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Servicios de polinización en el Bosque Tropical Seco

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1 Resumen

Los servicios ecosistémicos, como la polinización son de gran importancia para la seguridad alimentaria y el mantenimiento de los ecosistemas. A pesar de esta gran importancia, las poblaciones de algunos grupos clave como especies de abejas se encuentran en declive en ciertas regiones del mundo. La causa es multifactorial y entre estas causas destaca la pérdida del hábitat. El proceso de creación de nuevos terrenos dedicados para la agricultura seguirá en expansión, por lo que se estarán perdiendo áreas con hábitats naturales y seminaturales, principalmente en los trópicos. La conservación de los bosques tropicales será clave para la persistencia de las poblaciones de polinizadores que brindan el servicio de polinización a cultivos encontrados en los alrededores de hábitats naturales y seminaturales. El conocimiento de la interacción de los polinizadores con los cultivos nos permitirá crear estrategias de uso y conservación de los polinizadores a largo plazo. El objetivo de este estudio fue evaluar la importancia del Bosque Tropical Seco (BTS) como proveedor del servicio de polinización a cultivos dependientes de polinizadores. El estudio se realizó en el estado de Jalisco, en los cultivos aledaños a la reserva de la Biosfera Chamela-Cuixmala. La tesis se encuentra dividida en 3 capítulos, El primer capítulo trató sobre los servicios de polinización en dos cultivos económicamente importantes de la región costa de Jalisco (Cultivos de sandía y tomate verde), así como la influencia del paisaje en los polinizadores de los dos cultivos evaluados. En el segundo capítulo documenté la interacción estacional del servicio de polinización en cultivos de calabaza (*Cucurbita moschata*) y la dinámica del servicio durante la temporada lluviosa y seca del BTS. En el tercer capítulo detallo los sitios de anidamiento de la especie de abeja *Peponapis crassidentata*, uno de los principales polinizadores de cultivos de calabaza durante la temporada lluviosa del BTS. Finalmente, como conclusión, la conservación de los bosques tropicales secos debe ser considerado dentro de las actividades agropecuarias, así como también la biología de las especies que actúan como polinizadores de cultivos, para así asegurar las poblaciones de polinizadores que contribuyen a la productividad de los cultivos. El correcto manejo y conservación de los bosques contribuirán en la preservación de los servicios de polinización a largo plazo, servicio no solo útil para los cultivos, sino que también útil para el mismo mantenimiento de los bosques.

2 Abstract

Ecosystem services, such as pollination, are of great importance for food security and ecosystem maintenance. Despite this great importance, the populations of some key groups such as bee species are in decline in certain regions of the world. The cause is multifactorial and among these causes, the loss of habitat stands out. The process of creating new lands dedicated to agriculture will continue to expand, so areas with natural and semi-natural habitats, mainly in the tropics, will be lost. The conservation of tropical forests will be key to the persistence of pollinator populations that provide pollination service to crops found in the surrounding natural and semi-natural habitats. The knowledge of the interaction of pollinators with crops will allow us to create long-term use and conservation strategies for pollinators. The objective of this study was to evaluate the importance of the Tropical Dry Forest (TDF) as a pollination service provider to pollinator-dependent crops. The study was carried out in the state of Jalisco, Mexico, in the crops adjacent to the Chamela-Cuixmala Biosphere Reserve. The thesis is divided into 3 chapters. The first chapter dealt with pollination services in two economically important crops in the Jalisco coast region (watermelon and green tomato crops), as well as the influence of the landscape on the pollinators of the two evaluated crops. In the second chapter, I documented the seasonal interaction of the pollination service in pumpkin crops (*Cucurbita moschata*) and the dynamics of the service during the rainy and dry season of the BTS. In the third chapter, I detail the nesting sites of the bee species *Peponapis crassidentata*, one of the main pollinators of pumpkin crops during the TDF rainy season. Finally, in conclusion, the conservation of dry tropical forests must be considered within agricultural activities, as well as the biology of the species that act as crop pollinators, to ensure the populations of pollinators that contribute to the productivity of crops. The correct management and conservation of forests will contribute to the preservation of long-term pollination services, a service not only useful for crops but also useful for the same maintenance of forests.

3 Introducción y antecedentes

Los servicios ecosistémicos consisten en beneficios que los seres humanos obtienen de forma directa o indirecta de los ecosistemas. Entre estos servicios se incluyen los servicios de aprovisionamiento, por ejemplo: comida, agua, recursos maderables y fibra; servicios culturales, el cual brinda beneficios recreacionales, estéticos y espirituales; servicios de soporte como la formación de suelos, fotosíntesis y el ciclo de nutrientes y servicios de regulación, los cuales afectan el clima, inundaciones, enfermedades, desechos, la calidad del agua y la polinización de cultivos. (Costanza et al. 1997; Millennium Ecosystem Assessment 2005). Los seres humanos dependen completamente de los ecosistemas y los servicios que ellos proveen, como lo son; recursos alimenticios, agua, control de plagas, regulación del clima, plenitud espiritual y disfrute estético. Sin embargo, durante los últimos 50 años, la humanidad ha cambiado los ecosistemas de forma rápida y extensiva, debido a la necesidad creciente de obtener comida, agua, madera, fibras y combustibles. Este rápido crecimiento ha causado serios problemas a los ecosistemas y al mantenimiento de los servicios ecosistémicos (Wood, S. et al. 2000; Millennium Ecosystem Assessment 2005).

Los agroecosistemas son sistemas biológicos gestionados por humanos con el principal objetivo de producir alimento, así como otros bienes no alimenticios de valor social y monetario (Wood, S. et al. 2000), este tipo de ecosistemas ha llegado a ocupar el 38.4% de la superficie total terrestre sin hielo (FAOSTAT 2016). Recientemente se ha propuesto que los agroecosistemas pueden proporcionar servicios ecosistémicos, como el control de plagas, mantenimiento de la biodiversidad y polinización. Para poder obtener dichos bienes, los agroecosistemas dependen de los servicios ecosistémicos provenientes de ecosistemas naturales con poco manejo (Power 2010), como por ejemplo: la disponibilidad de agua, fertilidad del suelo y polinización (Wood, S. et al. 2000, Millennium Ecosystem Assessment 2005).

3.1 Sistemas agrícolas en expansión

A nivel mundial, los seres humanos han transformado diversos ecosistemas en sistemas agrícolas, con el objetivo de obtener diversos bienes (Ramankutty and Foley 1999). Esta tendencia continua en la actualidad y se ha estimado que los agroecosistemas ocupan el 38.4% de la superficie terrestre sin hielo (FAOSTAT 2016). Lamentablemente la conversión de los ambientes naturales a sistemas agrícolas ha traído como consecuencia la pérdida de biodiversidad y ha afectado los servicios ecosistémicos a nivel mundial (Millennium Ecosystem Assessment 2005). La agricultura es responsable de la extracción de aproximadamente el 70% de agua dulce y un tercio de las emisiones de gases de efecto invernadero (Foley et al. 2011), además amenaza con la extinción a un gran número de especies a comparación de otras actividades humanas (Green et al. 2005). Las actividades agropecuarias son la principal fuerza responsable de varias amenazas al ambiente, entre las que se incluyen el cambio climático, pérdida de biodiversidad, degradación del hábitat y cuerpos de agua (Millennium Ecosystem Assessment 2005; Power 2010; Foley et al. 2011). La agricultura ha removido o convertido el 70% de las praderas, 50% de las sabanas, 45% de los bosques templados caducifolios y el 27% de los bosques tropicales (Ramankutty and Foley 1999; Ramankutty et al. 2008). La agricultura se encuentra expandiéndose a los trópicos y se estima que más del 80% de las nuevas tierras de uso agrícola proviene de bosques intactos y con cierto grado de disturbio (Gibbs et al. 2010).

