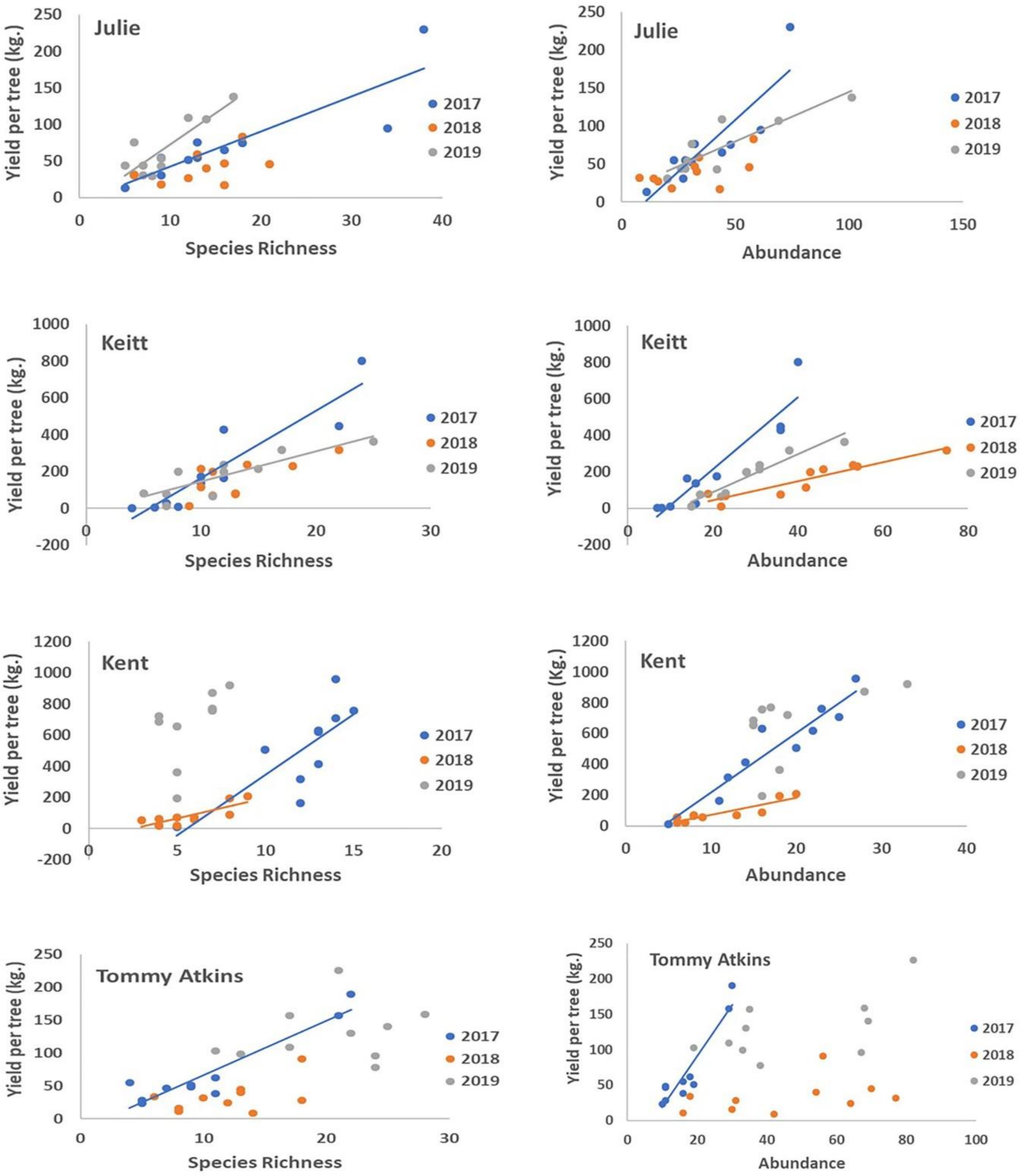

Figure 4.6. Regressions of fruit yield as a function of global species richness and global insect abundance for four fields (Julie, Keitt, Kent, Tommy Atkins) across the 3 years of the study. For each field GLM analyses detected significant relationships between yield and insect richness and abundance in some years but not others (Table 4). Lines represent the line of best fit from significant regressions at $p < 0.05$.

CHAPTER V

TESTING THE POLLINATION EFFICIENCY AND EFFECTIVENESS OF WILD POLLINATIONS OF MANGIFERA INDICA

ABSTRACT

The importance of maintaining pollinator diversity for agricultural production has gathered attention in recent decades given global pollinator declines. A prior study suggested that pollinator diversity was an important driver of fruit production in four mango cultivars (Julie, Keitt, Kent Tommy Atkins). The occurrence of functionally equivalent pollinators may be driving these observations. This study evaluated the efficiency and effectiveness (expected pollen deposition considering visitation rates) of the dominant pollinators and how these related to fruit yields in these four cultivars. Species-specific visitation rates where estimated with video cameras for each cultivar in 2018 and 2019. Pollen deposition of individual pollinators was evaluated with field experiments in 2019. When visitation rates were pooled we found, no significant differences in the average total visitation rate (pooled values of all insects) between 2018 and 2019 in any of the cultivars, but the visitation rates of individual species showed differences across years for some species. *Apis mellifera* and *P. vinetorum* were not detectable in the video feeds in 2018 but were present in 2019 in three of the four mango fields. Visitation rates for *Cochliomyia minima* were statistically higher in 2018 than in 2019 in Julie and those of *Chrysomya* sp were lower in 2018 relative to 2019. In 2018, *C. minima* insects visited Julie flowers more frequently than the other insect species and in 2019, *Chrysomya sp.* insects visited Kent flowers more frequently than any other insect species. The pollen deposition experiment in 2019, showed no significant differences among insect species in the mean pollen load deposited on stigmas on virgin flowers in any of the cultivars. When both components of pollination effectiveness (pollen deposition and visitation rate in 2019) were combined, there were significant differences in pollinator effectiveness only for Julie where *P. vinetorum* was expected to deposit more pollen grains than the other species. Results did not show significant correlations between the global (pooled) visitation rate, and species diversity estimated with video cameras and fruit yields. However, all pollinators showed significant correlations between their visitation rates and fruit yield in at least one cultivar but most significant correlations appeared in 2019 and only one in 2018. Diversity estimates from video

feeds as conducted were significantly lower that diversity estimates from net sweeping which may explain the lack of association between global diversity and fruit yields in this study. Augmenting the number of video cameras may increase detectability of insects and improve diversity estimates. The reduced detectability of some insects in 2018 may have resulted from effects of the hurricane events in 2017 as all insects are detectable in 2019. The hurricane events may also explain the lower number of significant correlations between species-specific visitation rates and fruit yields in 2018 relative to 2019. Even with some significant correlations, it should be emphasized that species-specific correlations were weak. Given that all pollinators seem equally functional for most cultivars (this study) and that fruit yields were not affected following the hurricane events (prior study), it is likely that fruit yields are stabilized across years by a consistent visitation rate by the overall pollinator community which includes pollinators not evaluated in this study.

INTRODUCTION

The loss of pollinator species diversity has been an important conservation issue of worldwide concern for some time (FAO, 2018; Costanza et al., 2014; Potts et al. ,2016). More than 80% of angiosperms species depend on animal pollination to produce seeds (Ollerton, 2011) and it has been argued that the global reduction in pollinators may threaten global economies and compromise global food security (Aizen et al., 2019). It is reported that 1,500 crops depend on animal pollination (Klein et al., 2007) and that animal-mediated pollination services to agriculture have a $361 billion value worldwide (Lautenbach et al., 2012). The economic importance of pollination services has been accompanied with surge in pollination research testing important ecological theories with the potential to support management (Winfree and Kremen, 2009). Ecological intensification is one of the management that help increased targeting of the specific species group providing the bulk of the services to a particular crop (Kleijn et al., 2019). The pollination by bees has been an important focus of pollination research to support crops (Patel et al., 2021) but an increasing number of studies have argued that taxonomic diversity can also be an important determinant of crop production (Brittain et al., 2013, Scott-Brown and Koch, 2020). Moreover, recent work has pointed out that most pollination research related to crop production, not only may be ecologically biased (mostly focused on bees and primarily on honeybees) (Rader et al., 2016, Scott-Brown and Koch, 2020) but also geographically biased with a large proportion of research on pollinator services in crop systems being conducted in temperate areas of the global north (Porto et al., 2020). The contextual nature of plant-pollinator interactions requires reducing the ecological and geographical biases in order to better serve the global management of pollinators in crop systems in the face of global pollinator declines.

The importance of pollinator diversity in crop systems has been at the forefront of pollination work with crop systems especially due to its potential to provide pollination redundancy in the face of environmental change (Winfree and Karem, 2009) or its potential to improve fruit production yields (Garibaldi et al., 2016). From a management perspective, understanding the role of pollinator diversity in crop pollination requires an understanding of the additive effects of various pollinators and how well they can replace each other following extinction or declines (Miñarro and Garcia, 2018). That being said, pollinator biodiversity may be particularly relevant to plant systems with generalized pollination systems where flowers can be successfully visited by a wider variety of pollinators relative to crops with more specialized systems where pollination can only be effectively executed by certain types of pollinators given their shapes and morphologies (Miñarro and Garcia, 2018).

Mangifera indica L. (Anacardiaceae) is a crop that is distributed in tropical and subtropical areas (Galán, 2009; Jiron and Hedström, 1985) where it is of great economic importance (Galán, 2009). This crop is visited by a taxonomically diverse pollinator fauna, although the composition and quantity of species seems to vary between the different geographical areas where it has been studied (Hsin Sung et al., 2006; Anderson et al., 1982; Bathia et al., 1995; Ramírez & Davenport, 2016; Singh,1988; Dag and Gazit, 2000; Wongsiri and Chen,1955). One study in the island of Puerto Rico reported changes in the relative abundance of pollinators visiting four cultivar of *M. indica* following the hurricane seasons of 2017 but these changes did not result in changes in fruit yields (Cabrera-Asencio and Melendez-Ackerman, 2021; Chapter IV). Results indicated that yields are positively influenced by pollinator biodiversity and suggested that some of the dominant pollinators be functionally redundant (equally effective) but not all (Cabrera-Asencio & Melendez-Ackerman, 2021; Chapter IV). In that study, the abundance of *Apis mellifera* did not seem as important at influencing fruit yields as their Dipteran counterparts.

The pollination effectiveness in crop systems of a given animal species will be a function of their pollen transfer efficiency and their visitation frequency (Kearns and Inouye, 1993; Rader et al. 2009). Pollinator efficiency is often viewed as the number of pollen grains deposited on a virgin stigma during a single flower visit (Ne'eman et al., 2010; see also Rader et al., 2009; Rader, et al.,2012; Garibaldi et al., 2013; King et al., 2013; Rader et al., 2013; Ballantyne et al., 2015). Meanwhile the visitation frequency is a function of the abundance of the pollinator and the number of flowers it visits in a given time interval (Herrera, 1987, 1989; Vazquez et al., 2005; Madjidian et al., 2008). The most effective insect pollinator would therefore be one that is expected to deposit the most amount of pollen when flower visits are considered. The effectiveness of a flower visitor can also be measured as the proportion of seeds set following a single visit to a flower (Phillips et al., 2018; Schemske & Horvitz, 1984; Sahli & Conner, 2007; Bruckman & Campbell, 2014; Barrios et al., 2016), but the relationship between pollen transfer and seed set can be complicated due to non-linear effects of multiple visits and unrelated post-pollination processes (Ne'eman et al., 2010) which why it is important to connect pollen deposition and yield when exploring the consequences of pollination effectiveness. More often than not the relationships between pollinator effectiveness as fruit yield are often less explored.

This study evaluated the pollination functionality of dominant flower visitors of four cultivars of *M. indica* taking advantage of prior studies in Puerto Rico that evaluated pollinator communities of this crop system and their links to fruit yields (Cabrera-Asencio and Meléndez-Ackerman 2021, Chapter IV). A major goal was to address if there were differences in pollination effectiveness among dominant flower visitors of these cultivars. A minor goal was to evaluate how different methods for estimating species diversity (via manual collection or video cameras) could influence results on the link between pollinator diversity and fruit yields. The use of conventional methods of observing pollinator activity in the field can be time- and cost-consuming (Pegoraro et al., 2020). Video recording has been proposed as an effective method to monitor pollinators, although there are issues with hardware reliability under field-conditions (Pegoraro et al., 2020). Studies that continue exploring the use of video systems are needed to establish monitoring standards. Results from this study provide important information about potential for pollinator redundancy and replacement than in turn could inform pollinator management and monitoring activities of mango crops to support the stabilization of fruit yields in the face of environmental change.

MATERIALS AND METHODS

Site of study: The field work was conducted at the Juana Diaz Agricultural Experimental Station (JDAES) (180 01'N, 660 31'W), located in the southern coast of the island of Puerto Rico. The station has an average temperature that varies from *22 °C* to 33 *°C and an* approximately total annual rainfall is 977.1 mm (Harmsen et al., 2014). This research center covers an area of 111.23 ha with a prime soil's classification (Beinroth et al., 2003) and maintains the only mango germoplasma in the island since 1968.

Study system: *Mangifera indica* L. is a perennial tree in the family Anacardiaceae. The flowers of the mango are produced in panicles with a main axis that branches into secondary, tertiary and sometimes quaternary axes (Coetzer et al., 1995; Goguey, 1997). The panicles produce both male and hermaphroditic flowers that contain a globose ovary with a single stigma (Ramirez and Davenport, 2010). In this study, we used plants from four mango cultivars Julie, Keitt, Kent, Tommy Atkins where flower traits, insect communities and dominant pollinators have been characterized (Chapter II, Chapter IV, Cabrera-Asencio and Meléndez-Ackerman 2021). At JDAES flowers of mango cultivars are visited by 50 insect species belonging to four different orders (Diptera, Hymenoptera, Coleoptera and Lepidoptera) of which four are dominant visitors: *Apis mellifera*, *Chrysomya* sp., *Cochliomyia minima* y *Palpada vinetorum* (Cabrera-Asencio and Melendez -Ackerman, 2021) (Appendice A).