Debido a la expansión de la agricultura en los trópicos, la biodiversidad se encuentra disminuyendo, agotando los servicios ecosistémicos críticos e incrementando las emisiones de gases de efecto invernadero (Foley et al. 2011). Se espera que estos problemas aumenten con los años, debido a que la expansión de las áreas agrícolas se incrementará en el futuro. Para el año 2050 se pronostica que el mundo necesite aumentar la producción de alimentos de 60 a 70% para poder alimentar a más de 9 mil millones de personas (FAOSTAT 2016). La intensificación y expansión de los sistemas agrícolas pondrán en riesgo la calidad y cantidad de los servicios ecosistémicos de los ambientes naturales, como el servicio de polinización, fundamental para la productividad de ciertas especies vegetales usadas en cultivos.

3.2 Servicio de polinización en los agroecosistemas

Se calcula que, a nivel mundial, el 87.5% de las especies de plantas con flores dependen de la polinización por animales para la reproducción sexual (Ollerton et al. 2011). Este importante servicio de regulación incrementa la productividad en los sistemas agrícolas y contribuye a mejorar aspectos nutricionales de los frutos (Klein et al. 2007b; Garibaldi et al. 2011; Brittain et al. 2014). La importancia de la polinización por animales en la agricultura es alta, aproximadamente el 75% de 115 especies de cultivos líderes en el mundo dependen de la polinización por vectores biológicos, y en el caso de México, la dependencia se eleva hasta el 85% de las especies vegetales usadas para alimentación humana (Klein et al. 2007b; Ashworth et al. 2009).

Entre los polinizadores animales se encuentran una gran variedad de grupos taxonómicos, como aves, murciélagos, polillas, mariposas, avispas, abejas y moscas juegan un papel importante en la producción de cultivos a nivel mundial, sin embargo, el principal grupo que lleva a cabo los servicios de polinización en los agroecosistemas son las abejas, donde se ha registrado como visitantes del 73% de las especies de cultivos que requieren de polinizadores (Buchmann and Nabhan 1997; Garibaldi et al. 2011, 2013; Winfree et al. 2011; Abrol 2012; Rader et al. 2015). Existen alrededor de 17000 especies de abejas en el mundo, entre estas especies, *Apis mellifera* ha sido ampliamente usada en una gran variedad de cultivos a nivel mundial, y se le atribuye la polinización de aproximadamente 90 cultivos en el mercado, además, la apicultura es una industria comercial grande donde se rentan las colonias de estas abejas para brindar los servicios de polinización a cultivos (Michener 2007; National Research Council 2007; Winfree et al. 2011; Abrol 2012).

Debido al amplio uso de *Apis mellifera* en la agricultura a nivel mundial, las estimaciones en el valor de la polinización se han calculado en base a la presencia de esta especie. En Estados Unidos han comprendido desde los \$150 millones hasta los \$18.9 mil millones de dólares al año (Abrol 2012). En este mismo país, se ha estimado que del año 1989 al 2000 ha habido un incremento del 36.3% en el valor de la polinización, atribuido a la inflación y demanda por alimentos provenientes de polinización animal como consecuencia del incremento en la población humana. (Morse and Calderone 2000). El

valor del servicio de polinización producto de polinizadores nativos puede ser mayor al proporcionado por *A. mellifera*, comprendiendo un valor de 3.07 mil millones de dólares, tomando en cuenta la producción de frutas y verduras en Estados Unidos (Losey and Vaughan 2006). A una escala global, el valor total anual de la polinización en la agricultura ha sido estimado en \$ 200 mil millones de dólares (Kearns et al. 1998) y se ha estimado una pérdida entre el 3 al 8 % en la producción mundial de cultivos en caso que los polinizadores disminuyeran o desaparecieran (Aizen et al. 2009).

3.3 *Apis mellifera* y la crisis en los servicios de polinización

Indiscutiblemente, la abeja *Apis mellifera* es uno de los principales polinizadores utilizados para la suplementación de la polinización en cultivos a nivel mundial y en México. Sin embargo, el depender de una sola especie de polinizador puede ser un movimiento riesgoso debido a diversos factores como;

La evidencia reciente indica que en Estados Unidos y Europa se ha registrado un decline en las poblaciones de *A. mellifera*, pudiendo tener consecuencias negativas en la seguridad alimentaria. Además de *A. mellifera*, otras especies de polinizadores pueden estar comprometidas de seguir las tendencias actuales. Se cree que el decline de abejas ha ocurrido por una causa multifactorial, en las que se distinguen tres categorías principales; parásitos y patógenos, estresantes ambientales y la degradación del hábitat (Potts et al. 2010b, a; Bianco et al. 2014; Goulson et al. 2015).

Parásitos y patógenos: Este grupo es considerado uno de los principales factores en la alta pérdida de abejas en varios países del hemisferio norte. Entre estos parásitos, destaca el acaro *Varroa destructor*, quien ha recibido mucha atención en el síndrome del colapso de las colmenas, además, *V. destructor* también es un vector para virus especializados en abejas. En particular se han registrado más de 24 especies de virus relacionados con *A. mellifera*. De entre estos, el virus de las alas deformadas ha sido de gran relevancia debido a su asociación con el acaro *V. destructor*. Se ha observado también que varios de estos virus pueden saltar de *A. mellifera* hacia poblaciones de abejas nativas, pudiendo

comprometer a los servicios de polinización, por lo que es necesario que se consideren estos virus para un apropiado manejo de las poblaciones de abejas (Tehel et al. 2016; McMenamin and Flenniken 2018).

Estresantes ambientales: El cambio climático puede provocar cambios temporales y espaciales en plantas y polinizadores, interrumpiendo la interacción entre planta y polinizador debido a cambios en la distribución de los interactuantes y en la fenología de las plantas, ocasionando pérdidas en varias especies de plantas, polinizadores y las interacciones entre estos (Memmott et al. 2007; Hegland et al. 2009). Las predicciones a futuro en general indican cambios y reducciones en la distribuciones de los polinizadores y las plantas, lo que puede comprometer la productividad de cultivos frutales y de hortalizas, poniendo en peligro a la seguridad alimentaria (Polce et al. 2014; Kerr et al. 2015; Elias et al. 2017).

Degradación del hábitat: Las actividades humanas ha traído consecuencias negativas a las poblaciones de ciertas especies de polinizadores, por un lado, tenemos a la amplia variedad de agroquímicos empleados para el control de plagas en cultivos y por otro lado encontramos un uso intenso de monocultivos y la eliminación de plantas ruderales y bosques para la facilitación de infraestructura humana, como áreas de cultivo controladas y zonas ganaderas. La amplia variedad de pesticidas usados en la industria agrícola implica la creación de cocteles tóxicos a los cuales las abejas *Apis mellifera* poder estar expuestas desde su etapa larvaria hasta su etapa adulta. Los neonicotinoides son un grupo de insecticidas que consisten en neurotoxinas capaces de afectar el sistema nervioso central de los insectos, son solubles en agua y altamente persistentes y pueden estar presentes en polen y néctar de las plantas silvestres alrededor de los cultivos (Tomizawa and Casida 2004; Krupke et al. 2012). La exposición crónica a largo termino ha resultado en una mortalidad de las abejas durante el invierno (Rondeau et al. 2014). También se ha observado efectos subletales de este pesticida en *A. mellifera* y abejorros del género *Bombus*, estos efectos subletales afectan el aprendizaje, forrajeo y la habilidad de regresar a la colmena, los cuales son esenciales para la supervivencia de las abejas (Yang et al. 2008; Mommaerts et al. 2009; Han et al. 2010; Henry et al. 2012; Feltham et al. 2014). Los

pesticidas, si bien han sido estudiados en abejas sociales, las abejas solitarias también pueden estar presentando un problema similar, debido a que se ha detectado la presencia de pesticidas en sus cuerpos, como el caso de la abeja especialista *Peponapis pruinosa* (Chan et al. 2019). Otro de los aspectos en la degradación del hábitat, son los monocultivos y la forma en que se realiza la agricultura moderna. Actualmente, las áreas tecnificadas de agricultura y en específico, los monocultivos solo ofrecen a los polinizadores una o pocas especies florales, siendo el néctar y polen poco variado en nutrientes necesarios para el desarrollo de las abejas (Goulson et al. 2015).