Visitation Rate and Insect Diversity – Flower insect visitors were recorded using four GoPro cameras (Hero 4) during the flowering period (six weeks) of each of the four cultivars in 2018 and 2019. Each day one camera was mounted on a tripod at a distance of 0.15 m from an inflorescence of one tree of each cultivar (one camera/tree; 4 trees/day) and it was left recording from 8:00 h to 14:00 h. The cameras were rotated each day from Monday through Friday to a different tree and this procedure was repeated every week for five other trees. At the end of each flowering period, all cultivars had 10 trees with video observations in three different dates which amounted to a combined number of observations of about 315 hours for each cultivar. Daily video feeds were observed to: a) identify all insect species that visited flowers, b) count the number of flowers visited by each insect species within a frame and c) estimate the total visitation rate (pooled flowers visitation rate regardless of the species) and the individual visitation rate (number of flower/hr/day) for each insect species. For each tree, I then estimated the average total insect visitation rate/tree and the average visitation rate/tree for each insect species based on the three observation days for each tree over the flowering period. For each cultivar, we tested for annual differences in the total visitation rate per tree and the cumulative number of insect species (species diversity) among cultivars using Wilcoxon Sign tests. For each year we also used Kruskal Wallis, to evaluate differences in visitation among the four dominant insect species (*Apis mellifera, Chrysomya* sp., *Cochliomyia minima* y *Palpada vinetorum)* within each cultivar. We ran Pearson correlations to evaluate relationships between total visitation rates (and insect-specific visitation rates) and fruit yields per tree estimated for a concurrent study. Data on pollinator diversity using a net sweeping method was also available from a prior study for the same cultivars and individuals evaluated here and for the same time periods of this study. (Cabrera-Asencio and Meléndez-Ackerman 2021, Chapter IV). This presented an opportunity to compare and validate diversity values estimated from the video feed with diversity values estimated from need sweeping using Wilcoxon Sign tests.

Pollination Efficiency and Effectiveness– In 2019, an experiment was carried out to determine the pollination efficiency of the four dominant insect visitors (*Apis mellifera, Chrysomya* sp., *Cochliomyia minima* y *Palpada vinetorum*) of flowers for each of the four *M. indica* target varieties. Two days before the experiment, two inflorescences with buds that were ready to open were selected and were covered with an organza bag to prevent the visitation of virgin flowers by insects. Soon after, a semi-open hermaphroditic flower was available in the inflorescence enclosure, the flower was tagged and the organza cover was quickly placed back over the inflorescence. An insect from one of the dominant pollinators was collected and inserted into one of the organza-covered inflorescences and kept inside until the insect visited the open flower (15 min to 1hr after placing the insect in the bag). Upon one single visit, the flower was collected and taken to the laboratory to count the number of pollen grains deposited on the visited stigma. A flower from the other unvisited inflorescences of the pair was also collected as a control for the bag manipulation. In the laboratory, the flower stigmas were removed from the flowers and these were mounted on a slide with a gelatin and glycerin medium (Kearns and Inouye procedure 1993) after which the number of pollen grains were counted. For each cultivar, the design had pollen counts for 2 visited flowers per tree which were then averaged and resulted in 10 values for the average number of deposited pollen grains per flower per tree per cultivar for each flower visitor. None of the control flowers had pollen grains, therefore these were not included in the analyses.

For each cultivar, I analyzed the difference in the average number of pollen grains deposited per stigma with Kruskal Wallis to test for differences in the average pollination efficiency of each visitor per cultivar. To obtain values of pollination effectiveness for each flower visitor (*Apis mellifera*, *Chrysomya* sp., *Cochliomyia minima* y *Palpada vinetorum*) for each tree, I multiplied the species-specific values of average pollen deposition for each tree by their average flower visitation rates. For each cultivar, differences in pollination effectiveness among flower visitors were tested with Kruskal Wallis.

RESULTS

Visitation rate- There were not significant differences in the average total visitation rate between 2018 and 2019 in any of the cultivars. (Fig. 1A). However, when analyzed by insect species *Apis mellifera* and *P. vinetorum* were not detectable in the video feeds in 2018 but were present in 2019 in three of the four mango fields (Table 5.1). Visitation rates for *C. minima* were statistically higher in 2018 than in 2019 in Julie and those of *Chrysomya sp.* were lower in 2018 relative to 2019. In 2018, *C. minima* insects visited Julie flowers more frequently than the other insect species and in 2019, *Chrysomya sp.* insects visited Kent flowers more frequently than any other insect species (Table 5.1). We found significant differences in the mean diversity of insect species visiting flowers with higher diversities in 2019 relative to 2018 for all cultivars (Fig. 5.1B).

Pollination Efficiency and Effectiveness -The pollen deposition experiment in 2019, showed no significant differences among insect species in the mean pollen load deposited on stigmas on virgin flowers in any of the cultivars (Table 5.2). When both components of pollination effectiveness (pollen deposition and visitation rate in 2019) were combined there were significant differences in pollinator effectiveness only for the field hosting Julie where *P. vinetorum* was expected to deposit more pollen grains than the other insect species (Fig. 5.2). Overall, In Julie, *Palpada vinetorum* is expected to deposit more pollen grains than the other insect species when visitation rates are considered (Fig. 5.2).

Visitation rate, insect diversity and fruit yields. Kendall correlations between the pooled visitation rates and fruit yields were not significant (Table 5.3). However, when considering individual species all pollinators showed significant correlations between their visitation rates and fruit yield in at least one cultivar but most significant correlations appeared in 2019 and only one was significant in 2018 (Table 5.4). In 2019 significant positive correlations were found between *Chrysomya sp* visitation rates and fruit yields in fields with Keitt and Kent, between *P. vinetorum* visitation rates and fruit yields in fields with Julie and Keitt and between *A. mellifera* visitation rates and fruit yields in the field with Keitt (Table 5.4). In 2018 only, visitation rates of *C. minima* were positively correlated with fruit yields in Tommy Atkins (Table 5.4). Kendall correlation analyses showed no significant differences between species diversity as estimated from video feeds for any of the cultivars evaluated (Table 5.3) but ANOVA analyses found significant differences in the estimates for species diversity generated from video feeds and from sweeping methods (Fig. 5.3, Table 5.5). In all cultivars the video method always showed lower insect diversity values than the sweeping method the (Fig. 5.3).

DISCUSSION

Mangifera indica is a crop system that attracts a wide range of pollinators species and taxa (Hsin Sung et al.,2006; Anderson et al., 1982; Ramirez and Davenport, 2016; Singh, 1988; Carvalheiro et al. 2010, 2012). From a management perspective, it is important to understand if all flower visitors are equally important or if some pollinators are more important than others. One study at the JDAES showed that mango fruit yields were positively correlated with the diversity of pollinators using net sweeping techniques even when some insect species dominate (Cabrera-Asencio and Meléndez-Ackerman 2021, Chapter IV). The combined results of that and this study suggest that even when some insect species dominate in terms of abundance, even the less common species are likely contributing pollination services and there is likely to be pollination redundancy. First, even when all dominant species were equally efficient at depositing pollen, their visitation rates were not always the best predictor for fruit yields. For all cultivars, fruit yields were consistent across years (Cabrera-Asencio and Meléndez-Ackerman 2021, Chapter IV) and so are the pooled insect visitation rates even when the visitation rates of dominant pollinators fluctuates considerably across years and species diversity as significantly different across years (this study). Even when all dominant pollinators are equally efficient at depositing pollen, Dipteran pollinators might be more consistent than *Apis mellifera* and may be providing some redundancy to this crop system over space and time. Visitation rates by Dipteran species were more frequently related to fruit yields than visitation rates by *A. mellifera*. All cultivars were consistently visited by at least two Dipteran species regardless of year where as *A. mellifera* was not detected by video feeds in three out of four cultivars in 2018. We know that *A. mellifera* was present at JDAES in 2018 from concurrent insect collections with net sweeping but in lower abundances than in 2019 most likely as a result of effects from the passage of hurricane Maria (Cabrera-Asencio and Meléndez-Ackerman 2021). Its absence from video feeds in 2018 and appearance in 2019 confirms the reduction-recovery pattern observed in that study and suggest that for 2018, this species most likely was not an important pollinator in 2018. Even when, other studies have listed *Apis mellifera* as a common flower visitor and a potential pollinator of mango (Wongsiri and Chen, 1995, Fajardo et al., 2008), some studies have suggested that these may not be effective pollinators of that crop (Carvalheiro et al., 2010; Carvalheiro et al., 2012; Jiron and Hedstrom,1986; Dag and Gazit, 2000). Here, I have shown that even when *Apis mellifera* may have the capacity to deposit as many pollen grains as the Dipteran species, it is not a temporally, nor a spatially consistent flower visitor of *M. indica*. From a managing perspective, ensuring dipteran diversity may be a better goal for maintaining fruit yields.

An interesting outcome was that *Palpada vinetorum* appeared to be a more effective pollinator of Julie in 2019. That resulted from significantly higher visitation rates of those insects to the Julie field which was not seen for the other fields. Spatial differences in floral resources available to mango pollinator have been shown to influence the reproductive success of mango crops elsewhere (Carvalheiro et al., 2012). Current data for these cultivars at JDAES on floral variation related to inflorescence size, flower production, flower nectar and UV reflectance (Chapter 2), do not show obvious consistent differences in characteristics (flower resources, visual cues) that would translate into differences in pollinator attraction between Julie and the other cultivars. However, Julie flowers emit a strong scent that is not obvious in the other cultivars and scent can serve as a floral attractant of insects (Dobson 2006). Therefore, future studies would be necessary to determine if differences in floral scent are the driver behind observed higher visitation rates to Julie by *P. vinetorum* relative to other cultivars.

A potential limitation of this study lies in the reduced number of video cameras available for quantifying visitation rates and pollinator diversity. Clearly, diversity estimate much lower to what was estimated with net sweeping and most likely actual visitation rates using video feeds were largely underestimated. With this system, identifying small pollinators can be difficult as that would require have the cameras up-close and there is a trade-off between the number of flowers that can be observed at once and the recording distance for the camera. All available studies of mango visitation elsewhere have used direct field observation or with net sweeping (Carvalheiro et al., 2012; Dag and Gazit, 2000; Jirom and Hedstrom, 1985; Huda et al., 2015; Corredor and Garcia, 2011; Kumar et al., 2018). Pegoraro et al. (2020) recommends the used of several camera systems to record large pollinators. Possibly for mango, multiple cameras (more than one) at different distances in a tree should be recommended to better determine all large and small visitors. It should be noted that video identification requires entomological expertise that may not be as readily accessible as it was for this study and that it requires a lot of processing time (more than one day for to upload the videos to the computer every day to take videos and more than 9 months to view the videos).

The decline in the diversity and abundance in wild insect pollinators in many agricultural landscapes may lead to reductions in agricultural yields (Garibaldi et al., 2013). *Mangifera indica* seems to be a generalist flowering crop species, visited by equally functional pollinators and where diversity appears to be important for maintaining fruit yields. AT JDAES, maintaining the diversity of visiting insects should be a priority. Insect monitoring programs should be implemented to keep track of this diversity as well as research to understand the drivers of this diversity and how well it responds to environmental fluctuations. Research to understand how these insects are attracted to other plant species in the vicinity of the *Mangifera* germplasm, and their overall habitat requirements may help us devise landscape-level strategies to increase and manage their populations. These strategies may be useful to increase fruit yields or to stabilize populations following unforeseen pollinator declines.

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Table 5.1. Visitation rate of Apis *mellifera, Chrysomya sp.*, *Cochliomyia minima, Palpada vinetorum* for each cultivar of *Mangifera indica* (Julie, Keitt, Tommy Atkins, Kent) during 2018 and 2019. For each year and each cultivar, H and p statistics are provided for Kruskal-wallis tests evaluating differences among insect between species in flower visitation rates. Visitation rate values in bold indicate significant value differences across years using Wilcoxon Signed Rank Tests at $\boldsymbol{\alpha} =0.05$.

Visitor	Cultivar	Visitation rate 2018 mean ± sd	Visitation rate 2019 mean ± sd
Apis mellifera	Julie	--	1.78 ± 2.55
Chrysomya sp		1.21± 1.34	0.10 ± 0.11
Cochliomyia minima		3.35 ± 3.68	0.23 ± 0.31
Palpada vinetorum		---	3.94 ± 5.13
		H=14.63, p=0.0008	H=2.04, p=0.54
Apis mellifera	Keitt	0.21± 0.46	1.15 ± 1.84
Chrysomya sp		0.57 ± 1.21	0.10 ± 0.14
Cochliomyia minima		2.86 ± 3.80	0.23 ± 0.09
Palpada vinetorum		0.05 ± 0.10	1.84 ± 3.11
		H= 2.45, p=0.55	H= 5.03, p=0.17
Apis mellifera	Kent	--	0.04 ± 0.06
Chrysomya sp		0.04 ± 0.09	2.05 ± 1.79
Cochliomyia minima		0.53± 0.62	0.17 ± 0.20
Palpada vinetorum		--	0.64 ± 0.72
		H= 0.00, p=0.99	H=8.88, p= 0.03
Apis mellifera	Tommy Atkins	--	1.25 ± 1.13
Chrysomya sp		0.73± 1.02	0.37 ± 0.32
Cochliomyia minima		3.66 ± 3.21	0.54 ± 0.35
Palpada vinetorum		--	0.18 ± 0.28
		H=3.0, p= 0.1143	H= 5.91, p= 0.1146

Table 5.2. Kruskal wallis for the pollination efficiency (pollen grains/pistil) of insect flower visitors (*Apis mellifera, Chrysomya sp., Cochliomyia minima, Palpada vinetorum*) for four cultivars of *Mangifera indica* (Julie, Keitt, Tommy Atkins, Kent) determined from single visit experiments conducted in 2019**.**