Las especies de planta nativas comparten historias evolutivas con sus polinizadores nativos a través de relaciones mutualistas, por lo que es razonable asumir que los polinizadores nativos deben de ser más eficientes para brindar el servicio de polinización que aquellos polinizadores comerciales introducidos, además, en algunos casos, estos polinizadores nativos resultan más efectivos al momento de llevar el servicio de polinización de ciertos cultivos (Winfrey et al. 2007; Ashworth et al. 2009; Garibaldi et al. 2013). Un ejemplo de este caso es el uso de abejas del género *Bombus* para la polinización de tomate (*Lycopersicum esculentum*), el polen de las flores de tomate y otras plantas pertenecientes a la familia de las Solanáceas se libera a través de la vibración de las anteras poricidas, vibración que no se ha observado en *A. mellifera* y algunas abejas meliponas. (Free 1993) y que las abejas del género *Bombus* y otras abejas solitarias son capaces de realizar, permitiendo la polinización por vibración sonora. Otro estudio detalla el servicio de polinización con abejas nativas y arándanos (*Vaccinium corymbosum*) y abejas nativas y calabazas (*Cucurbita* spp), en donde se detalla que las abejas nativas son mejores polinizadores a comparación de *A. mellifera* en áreas donde las abejas nativas son abundantes (Tepedino 1981; Canto-Aguilar and Parra-Tabla 2000; Isaacs and Kirk 2010; Broussard et al. 2011; Gibbs et al. 2016).

Finalmente, ante las amenazas que enfrenta *A. mellifera* y su poca eficacia para ciertos cultivos, diversos países han explorado alternativas con otras especies de polinizadores para emplearlos de forma comercial. En México actualmente se encuentran disponibles de forma comercial *Bombus impatiens* (Torres-Ruiz et al. 2013). Se ha llegado

a experimentar con un polinizador nativo en México, *Bombus ephippiatus*, siendo igual de eficaz que *B. impatiens* en cultivos de jitomate, sin embargo, para experimentación de polinizadores comerciales, los nidos se han obtenido de forma silvestre (Torres-Ruiz and Jones 2012). Por lo que los bosques representan reservorios de polinizadores silvestres que pueden llegar a ser polinizadores eficaces de varios cultivos.

3.4 Los bosques como proveedores del servicio de polinización hacia los agroecosistemas

Con el fin de producir bienes utilizados de forma directa o indirecta por humanos, los agroecosistemas dependen de servicios ecosistémicos como la polinización, los cuales provienen de forma gratuita de entornos naturales con poco manejo (Power 2010). La reducción de las áreas naturales y la simplificación del paisaje ha traído cambios negativos en la abundancia y riqueza de polinizadores en los agroecosistemas, principalmente debido a una drástica disminución en la disponibilidad recursos para los polinizadores, así como un menor número de sitios de anidamiento y de reproducción, poniendo en riesgo a los servicios de polinización y la productividad de los cultivos dependientes de polinizadores (Ricketts et al. 2008; Petersen and Nault 2014; Vanbergen 2014).

Los ambientes agrícolas representan hábitats de baja calidad para los polinizadores, debido a que estos sistemas no proveen de los recursos suficientes para la sobrevivencia, como sitios para anidar, forrajear y otras condiciones físicas para la persistencia de los polinizadores (Heard 1999), además de que la distancia de los hábitats naturales a los cultivos puede afectar de forma negativa la abundancia y riqueza de polinizadores (Ricketts et al. 2008). Los bosques pueden ser considerados reservorios de visitantes florales que pueden estar contribuyendo de forma significativa a los servicios de polinización requeridos para ciertos cultivos (Blanche et al. 2006; Flores et al. 2012) por lo que el mantenimiento de los ambientes naturales pueden incrementar la tasa de visita de los polinizadores a los cultivos (Carvalho et al. 2010) y la productividad de los cultivos se puede ver beneficiada al estar cercanos a los hábitats ideales de los polinizadores (Roubik 2002).

Los estudios referentes al impacto de la cercanía de los bosques en el servicio de polinización de cultivos son escasos. Se ha llegado a demostrar que la productividad en los cultivos cercanos a los ambientes naturales puede llegar a ser altos a comparación de cultivos aislados o lejanos del bosque. En Brasil, las huertas cercanas o adyacentes a los bosques llegan a producir más granos de café (*Coffea arabica*), llegando a incrementar un promedio de 14.6% en la productividad debido a la polinización por insectos (Marco and Coelho 2004) y los cultivos de la nuez de la india (*Anacardium occidentale*) próximos a un fragmento más conservado de bosque, presenta mayor diversidad y abundancia de visitantes florales que el área próxima al fragmento más perturbado, esto se ve reflejado en el éxito reproductivo (Flores et al. 2012). En huertas de macadamia (*Macadamia integrifolia*) y ojo de dragón (*Dimocarpus longan*) cercanos a un bosque tropical lluvioso en Australia se demostró que la proximidad al bosque tropical lluvioso puede mejorar las tasas de polinización (Blanche et al. 2006).

Actualmente la agricultura se está expandiendo a los trópicos y se estima que más del 80% de las nuevas tierras de uso agrícola proviene de bosques intactos y con cierto grado de disturbio (Gibbs et al. 2010) y los polinizadores tropicales, particularmente en el grupo de las abejas, se ha encontrado evidencia que son muy susceptibles a los cambios en el paisaje (Ricketts et al. 2008). Sin embargo, para muchos sistemas tropicales se desconoce la relevancia de los ambientes naturales en el servicio de polinización.

3.5 El Bosque Tropical Seco como proveedor de servicios de polinización

Uno de los sistemas tropicales más extensos del mundo lo comprende el Bosque Tropical Seco (BTS) abarcando más del 40% de los bosques tropicales en el mundo, y de la vegetación en Mesoamérica (Murphi and Lugo 1986). Únicamente una tercera parte de estos bosques son continuos, siendo el resto vegetación abierta, fragmentos de bosques y una parte considerable bosque secundario (Millennium-Ecosystem-Assessment 2005). El BTS presenta una amplia distribución a nivel mundial (Trejo and Dirzo 2000), se estima que cubre 1, 048,700 km² de la superficie terrestre (Miles et al. 2006). En el continente Americano el BTS se distribuye del noroeste de México al norte de Argentina y suroeste de

Brasil, en áreas separadas de diferentes tamaños (Linares-Palomino et al. 2011). En México estos cubren alrededor del 60% del área ocupada por los bosques tropicales (Trejo and Dirzo 2000), y se ubican principalmente en la Vertiente del Pacífico Mexicano (Miles et al. 2006).

El conjunto de tipos de vegetación que conforman al BTS son propios de climas cálidos, dominados por especies arbóreas y arbustivas que pierden sus hojas en la época seca del año durante un lapso variable de tiempo, por lo general oscila alrededor de seis meses. Una de las principales características de este tipo de sistemas son las fluctuaciones en la disponibilidad de recursos, manejado por la presencia de agua, la cual está determinada por la época seca y húmeda (Murphy and Lugo 1986; Trejo and Dirzo 2000, Rzedowski 2006) .

(Janzen 1988), califica a los BTS como uno de los bosques más amenazados en comparación con otros tipos de bosques tropicales. Debido a las características de este ecosistema, estos bosques están entre los ecosistemas más utilizados, perturbados y menos conservados en Mesoamérica (Ewel 1999, Quesada & Stoner 2004), además de estar amenazados debido al cambio de uso del suelo (Sanchez-Azofeifa *et al.* 2003).

En la costa de Jalisco, México se ubica la Reserva de la Biosfera Chamela, Cuixmala, decretada en 1993 con el fin de proteger principalmente el BTS, así como humedales de la costa central del país (Ceballos et al. 1999). La zona costa de Jalisco, lugar donde se ubica la reserva, se compone principalmente de BTS, comprendiendo un 56.1% de cobertura total, mientras que las zonas de agricultura y pastoreo abarcan un 25.8% de la cobertura (Sánchez-Azofeifa et al. 2009). En la reserva se han documentado una gran cantidad de especies de plantas vasculares, registrándose 1149 especies pertenecientes a 125 familias (Lott 2002). Para la reserva de la biosfera Chamela-Cuixmala se ha registrado un total de 238 especies de abejas en 81 géneros y cinco familias, siendo Apidae la más diversa en especies con 102, Megachilidae y Halictidae con 52 y 46 especies respectivamente (Ayala 2004). Entre estas abejas se destaca *Apis mellifera*, debido a su extenso uso en zonas apícolas, cultivos y el establecimiento de poblaciones dentro del BTS.

Poco se conoce acerca de los servicios ecosistémicos provistos por el BTS, a pesar de su gran cobertura dentro de los sistemas tropicales (Murphy & Lugo 1986) y de que es la

base para la forma de vida de millones de personas a nivel mundial (Maass et al. 2005). En particular, dentro de los servicios ecosistémicos de regulación, la polinización en conjunto con la regulación de plagas ha recibido poca atención en los estudios realizados en Latinoamérica (Balvanera et al. 2012).

En México cerca de la mitad de la población vive por debajo de la línea de pobreza (Torres 2001). La forma de vida de mucha de esta gente depende de los servicios de polinización para obtener alimento (Ashworth et al. 2009), en adición a esto, México es uno de un centro de origen de plantas domesticadas, donde poblaciones de cultivos silvestres y domesticados coexisten (Bye and Linares 2000). Se han registrado alrededor de 316 especies cultivables, de las cuales alrededor del 80% depende de alguna medida de los polinizadores por animales para una producción eficiente donde los polinizadores silvestres nativos pueden estar jugando un papel importante (Ashworth et al. 2009).

La polinización es un servicio de gran importancia para los agricultores, quienes siembran principalmente hortalizas (Maass et al. 2005). En los cultivos mexicanos este servicio ha sido brindado principalmente por *Apis mellifera*, en donde se ha empezado a establecer la renta de colmenas de *A. mellifera* para cubrir los requerimientos de polinización de los cultivos, convirtiéndose en el objetivo principal en estados como Sinaloa, Chihuahua y Coahuila, quedando como actividad secundaria la producción de miel (SAGARPA 2008). En México existe una gran diversidad de especies vegetales usadas en cultivos y que dependen en algún grado de los servicios de polinización (Ashworth et al. 2009), sin embargo se emplea la renta de *A. mellifera* para la mayoría de los cultivos orientados a obtener productos de calidad y destinados a la exportación, como el pepino, berenjena, calabacita, sandía, melón, cártamo, manzana, fresa, aguacate, cítricos, entre otros (SAGARPA 2008).

Algunos de los principales cultivos encontrados en la región de estudio son: sandía, calabaza, pepino, chayote, melón, tomate, jitomate, chile, papaya y mango. La mayoría de estos dependen en algún grado de polinizadores y los principales polinizadores pertenecen al grupo de las abejas (**Cuadro 1.** Principales cultivos en el sitio de estudio, la dependencia a los polinizadores de acuerdo a (Klein et al. 2007a) y los principales polinizadores reportados en la literatura.).

Cuadro 1. Principales cultivos en el sitio de estudio, la dependencia a los polinizadores de acuerdo a (Klein et al. 2007a) y los principales polinizadores reportados en la literatura.

Cultivo	Dependencia*	Sistema reproductivo ⁺	Principal polinizador
<i>Citrullus lanatus</i>	E	>M/AC	Abejass
<i>Cucurbita moschata, C. pepo</i>	E	M/AC	Abejass
<i>Cucumis sativa</i>	G	M-AM/AC	Abejass
<i>Sechium edule</i>	E	M/AC	Abejass
<i>Cucumis melo</i>	E	M- AM/AC	Abejas
<i>Physalis ixocarpa</i>	?	H/AI	?
<i>Solanum lycopersicum</i>	L	H/AC	Abejass
<i>Capsicum annum</i>	L	H/AC	Abejas y Sirfidos
<i>Carica papaya</i>	L	D, M, H/AC	Abejas, trips, polillas grandes, colibríes, mariposas
<i>Manguijera indica</i>	G	AM/AC	Abejas, moscas, hormigas, avispa

*Dependencia de acuerdo con Klein et al. 2007: E= Esencial, G= Grande, L= Pequeña.

⁺Sistema reproductivo: M= Monoico, AM= Andromonoico, H= Hermafrodita, D= Dioico / AC= Autocompatible, AI= Autoincompatible.

Conservar los polinizadores nativos en México es una prioridad para asegurar los servicios de polinización que mantienen la diversidad de alimentos y de plantas con los beneficios asociados para el modo de vida de una gran cantidad de personas (Ashworth et al. 2009). Sin embargo las altas tasas de deforestación y fragmentación de los bosques,

pueden representar un serio problema a la fauna polinizadora y por lo tanto a los servicios de polinización (Quesada and Stoner 2004).

El BTS cuenta con una gran cantidad de recursos florales y de diversas especies de polinizadores que pueden brindar el servicio de polinización a cultivos aledaños a este tipo de sistema, sin embargo, las altas tasas de deforestación de los bosques tropicales y la amenaza de un escenario de pérdida de polinizadores de muchos cultivos hacen que los estudios enfocados a describir los servicios de polinización en áreas agrícolas cercanas a bosques conservados, sean de gran importancia para la seguridad alimentaria y la conservación de los bosques tropicales.

4 Objetivos e hipótesis

4.2 Objetivo general

Evaluar la importancia del Bosque Tropical Seco (BTS) como proveedor del servicio de polinización a cultivos dependientes de polinizadores.

Hipótesis: El BTS aporta de sitios de anidación y provee de recursos florales a los polinizadores que se encuentran brindando el servicio de polinización a cultivos de especies dependientes de polinizadores, por lo que se espera que, los servicios de polinización se incrementen en los agroecosistemas con coberturas extensas de bosque.

5 Descripción de los capítulos

La tesis se encuentra estructurada en tres capítulos en donde se aborda la pregunta central del objetivo general. En el primer capítulo trato sobre los servicios de polinización en dos cultivos económicamente importantes de la región costa de Jalisco, así como la influencia del paisaje en los polinizadores de los dos cultivos evaluados. En el segundo capítulo documenté la interacción estacional del servicio de polinización ocurrido en cultivos de calabaza (*Cucurbita moschata*) y la dinámica estacional durante las dos temporadas principales del bosque tropical seco. El tercer capítulo detallo tres sitios de anidamiento de la abeja *Peponapis crassidentata* asociados con las cañadas de los bosques tropicales. La especie es uno de los principales polinizadores de cultivos de calabaza durante la temporada lluviosa del bosque tropical seco. Estos dos últimos capítulos se encuentran publicados en revistas científicas internacionales indexadas. Finalmente, incluyo una discusión general donde retomo las discusiones de ambos y el objetivo principal de la tesis.

6 Capítulo I: Influencia del paisaje en la abundancia y diversidad de polinizadores y en la producción de frutos de sandía (*Citrullus lanatus*) y tomate verde (*Physalis ixocarpa*) en la región costa de Jalisco

Pollination services on crops of *Citrullus lanatus* (watermelon) and *Physalis ixocarpa* (green tomato) in the coastal region of Jalisco, Mexico.

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Highlights

- *Citrullus lanatus* and *Physalis ixocarpa* are economically important crops in Jalisco.
- *Apis mellifera* is the principal flower visitor of both crops.
- *Citrullus lanatus* and *Physalis ixocarpa* relies in pollinators for crop production
- Landscape play an important role in presence of *A. mellifera* and native pollinators.