Visitor	Cultivar	Grains pollen/pistil mean± sd	H	P
Apis mellifera	Julie	1.89 ± 1.27	5.90	0.06
Chrysomya sp		1.29 ± 0.61		
Cochliomyia minima		1.50 ± 0.86		
Palpada vinetorum		2.41 ± 1.58		
Apis mellifera	Keitt	1.60 ± 0.83	4.63	0.09
Chrysomya sp		1.15 ± 0.38		
Cochliomyia minima		1.41 ± 0.71		
Palpada vinetorum		1.94 ± 1.30		
Apis mellifera	Kent	1.82 ± 0.73	2.40	0.39
Chrysomya sp		1.50 ± 0.71		
Cochliomyia minima		1.44 ± 0.63		
Palpada vinetorum		1.90 ± 1.37		
Apis mellifera	Tommy Atkins	1.30 ± 0.48	3.45	0.18
Chrysomya sp		1.14 ± 0.36		
Cochliomyia minima		1.53 ± 0.64		
Palpada vinetorum		1.67 ± 0.90		

Table 5.3. Kendall correlation coefficients for the comparison of Global visitation rate vs yield and Global species diversity vs. yield in videos for each cultivar during 2018 and 2019. Values in bold indicate significant value with α =0.05 when corrected for multiple comparisons.

Insect Species	Year	Cultivar	τ	p
Global visitation rate	2018	Julie	-0.1556	0.5312
		Keitt	0.1380	0.5860
		Kent	0.3043	0.2655
		Tommy Atkins	-0.0449	0.8575
	2019	Julie	0.4420	0.0833
		Keitt	0.0460	0.8559
		Kent	0.4420	0.0833
		Tommy Atkins	0.2247	0.3692
Global species diversity	2018	Julie	0.0994	0.7071
		Keitt	-0.0563	0.8371
		Kent	0.2254	0.4109
		Tommy Atkins	-0.0830	0.7638
	2019	Julie	0.8990	0.1071
		Keitt	0.1491	0.5730
		Kent	0.4166	0.1119
		Tommy Atkins	0.3381	0.2174

Table 5.4. Kendall correlation coefficients for the comparison of visitation rate video vs yield for each cultivar during 2018 and 2019 for dominant insect species in videos. Values in bold indicate significant value with $\alpha = 0.05$ when corrected for multiple comparisons.

Insect Species	Year	Cultivar	r	p
Apis mellifera	2018	Julie	N	N
		Keitt	0.8367	0.0522
		Kent	N	N
		Tommy Atkins	N	N
	2019	Julie	0.7379	0.0770
		Keitt	0.7487	0.0235
		Kent	-0.1195	0.7815
		Tommy Atkins	0.6708	0.1172
Chrysomya sp.	2018	Julie	0.4000	0.3272
		Keitt	0.7379	0.0770
		Kent	N	N
		Tommy Atkins	0.6000	0.1416
	2019	Julie	0.7379	0.0770
		Keitt	0.9487	0.0230
		Kent	0.8889	0.0143
		Tommy Atkins	0.4472	0.2963
Cochliomyia minima	2018	Julie	0.4000	0.3272
		Keitt	0.8000	0.0500
		Kent	-0.1195	0.7815
		Tommy Atkins	0.8007	0.0143
	2019	Julie	0.8819	0.0459
		Keitt	0.5270	0.2065
		Kent	0.7379	0.077
		Tommy Atkins	-0.1179	0.7883
Palpada vinetorum	2018	Julie	N	N
		Keitt	0.6325	0.1573
		Kent	N	N
		Tommy Atkins	N	N
	2019	Julie	0.9487	0.0230
		Keitt	0.8695	0.0143
		Kent	0.4001	0.3272
		Tommy Atkins	0.3536	0.4205

Table 5.5. Anova analyses for two different methods (video and sweeping) for observed diversity of visitors of flowers in each different cultivar of Mangifera indica (Julie, Keitt, Kent, Tommy Atkins) in two different years 2018 and 2019.

A. Julie	Anova	Diversity with different methods	
	Model	F	p
	Year	0.92	0.3427
	Method	100.55	0.0001
	Year * Method	1.31	0.2599

B. Keitt	Anova	Diversity with different methods	
	Model	F	p
	Year	1.11	0.2991
	Method	109.84	0.0001
	Year * Method	2.16	0.8688

C. Kent	Anova	Diversity with different methods	
	Model	F	p
	Year	47.48	0.0001
	Method	140.61	0.0001
	Year * Method	12.43	0.0012

D. Tommy Atkins	Anova	Diversity with different methods	
	Model	F	p
	Year	21.95	0.0001
	Method	108.66	0.0001
	Year * Method	5	0.0316