Abstract

Bees are one of the main groups of organisms responsible for carrying out the pollination service for crops used for human consumption. However, a decline in bee populations has been reported globally due to various biotic and abiotic factors. One of the main factors is the loss of habitat that has the highest rates of deforestation in tropical forests. We evaluated the pollination services on crops of *Citrullus lanatus* (watermelon) and *Physalis ixocarpa* (green tomato) in the Tropical Dry Forest of Jalisco, Mexico. We described the composition of the community of pollinators and their frequency of visits to the crops, we evaluated the contribution of the main pollinators to the reproductive success of the crops and the dependence of these crops on pollination by animals, and finally, we evaluated the influence of the landscape to the abundance and richness of pollinators of *P. ixocarpa* and *C. lanatus*. We selected *C. lanatus* and *P. ixocarpa* crops, because they are pollinator-dependent crops and are grown every year in the region. During the years 2008 and from 2014 to 2017 we selected several plots of *C. lanatus* and *P. ixocarpa*. In each plot we surveyed a 50 meter transect and we recorded the floral visitors to each flower and the fruit-set under different treatments (open pollination, pollinators excluded and one visit of pollinator). We plotted buffer zones of each plot sampled in a radius of up to 2000 km. In each area we characterized the landscape with satellite images. We performed linear regressions between the abundance of bees and the forest cover and distance to each plot. In addition, we use the INVEST pollinator model and compared it with the abundance obtained in the field. For year 2008. We found a positive relationship between the abundance of bees and the cover and distance of the forest, we also found a positive relationship between the results of the INVEST model with the abundance we observed in

the field. The results indicate that the conservation of natural areas is important for the maintenance of pollinators and the assurance of food safety.

Keywords: Bees, *Apis mellifera*, Tropical Dry Forest, food safety.

1. Introduction

Approximately 70% of the leading crops species of the planet rely on animal pollinators to ensure crop productivity. This percentage is even greater in countries like Mexico, where approximately 85% of crop species depend on pollination by animals (Ashworth et al., 2009). Bees are the principal group of flower visitors in pollinator dependent crops species, representing 62% of the flower visits on crops; the remaining 38% consists mainly of other insect groups like flies, butterflies, moths, ants, wasps and some beetle families (Buchmann and Nabhan, 1997; Klein et al., 2007; Rader et al., 2015).

Among bees, the commercial european bee, *Apis mellifera*, is the most widely used to ensure pollination of 90 species of commercial crops worldwide (Abrol, 2012; National Research Council, 2007; Rader et al., 2015; Winfree et al., 2011). Native bees are also important pollinators of certain crops, representing 23% of the floral visitors worldwide; since native bees and plants share an evolutionary history, native bees can be the main pollinator of some species of crops, such as pumpkins and blueberries (Garibaldi et al., 2013; Hurd et al., 1974; Javorek et al., 2002; Rader et al., 2015; Tepedino, 1981; Winfree et al., 2007; Delgado-Carrillo et al., 2018). Despite the importance of bees in crop pollination and food security, some species of bees are threatened around the world. For example, the phenomenon called colony collapse disorder in *Apis mellifera* represents a major problem in the USA and Europe (De la Rúa et al., 2009; National Research Council, 2007; Oldroyd, 2007; Potts et al., 2010a, 2010b).

Agricultural environments represent low quality habitats for pollinators because these systems do not provide sufficient resources for bee survival, such as nesting, foraging

sites and other physical conditions for the persistence of pollinators. However, unmanaged habitats near agricultural fields, such as natural forests, remnants of natural or seminatural habitats represent important habitats for the persistence of pollinators offering nesting and foraging sites as well as buffer zones against the loss of pollinators (Chacoff and Aizen, 2006; Heard, 1999; Holzschuh et al., 2012). It has been shown that the proximity, shape, and size of natural habitats to crops can affect the abundance and richness of pollinators (Joshi et al., 2016; Ricketts et al., 2008; Zurbuchen et al., 2010). For these reasons, natural habitats can be considered reservoirs of floral visitors who may be contributing significantly to the pollination services required by certain crops (Blanche et al., 2006; Flores et al., 2012). The maintenance of natural environments can increase visitation rates of pollinators to flower crops (Carvalho et al., 2010) and the productivity of crops can benefit from being close to the ideal pollinator habitats (Carvalho et al., 2010; Joshi et al., 2016; Roubik, 2002).

Although the enormous importance of natural forests for the persistence of pollinating bees, agroecosystems are expanding without maintaining natural areas nearby. It is estimated that more than 80% of new agricultural land comes from intact and disturbed forest, and evidence indicate that tropical pollinators, particularly bees, are very susceptible to changes in the landscape (Gibbs et al., 2010; Ricketts et al., 2008). However, the relevance of natural environments for pollination services is unknown for many tropical agroecosystems. One of the most extensive tropical systems in the world is the Tropical Dry Forest (TDF), which covers more than 40% of the world's tropical forests (Murphy and Lugo, 1986). Only a third of the area these tropical forests occur in continuous landscapes, and the rest is found in fragmented forests, in which most of it is secondary forest

(Millennium Ecosystem Assessment, 2005). TDF is one of the most threatened ecosystems in comparison to other types of tropical forests, and it is among the most used, disturbed and least conserved ecosystems in Mesoamerica (Ewel, 1999; Janzen, 1988; Quesada et al., 2009; Quesada and Stoner, 2004).

We evaluated the pollination services on crops of *Citrullus lanatus* (watermelon) and green tomato (*Physalis ixocarpa*) in the coastal region of Jalisco, Mexico. Specifically, we described the composition of the community of pollinators and their frequency of visits to the crops. We evaluated the contribution of the main pollinators to the reproductive success of the crops and the dependence of these crops to the pollination by animals. Finally, we evaluated the influence of the landscape to the abundance and richness of pollinators of *P. ixocarpa* and *C. lanatus*. We predict that the reproductive success of the crops will be less in flowers without visits and in plots with a low floral abundance of visitors compared to those flowers and plots with a high abundance of floral visitors. We also predicted that plots near the forest would have a greater richness and abundance of pollinators compared to those plots with little coverage or that are away from the forest.

2. Material and methods

Study site

We conducted the study in the southwest coast of Jalisco, Mexico, in crop fields located in the municipalities of La Huerta and Cihuatlan. Into La Huerta is the Chamela-Cuixmala Biosphere Reserve, located in Jalisco coast, México. The coastal area of Jalisco, where the reserve is located, is composed mainly of TDF, comprising 56.1% of total coverage, while the agriculture and grazing areas cover 25.8% of the coverage (Sánchez-

Azofeifa et al., 2009). Within the Costa de Jalisco region, there are several sites used as agricultural fields. Among these fields, we can find crops of *Citrullus lanatus* and *P. ixocarpa*, grown mainly during the dry season of TDF. *C. lanatus* is highly dependent on pollinators and *P. ixocarpa* may be very dependent on pollinators as well because both species are self-compatibility. Due to the few studies of pollinators in crops in TDF ecosystems and the frequent use of crops like *C. lanatus* and *P. ixocarpa*, the region provides an important environment to study the relationship of the landscape with pollination services to economically important crops. The climate of the study area is predominantly warm sub humid with summer rains, with a mean annual temperature of 20 to 28 °C, and a mean annual precipitation of 600 to 2000 mm. Crop fields are located along the river basins of the region and these crops areas are expanded during the dry season when rivers become completely dry or with a low flow of water in the tropical dry forest. By cultivating in the dry season, farmers prevent the risk of losing crop production due flooding areas or due to the high abundance of herbivore insects during the wet season of the tropical dry forest (Cuevas-Reyes et al., 2006; Dirzo and Domínguez, 2002). The dry season period is from November and until the end of May. Several crops dependent on pollinators are cultivated in the dry season, such as watermelon (*Citrullus lanatus*), green tomato (*Physalis ixocarpa*), chili (*Capsicum annum*), cucumber (*Cucumis sativus*), chayote (*Sechium edule*), papaya (*Carica papaya*), squash (*Cucurbita moschata* and *C. pepo*) and tomato (*Solanum lycopersicum*). *C. lanatus* and *P. ixocarpa* crops are economically important in the region. According to the Servicio de Información Agroalimentaria y Pesquera (SIAP, 2017), during the years 2008 to 2016, in the municipalities of Cihuatlán and la Huerta, *C. lanatus* crops generated 29.3% and 77% and

P. ixocarpa crops generated 8.4% and 2.27% of the total value of crops cultivated in the dry season.

Study crops

Citrullus lanatus (Watermelon): *C. lanatus* is native to Northeastern Africa and this vegetable crop has been cultivated worldwide since ancient times and is among the most widely grown. Flowers are solitary 2-3 cm in diameter, with five light yellow petals. *C. lanatus* is a monoecious plant with self-compatible unisexual staminate and pistillate flowers. Some cultivars of *C. lanatus* have hermaphrodite flowers (Pisanty et al., 2015). *C. lanatus* strongly depend of pollination by bees. Fruit shape is often spherical but can be globular, oval or oblong (Klein et al., 2007; Paris, 2015). In our study, we used seedless *C. lanatus* as recipient plants and seeded *C. lanatus* as pollen donors in a proportion 3:1, respectively. Seeded *C. lanatus* are required as pollen donors because seedless *C. lanatus* produce non-viable staminate flowers, seedless *C. lanatus* only produce fruit if pistillate flowers are pollinated with viable pollen from seeded plants (Bomfim et al., 2015; Sugiyama and Morishita, 2000; Walters, 2005).

Physalis ixocarpa (Green tomato): The genus *Physalis* and its fruits have been used in the kitchen since pre-Columbian era. There are about 90 species within the genus *Physalis*, and of these, 70 species are found in Mexico, country that is cataloged as center of origin of *Physalis* (D'Arcy, 1991; Martinez, 1998; Vargas-Ponce et al., 2011). The main species of *Physalis* cultivated in Mexico is *P. ixocarpa*, and it is the fifth vegetable of economic importance in the country. The flowers are hermaphrodite, solitary and come from the dichotomy of the branches. The flowers open before the dehiscence of the anthers and pollination occurs mostly by insects, probably bees. The fruit is a fleshy, globose berry

of variable size and, it is wrapped by the calyx that is broad, with a characteristic of persistence even after the ripening of the fruit. The berry is small, smooth and green to yellow color. The species is classified as self-compatible. It has been documented that plants in greenhouses have a lower fruit production compared to plants in open fields (Cobaleda-Velasco et al., 2013; Mulato-Brito et al., 2007; Peña-Lomelí et al., 2018, 2014).

From the three municipalities studied site in 2017, the total cultivated area was 14,669.32 ha and the total income of the agricultural income production was 27,592,080 dollars. The income of *Citrullus lanatus* production comprised on average 29% from a total of agricultural income production and 7% of the cultivated area. Income of *Physalis ixocarpa* production comprised on average 6% from a total of agricultural income production and 7% of the cultivated area (SIAP, 2017).

Selection of the Citrullus lanatus and Physalis ixocarpa plots

To select the study plots, we first searched in river basins of the study region for crops plantations during the dry seasons of the year 2008, and years 2014 to 2017. We then selected crops only at the peak of the flowering stage (low quantity of fruits and high quantity of flowers per plant). We sampled each plot only on sunny to mid cloudy days and after three days of any application of agrochemicals. We georeferenced each surveyed plot. We carried out all activities from 900 to 1200 hrs.

Contribution of the main pollinators: Frequency and duration of visits

To measure the frequency and duration of visits of the main pollinators, we selected seven plots in three years (2015 with 2 plots, 2016 with 2 and 2017 with 3) for *Citrullus lanatus*, and for *Physalis ixocarpa* four plots in two years (2015 and 2016 with 2 plots for each surveyed year). Each plot was different between years. In each plot, we filmed 2 to 10 plants per plot and one flower per plant. In *C. lanatus* plants, we filmed pistillate and staminate flowers; and hermaphrodite flowers in *P. ixocarpa* plants. We only registered the floral visitors that touched the reproductive organs of each flower. For each floral visit, we recorded the pollinator species (to the lowest possible taxonomic level), time of arrival to the flower and duration of the visit. We calculated visitation rates (visits per flower per hour) for each pollinator taxa (i.e. for each flower filmed). We evaluated the foraging behavior of pollinators by assessing the duration in minutes of each pollination event. Time of arrival to the flower was used to obtain the mean number of pollinator visits per flower by the time of the day. To determine whether pollinator visitation rates and duration of pollinator visits differed between pollinator species, and in the case of *C. lanatus*, flower gender, we used the GLIMMIX procedure in SAS version 9.4 (SAS, 2014). We conducted generalized linear mixed models to analyze: (1) the effect of pollinator species (fixed effects) on pollinator visitation rates, and duration of individual pollinator visits (response variables), in the case of *C. lanatus* crops, the effect of flower gender, pollinator species, and their interaction on the same response variables. We included year and field plot as a random effect in the model of both analyses. We specified a Poisson distribution and a log link function for both response variables. In both analyses, we specified the ILINK option of the LS-MEANS statement to obtain back-transformed least square means and a Tukey adjustment for multiple comparisons.

Contribution of the main pollinators: Pollen loads on pollinators' bodies

To determine the capacity of different floral visitors to carry pollen of *Citrullus lanatus* and *Physalis ixocarpa*, we captured pollinators visiting flowers in the year 2015. In the case of *C. lanatus*, we captured pollinators in staminate flowers; and in *P. ixocarpa*, we captured them in hermaphrodite flowers (in both crops species, we captured 3-15 individuals of each pollinator species), and we placed them in separate vials. We removed pollen from each captured pollinator, dabbing one piece of fuchsin gel over four different parts of the pollinator body, maintaining the samples separated: back, head, ventral abdomen, and ventral torso. We did not remove pollen from specialized structures for pollen transport (i.e. corbiculae, scopae). We deposited each pollen sample on a slide and counted the number of pollen grains of *C. lanatus* using a stereoscopic microscope and the Zen program V 1.1.2 (Zen 2012). Because of the difficulty to see *P. ixocarpa* pollen grains under a stereoscopic microscope, we selected and counted five regions of the slide with a magnification of 40X using an optical microscope and we averaged the number of pollen grains of each individual slide. To evaluate the capacity of different floral visitors to carry pollen of *P. ixocarpa* and *C. lanatus*, we performed a generalized linear mixed model with GLIMMIX procedure in SAS version 9.4 (SAS, 2014). The model included pollinator species as a fixed effect and the total of the four regions of pollen obtained by individual pollinator as a response variable. This analysis used a Poisson distribution and a log link function, the ILINK option of the LS-MEANS statement was used to obtain back transformed least square means, and field plot was included as a random effect in the model.

Contribution of the main pollinators: Pollinator efficiency experiments on Citrullus lanatus and Physalis ixocarpa

Citrullus lanatus (Watermelon): To evaluate the contribution of the main pollinators to the reproductive success of the crops and the dependence of these crops on pollination by animals, we conducted exclusion experiments in seven plot crops in 2014. In each plot we had two treatments. **Treatment 1:** This was the open pollination treatment and consisted of marked flowers with visits from any pollinators. In this treatment we only marked virgin flowers, one flower per plant, and we allow the visit of any pollinator. **Treatment 2:** This was the exclusion treatment and consisted of bagged virgin flowers without pollinator visits. We first bagged all flowers of this treatment one day before anthesis to ensure the virginity. Then, after three days of anthesis, we removed the bag and we marked the flower. In each treatment, we marked ten plants per plot and one flower per plant.