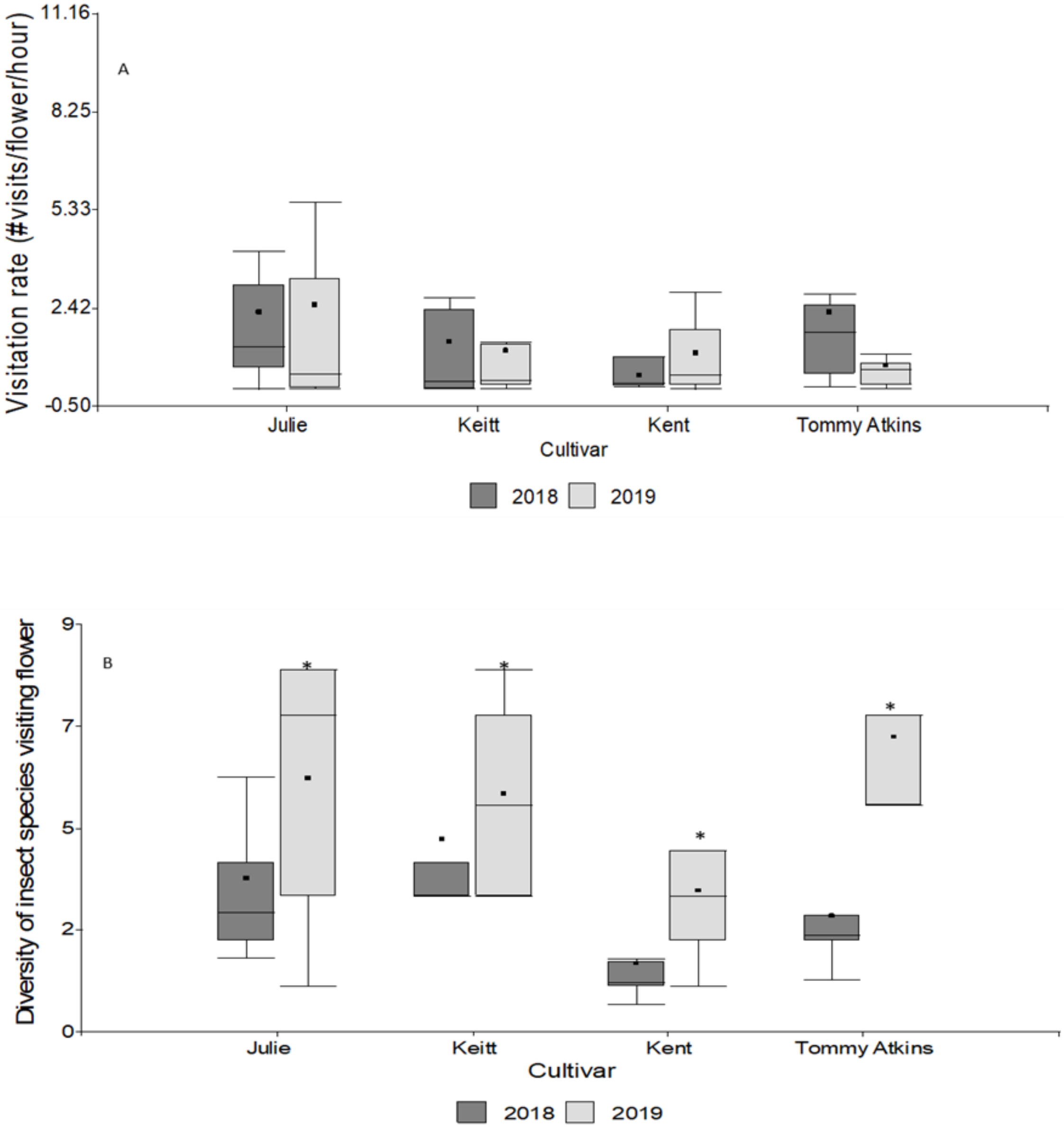

Figure 5.1. Global (polled) visitation rates (mean number of visits per flower per hour by observation days and species diversity) estimated from video feeds for each mango cultivar (Julie, Keitt, Kent, Tommy Atkins) during 2018 and 2019. A. Wilcoxon tests for each cultivar evaluating between year differences in visitation rate yielded all *p's* > 0.05. B. Results for Wilcoxon tests evaluating differences in mean insect diversity for each cultivar (Julie=W=110, p< 0.04; Keitt= W=101, p<0.04; Kent= W=67, p<0.003; Tommy Atkins= W=74, p< 0.01 yielded significant differences for (p<0.05).

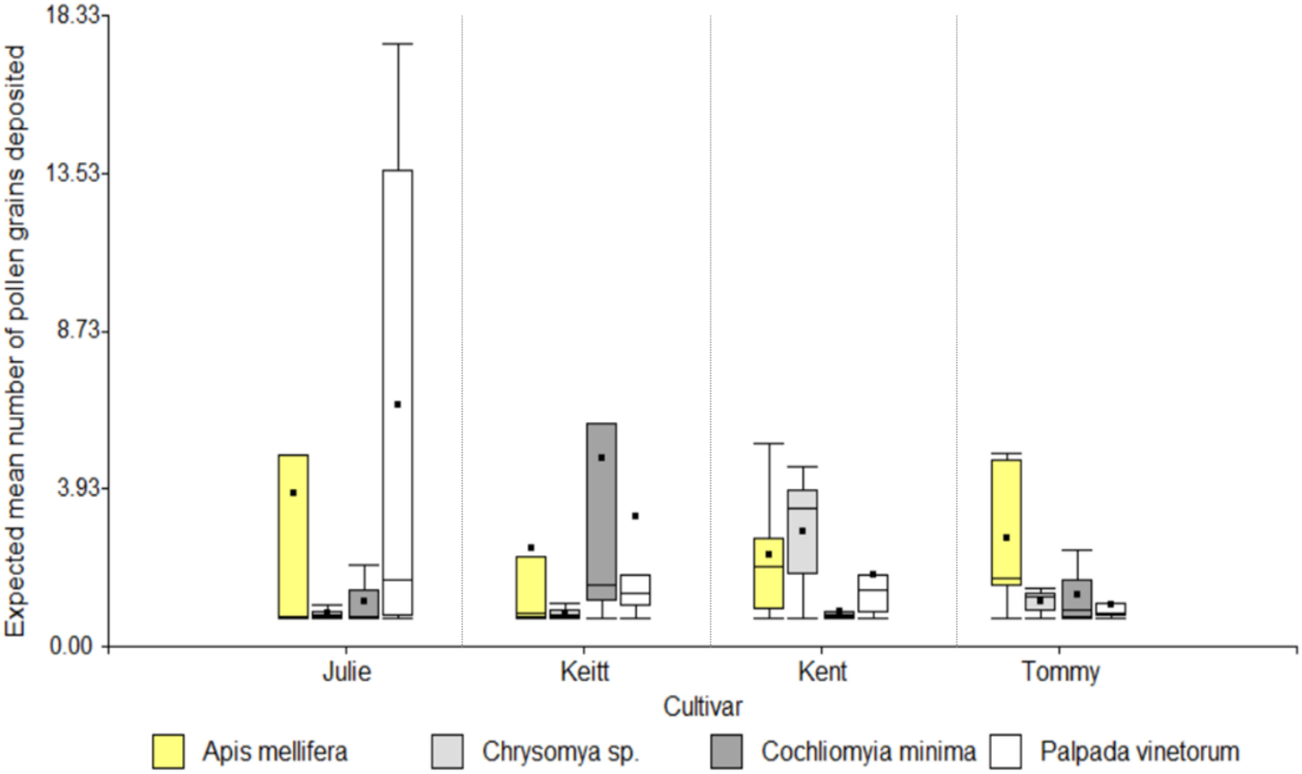

Figure 5.2. Pollination effectiveness of four visitors of mango flour on four cultivars. Effectiveness was calculated as the expected number of pollen grains deposited on stigmas based on empirical pollen depositions corrected for known visitation rates. Kruskal Wallis for each cultivar tested for differences in mean pollination effectiveness among different insect visitors (*Apis mellifera*, *Chrysomya sp.*, *Cochliomyia minima*, *Palpada vinetorum*). The tests were significant only in Julie (H=7.00, p =0.0362) were *Palpada vinetorum* showed to be a more effective pollinator relative to the others three insect species. For all other Mangifera *indica* cultivars Kruskal Wallis tested we're not significant (Keitt=H> 6.49, p = 0.09; Kent=H> 6.51, p = 0.08; Tommy= 2.98, p=0.38).