In 2016 and 2017 we added two more treatments to the experiments of *Citrullus lanatus*. **Treatment 3:** This was the exclusion treatment with one pollinator visit. The pollinators were *Apis mellifera* and *Trigona fulviventris* and we treated them as independent events. Similar to the treatment 2, we bagged all flowers one day after anthesis, then we removed the bag the day of anthesis and we allowed one visit of the pollinator. After the visit, we bagged the flower again and we removed the bag after three days of the anthesis. **Treatment 4:** This was the pollen supplementation treatment. Because of the use of seedless *C. lanatus* in the region, and the implication of sterile pollen in crops, in 2017 we tested on four plots, 10 to 20 individuals per plot. At same sites, we performed the

treatment 1. One day prior anthesis, we bagged one pistillate seedless flower and one staminate pollen viable flower per individual plant. The day of the experiment, we mixed pollen from individuals with viable pollen (marked by the farmer), and with the help of a small brush, we put the mix on pistillate flowers until we covered the stigma completely. Right after the experiment, we bagged the flower, and three days after the experiment we removed the bag. In all treatments, after two weeks, we counted the fruits as successful flowers.

***Physalis ixocarpa* (Green tomato):** To evaluate the contribution of the main pollinators to the reproductive success of *P. ixocarpa* and the dependence of this crop to pollination by animals, we conducted exclusion experiments in eleven plots of *P. ixocarpa* in 2014. We had two treatments in each plot, and in each treatment, we had ten plants per plot and one flower per plant. **Treatment 1:** This was the open pollination treatment and consisted of marked flowers with visits from any pollinators. In this treatment we only marked virgin flowers, one flower per plant, and we allow the visit of any pollinator. **Treatment 2:** This was the exclusion treatment and consisted of bagged virgin flowers without pollinator visits. We first bagged all flowers of this treatment one day before anthesis to ensure the virginity. Then, after three days of anthesis, we removed the bag and we marked the flower.

In 2016 we performed another two treatments in four plots. **Treatment 3:** This was the exclusion treatment with one pollinator visit. The treatment consisted in one visit of *Apis mellifera*. All flowers in this treatment were bagged one day before anthesis, we removed the bag the day of anthesis and we allow one visit of the pollinator. After the visit, we bagged again the flower and we removed the bag after three days of the anthesis.

Treatment 4: This was the emasculated treatment. Because *Physalis ixocarpa* is reported as self-incompatible and with no apomixis, we tested both processes with emasculated, and bagged flowers. The flowers were emasculated and bagged one day before the anthesis and the bag was removed three days after the anthesis. In all treatments, after two weeks, we counted the fruits as successful flowers.

For both crops species, we compared the relative contribution of the treatments to fruit set. We performed generalized linear models with GENMOD procedure in SAS version 9.4 (SAS, 2014). The model used pollination treatment as the independent variable with the proportion of flowers that developed into fruit as a response variable. The analysis used a binomial distribution and a logit link function for fruit set. We used the Tukey-adjusted P-values for multiple comparisons.

Finally, for both crop species, we evaluated the effect of pollinator abundance on fruit set in open pollination treatments. In each plot, we draw a random transect of 50 meters long. In the transect, we walked for 10 minutes and we registered the diversity and abundance of the flower visitors (only visitors that touched the reproductive parts of the flowers). A simple linear regression was performed with the REG procedure in SAS version 9.4 (SAS, 2014) to predict the fruit set of open treatments based on the abundance of pollinators.

Influence of the landscape

To evaluate the influence of the landscape on the community of pollinators of *Physalis ixocarpa* and *Citrullus lanatus* crops, we correlated the abundance of pollinators with land cover metrics type-associated nesting and forage suitability across the three

studied municipalities. We evaluated the abundance of pollinators in several plots for different years: for watermelon we sampled five plots in 2008, 11 plots in 2014, nine plots in 2015, six plots in 2016 and eight plots in 2017; and for *P. ixocarpa* crops we sampled 16 plots in 2008, 14 plots in 2014, 10 plots in 2015, seven plots in 2016 and four plots in 2017. In each sampled plot we draw a random transect of 50 meters long, in each transect we walked for 10 minutes and we registered the diversity and abundance of the flower visitors (only visitors that touched the reproductive parts of the flowers). To create a land cover map, we classified the land cover based on the amount and location of nesting and floral resources of bees. Then, we estimated the forest cover at different radios and distance of the plot to the forest in each plot as an explanatory variable.

The estimation of the land cover metrics as explanatory variables was based on the classification that was made by visual interpretation of SPOT5 (5 and 10 m) images from 2007, Rapideye (5 m) from 2012 and Planet from (3 m) 2017 (Planet, 2017). Visual interpretation is a method of classification that depends on the knowledge of the interpreter, to identify the types of coverage and digitize through a chiseled tool the limits of each of these coverages. All images were selected between February and May of each mentioned year. In the case of the SPOT5 images, a fusion process was carried out between the 5-meter resolution panchromatic band and the multispectral bands to increase the spatial resolution to 5 meters. Acquisition dates represented the time closest to the sampling period of the bees and correspond to the dry season of the year when there is the greatest differentiation in the leaf phenology of the forests (Kalácska *et al.*, 2005; Sánchez-Azofeifa *et al.*, 2009; Gallardo-Cruz *et al.*, 2012). To avoid spurious changes in land cover map, we decided to carry out an interdependent classification process, starting in 2007 and updating

after 2010 and 2018. For the classification, the first three bands of SPOT and RapidEye sensors with a spatial resolution of 5 m were analyzed. The images were classified into 9 land use and land cover classes: (1) secondary forest (e.i., including pastures and early forest succession stage), (2) deciduous tropical forest of advanced successional age (including intermediate and late successional stages, (3) riparian forest (including both gallery forest along large rivers and vegetation set along the banks of streams and rivers), (4) mangroves, (5) seasonal crops (i. e. , corn, green tomato, tomato, chili, melon, watermelon, cucumbers, squash), (6) orchards (ie mango, papaya, coconut or citrus), (7) exposed soil (including paved roads), (8) water and (9) urban settlements. Image processing was done in ArcGIS 10.2 (ESRI, 2012) and Qgis (QGIS Development Team, 2009) at a scale of 1: 15000. From the centroid of each of the agricultural plot examined where the pollinator data were recorded, a buffer area with a radius of 2000 m was generated. We decided to evaluate buffer areas up to 2000m because this represent the maximum flight distance recorded for *Apis mellifera* and at that distance, the proportion of forest can be a reliable predicted bee abundance (Greenleaf et al., 2007; Groff et al., 2016). The validation sample was determined following the methodology proposed by Olofsson et al. (2014). We selected 553 random points from the land cover map, and then, we carry out verifications on images in each point using Google Earth, for this task we designated interpreters who had not been involved in the construction of the coverage maps. The overall accuracy of the classifications for 2007 and 2012 is 94%, while for 2017 it is 96%. We obtained this calculation using the AccurAssess plugin for Qgis (Mas, et al, 2014).

Around each plot centroid where the abundance of pollinators was measured, we calculated circular buffer areas of 500m, 750m, 1000m, 1250m, 1500, 1750m and 2000m.

Using the classified LULC maps for 2007, 2012 and 2017, we estimated the following LULC metrics: (1) percentage of forest cover -including mangroves, tropical deciduous forest (secondary and late forest) and riparian vegetation- for each buffer area surrounding *Physalis ixocarpa* and watermelon crops, and (2) distance to the different classes of forests (i.e. secondary forest, deciduous tropical forest of advanced successional age and riparian forest).

We generated linear regressions to evaluate the relationships between the percent of forest cover and the distance to the different classes of forests vs. the following variables: (1) abundance of *Apis mellifera*, (2) abundance of native species, (3) total abundance. Normality of the data was verified with Shapiro-Wilk test. All variables were squared root transformed for the adjustment of linear regressions. We used free access environment R to conduct the spatial and statistical analysis (v 3.2.2, R Core Team, 2015).