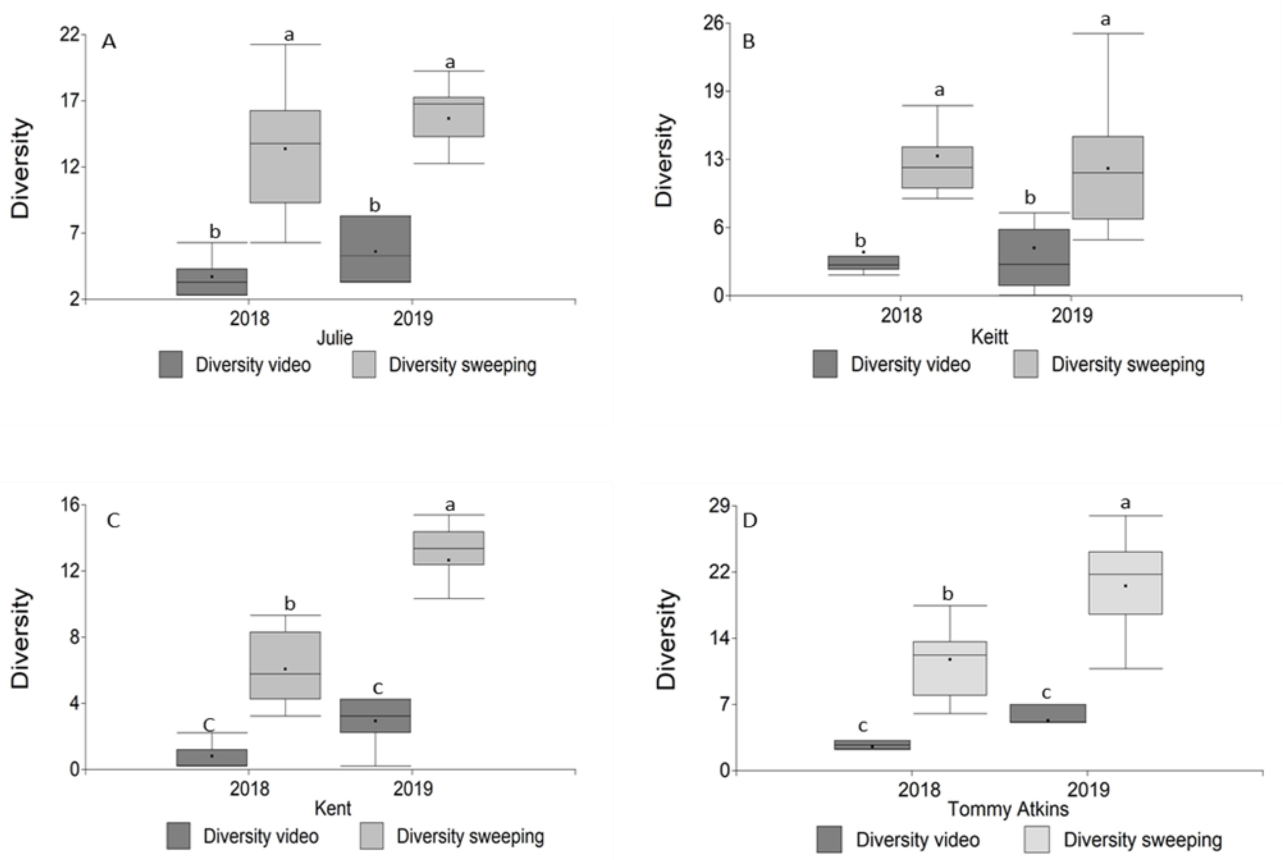

Figure 5.3. Compare two different methods (video and sweeping) for observed diversity of insect visitors in each cultivar of Mangifera indica (A) Julie, (B) Keitt, (C) Kent, (D) Tommy Atkins during two years 2018 and 2019. Different letters in the two methods within each cultivar indicate significant differences (at $\boldsymbol{\alpha} = 0.05$) among years following two-way ANOVA (Table 5).

CHAPTER VII

GENERAL CONCLUSIONS AND RECOMENDATIONS

In this dissertation, I explored different aspects of how biotic and abiotic factors may influence the successful pollination of *Mangifera indica* flowers and subsequent fruit production in four different cultivars (Julie, Kent, Keitt, Tommy Atkins). In agreement with other studies, we found that inflorescence and flower production is highly variable but fruit yields are consistent across years even following a hurricane event. Results suggest that fruit production needs animal pollination and that, in this system, pollination is carried by a variety of insect species (50) and that overall insect diversity and abundance is important for fruit production. This study showed that dominant pollinators are equally capable at pollinating stigmas in all cultivars but that depositing pollen. Results also showed that the abundance of dominant pollinators and the composition of pollinator assemblages that visit these cultivars vary in time and space. Interannual variation in pollinator assemblages did not result in changes in fruit yields but instead were consistent even after the passage of Hurricanes Irma and Maria which let to plant defoliation. Spatial variation in pollinator assemblages does not align with differences in floral or inflorescences characters measured. A hypothesis is that unmeasured factors (floral scents or other plant species) may be causing the observed spatial variation in pollinator assemblages. Open flower pollinations have better reproductive success that artificial self and cross pollinations within a cultivar. In agreement with other studies (Geetha et al., 2016; Gehrke et al., 2011; Issarakraisila and Considine,1994) pollen germinability is related to temperature in a non-linear fashion. Cultivars show their own optimal temperature ranges for germination and pollen germination decreases below and above that optimum range. However, it was shown that for all cultivars but the negative effects of high temperatures are manifested in pollen from male versus pollen from hermaphroditic flowers. Specifically, pollen from hermaphroditic flowers has much lower germination rates at high temperatures than pollen from male flowers. A hypothesis is that differences in allocation between male and female flower organs in hermaphroditic flowers leads to those patterns. This work evaluated insect visitation rates and insect diversity per tree using video cameras and net sweeping. While video cameras allow to calculate visitation rates on a per flower basis, species diversity was underestimated when compared with net sweeping. One possibility for the lower values using video camera was the low number of cameras per tree (one) used in this study.

The above information can help guide the development of management strategies for different goals (ensuring fruit production, development of breeding programs) in the face of expected pollinator and climate variation. Here I list a number of recommendations that may be used to support the management of this and another mango germplasm:

- AT JDAES, there are 84 mango cultivars but only 4 have been evaluated in terms of their floral and pollination biology. The floral biology of the other cultivars present at JDAES should continue to have a baseline record the differences of each cultivar present. This will allow to have morphological, breeding system and pollen germinability information of all cultivars in the germplasm.

-Evaluating the pollen viability under different temperature environments for other cultivar is important in order to identify strains that may be more tolerant to temperature increases. These studies should evaluate the actual links between germinability and fruit production. In the literature it is mentioned that crops that grow wild in warm areas should be selected to determine how tolerant to heat stress they are. Once selected, breeding programs should begin, seeking to maintain heat-tolerant genotypes (Sita et al. 2017; Salem et al. 2007). Implementing a heat tolerance improvement program will help us create a management plan for our germplasm material to avoid the possible extinction of some cultivars.

- The use of DNA markers studies can help clarify the origin of pollen donors from open pollinations. This is being used in other countries such as Australia (Kuhn et al. 2017) Israel (Degani et al.1990,1992) and India (Salvi et al. 2019) that are working with DNA markers seeking to ensure which parents are to obtain new crosses.

-It is important to study factors that may influence the pollinator assemblages in space and time. One pollinator appears to visit the cultivar Julie over others. Studies that evaluate the chemical composition of floral fragrances of the different cultivars can help test this hypothesis and could help identify chemical attractants for pollinators. For example, in the Cucurbita sp crop, they found that the volatile of cinnamaldehyde in flowers attracted only the pollinator Peponapis pruinose and not other herbivores (Andrews and Adler, 2007). Another plant is Ceropegia dolichopylla that has an essence in the flowers that is described as meaty and earthy and this attracts only a milichiid flies that are its pollinators (Heiduk et al., 2010).

-Maintaining the diversity of pollinators should be a more important management goal than managing *Apis mellifera*. In our study, Apis mellifera pollination is very variable in space and time and Dipterans appear to be more consistent. Studies to understand the life cycles of these Diptera flower visitors and their habitat requirements is necessary to be able to increase their populations when needed.

-It would be necessary to evaluate the floral resources of the surrounding vegetation and their role in attracting wild pollinators to change areas. Studies carried out in Africa (Carvalheiro et al. 2010, 2012) recommend having patches of vegetation that attract mango visitors who are in that geographic location. The extent by which the surrounding vegetation has influenced the observed pollinator assemblages here is unknown and such information is needed in order to manage for pollinators using landscape-level approaches. It is necessary to evaluate the insecticide applications that are carried out in the mango germplasm to avoid impacts on the diversity of wild pollinators. Other works in mango with visitors have indicated how pesticides can reduce the populations of flower visit (Carvalheiro et al.,2012; Quenaudon,2019),

-For cultivars with pollen germination that is susceptible to temperature increases geographical areas on the island should be evaluated where the cultivars could be relocated to maintain this historical germplasm that may be at risk. One possibility are areas on the north of the island.

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APPENDICES

Appendix A. Supplementary material for CHAPTER IV and CHAPTER V.

Table A.1. Species visitors in four cultivars Julie, Keitt, Kent, Tommy Atkins, in three years in Puerto Rico with entomological: Family, Order, new record, new report, new specie, others country, literature.

Species visitors	Family	Orden	New record of flowers visitors in cultivars mango P.R [1] [2] [3]		Flower visitors in mango in others Country	Literature
Chrysomia megacephala	Calliphoridae	Diptera	X	J, Kei, Ken, TA[3]	India; Taiwan	Sung et al.2006
Chrysomia rufifacies	Calliphoridae	Diptera	X	J, Kei, Ken, TA	Costa Rica	Jiron & Hedström 1985
Cochliomyia minima	Calliphoridae	Diptera	X	J, Kei, Ken, TA		
Lucilla eximia	Calliphoridae	Diptera	X	Kei, Kent, TA		
Chloropidae sp	Chloropidae	Diptera	X	Kei		
Villa lateralis	Bombillidae	Diptera	X	J, Kei, Ken, TA		
Musca domesticae	Muscidae	Diptera		J, Kei, TA	Australia; Brazil; India, Israel; India; Taiwan;	Anderson et al. 1982; Sung et al. 2006
Lepidodexia pacta	Sarcophagidae	Diptera	X	J, Kei, TA		
Peckia sp.	Sarcophagidae	Diptera	X	J, TA		
Hermetia illucens	Stratiomyiidae	Diptera	X	Kent	Costa Rica	Jiron & Hedström 1985
Architas marmoratus	Tachinidae	Diptera	X	Kent		
Gonia crassicornis	Tachinidae	Diptera	X	J, Kei, TA		
Linnaemya fulvicauda	Tachinidae	Diptera	X	Kei		
Ptilodexia rufianilis	Tachinidae	Diptera	X	Ken		
Euxesta stigmatias	Ulidiidae	Diptera	X	J, Kei, Ken, TA		
Physiphora clausa	Ulidiidae	Diptera	X [1]	J, Kei, Ken, TA		
Allograta radiata	Syrphidae	Diptera	X	J, Kei, Ken, TA		
Copestyllum pallens	Syrphidae	Diptera	X	Kei, Ken, TA		
Copestyllum vacuum	Syrphidae	Diptera	X	J, Kei		
Lepidomya calopus	Syrphidae	Diptera	X	J, Kei, Ken, TA		
Monoceromyia wolcotti	Syrphidae	Diptera	X [1]	Kei, Ken, TA		
Ornidia obesa	Syrphidae	Diptera	X	Kei, Ken, TA	Costa Rica	Jiron & Hedström 1985
Palpada albifrons	Syrphidae	Diptera	X	J, Kei, Ken, TA		
Palpada furgata	Syrphidae	Diptera	X	Kei		

Palpada vinetorum	Syrphidae	Diptera	X	J, Kei, Ken, TA	Brazil	Siqueira et al. 2008;
Psedodorus clavatus	Syrphidae	Diptera	X	J, Kei, Ken, TA		
Apis mellifera	Apidae	Hymenoptera		J, Kei, Ken, TA	Africa; Brazil; India; Israel; South Africa; Taiwan; Philippines; Miami EEUU	Bhatia et al. 1995; Dag & Gazit 2000; Carvalheiro et al.2010; Fajardo et al.2008; Kumar et al. 2012; Popenoe 1917; Siqueira et al. 2008; Sung et al.2006; Reddy et al. 2017
Exomalopsis similis	Apidae	Hymenoptera	X	J, TA		
Hopliosoides niger	Crabronidae	Hymenoptera	X	Kei, TA		
Liris fulginosus	Crabronidae	Hymenoptera	X	J, Kei, Ken, TA		
Liris labiatus	Crabronidae	Hymenoptera	X	TA		
Ophionellus sp.	Anomalonidae	Hymenoptera	X[1][2]	J		
Monomorium floricola	Formicidae	Hymenoptera	X	Ken		
Pepsis marginata	Pompilidae	Hymenoptera	X	J, Kei, Ken, TA		
Pepsis rubra	Pompilidae	Hymenoptera	X	Kei		
Pepsis ruficornis	Pompilidae	Hymenoptera	X	J, Ken		
Pachodynerus guadulpenis	Vespidae	Hymenoptera	X	TA		
Chilocorus nigritus	Coccinellidae	Coleoptera	X	J, Ken, TA		
Coleophora inegualis	Coccinellidae	Coleoptera	X	Kei, Ken		
Cycloneda sanquinea	Coccinellidae	Coleoptera	X	J, Kei, Ken		
Hippodamia convergens	Coccinellidae	Coleoptera	X	J		
Olla nigrum	Coccinellidae	Coleoptera	X	Ken, TA		
Cryitocephalus irroratus	Chrysomelidae	Coleoptera	X	Kei		
Thonalmus chevrolati	Lycidae	Coleoptera	X	Ken		
Pannoquina lucas woodruffi	Hesperiidae	Lepidoptera	X	J, Kei		
Electroatrymon angelica	Lycaenidae	Lepidoptera	X	J, Kei, Ken, TA		
Junonia genoveva neildi	Nymphalidae	Lepidoptera	X	J, Ken		
Ascia monueste eubotea	Pieridae	Lepidoptera	X	Ken		
Phidotricha erigens	Pyralidae	Lepidoptera	X	J, Kei, Ken		
lep. sp. unknow	Geometridae	Lepidoptera	X	J		

[1] New report species for Puerto Rico

[2] New species

[3] Cultivars: J=Julie, Kei=Keitt, Ken=Kent, Tommy Atkins=TA