Use of InVEST Natural Capital Project Program

The InVEST pollination model is a landscape computer algorithm that estimates an index of bee abundance across a landscape, this prediction is based on nesting resources within the focal cell and floral resources surrounding the cell and within pollinator flight ranges. The inputs required for the model are a) a land use and land cover (LULC) map, b) land cover attributes, and c) guilds or species of pollinators present, their nesting biology and flight ranges (Lonsdorf et al., 2009; The Natura Capital Project, 2016). We obtained the information as follows;

a) LULC maps: We used the LULC maps from the influence of landscape section.

b) Land cover attributes: For the evaluation of land cover suitability for bee floral resources of each land cover and land use classes, we reviewed the literature of the plant species that are flowering during the months January to March in the tropical dry forest. We obtained the percentage of the flowering plants during the sampling months in relation to the angiosperms found in each type of coverage during the sampled months (Appendix 1).

c) Species of pollinators, nesting biology and flight ranges: We used the list of species of pollinators presented in video recordings and in transects of the previously steps. We estimated foraging distance of each bee from intertegular distance in mm from individuals captured in pollen counts. We recorded 2 to 20 measures per specie and we used the typical foraging range in meters with a regression used by Greenleaf et al., 2007. (Appendix 2). We reviewed the literature to determine the nesting suitability (cavity or ground) of each bee specie (Appendix 3).

A simple linear regression was performed with the REG procedure in SAS version 9.4 (SAS, 2014) to predict the results of the INVEST model with the real abundance of pollinators in transects of *Citrullus lanatus* and *Physalis ixocarpa* crops.

3. Results

Contribution of the main pollinators: Frequency and duration of visits

Citrullus lanatus (watermelon): We observed eight species of bee pollinators on flowers of *C. lanatus* and *Apis mellifera* was the main visitor. We registered *A. mellifera* in 93.3% and 94.8% of the total visits to pistillate and staminate flowers, respectively (Fig. 1). Peak visitation occurred between 9:00 and 11:00 hours (Fig. 2a). We did not find significant differences on pollinator visitation rates in flower gender ($F_{(1,40)} = 0.56$, $P = 0.45$) nor the

interaction between flower gender and pollinator species ($F_{(5,40)} = 1.27, P = 0.29$), we found significant differences in pollinator species ($F_{(7,40)} = 17, P \geq 0.0001$), with *A. mellifera* as the main visitor. We did not find significant differences on duration of individual pollinator visits in flower gender ($F_{(1,117)} = 0.09, P = 0.77$), pollinator species ($F_{(5,117)} = 0.42, P = 0.8$) and the interaction between flower gender and pollinator species ($F_{(4,117)} = 0.1, P = 0.98$), duration of pollinators in flowers was in average of 0.15 ± 0.16 minutes.

Physalis ixocarpa (Green tomato): We observed five species of bees on flowers of *P. ixocarpa*, *Apis mellifera* was the main visitor, accounting the 94.7% of the visits (Fig. 1). Peak visitation occurred between 10:00 and 12:00 hours (Fig. 2b). We found significant differences on pollinator visitation rates in pollinator species ($F_{(7,33)} = 156.42, P \leq 0.0001$; Fig. 3). We did not find significant differences on duration of individual pollinator visits in pollinator species ($F_{(4,1008)} = 0.22, P = 0.9$), duration of pollinators in flowers was in average of 0.15 ± 0.12 minutes.

Contribution of the main pollinators: Pollen loads on pollinators' bodies

Citrullus lanatus (watermelon): We captured 33 individuals of seven species of bees in male flowers of *C. lanatus*. All captured bees have pollen on their bodies. We found significant differences in the pollen count of the pollinator taxa ($F_{(6,21)} = 592, P \leq 0.0001$; Fig. 4).

Physalis ixocarpa (Green tomato): We captured 45 individuals of eight species of bees. All captured bees have pollen on their bodies. We found significant differences in the pollen count of the pollinator taxa ($F_{(7,33)} = 156.42, P \leq 0.0001$; Fig. 5).

Contribution of the main pollinators: Pollinator efficiency experiments on Physalis ixocarpa and Citrullus lanatus

Citrullus lanatus (watermelon): For treatment 1, with any visit of pollinators, we marked 11 plots with 142 female flowers in total and only 41 successfully produced fruits. For treatment 2, without pollinator visits, we marked 4 plots with 40 plants, one flower per plant, and we did not obtain fruits. For treatment 3 we marked 2 plots with 10 flowers with one visit of *Apis mellifera* and 10 flowers with *Trigona fulviventris*. We did not obtain fruits for flowers with one visit of the two species of pollinators. For treatment 4 we worked in 4 plots and 65 plants, each plant with one flower. We obtained 34 fruits. We found significant differences between treatment 1 (open pollination) and treatment 4 (manual pollination), where manual pollination doubles the fruit set compared with respect to the fruit set of open pollination ($\chi^2 = 18.92$, $df = 1$, $P \leq 0.0001$; Fig. 6). We predicted the fruit set of open treatments based on the abundance of pollinators, but we do not find a significant regression ($F_{(1,9)} = 0.07$, $P = 0.8$, $R^2 = 0.007$).

Physalis ixocarpa (Green tomato): We worked in 10 plots for treatment 1 with no visits of pollinators, we marked 10 plots and 169 plants, one flower per plant, we only had 68 successful fruits. For treatment 2 (exclusion treatment), we marked 10 plots and 167 plants, one flower per plant, we only obtained 19 successful fruits. For treatment 3, we worked in two plots and 21 plants, one flower per plant, we only obtained two fruits. For treatment 4, we worked in four plots and 74 plants, one flower per plant, we only obtained one fruit. We found significant differences between treatments, where open pollination was higher than the other treatments, and in the absence of pollinators, *P. ixocarpa* crops can reduce their

production by 65% ($\chi^2 = 65.94$, $df = 3$, $P \leq 0.0001$; Fig. 7). We predicted the fruit set of open treatments based on the abundance of pollinators, and we found a significant regression ($F_{(1,8)} = 17.63$, $P = 0.003$) with a R^2 of 0.68. Predicted fruit set of open pollination is equal to $0.28 + 0.003$ (pollinator abundance) when pollinator abundance is measured by transect of 50 m.

Influence of the landscape

For *Citrullus lanatus* crops, we sampled a total of 39 plots in five years, and we observed 15 pollinator species (Appendix 4). For *Physalis ixocarpa* crops, we sampled a total of 51 plots in five years and we observed 19 species of pollinators (Appendix 4). In crops of *Physalis ixocarpa* and for year 2008, we found a significant positive regression in the distance to any kind of forest ($P = 0.03$, with a R^2 of 0.55) and to late forest ($P = 0.05$, with a R^2 of 0.49) with the total abundance of pollinators, and a significant positive regression in forest cover with the total abundance of pollinators at the radio distance to the focal crop of 1500m ($P = 0.01$, with a R^2 of 0.6), 2000m ($P = 0.05$, with a R^2 of 0.49), 2250m ($P = 0.03$, with a R^2 of 0.54) and 2500m ($P = 0.02$, with a R^2 of 0.55). We did not find significant differences with distance to the forest and forest cover in the years 2014 and 2016. We did not find significant differences in landscape metrics in *Citrullus lanatus*.

Use of InVEST Natural Capital Project Program

We obtained the per-pixel total abundance of 12 pollinators species for three years. We found a significant positive regression between the abundance predicted by the model and the real abundance for the year 2008 and with the observed abundance of pollinators ($F_{(1,19)} = 5.41$, $P = 0.03$, with a R^2 of 0.22) and observed abundance of social bees ($F_{(1,8)} = 5.37$, $P =$

0.03, with a R^2 of 0.22). We did not find a significant regression with the predicted abundance and real abundance for 2014 and 2016.

4. Discussion

In this study, we show for *Citrullus lanatus* and *Physalis ixocarpa*, *Apis mellifera* is the most important flower visitor. Africanized populations of *A. mellifera* are well established in the study region, besides that, farmers used hives of *A. mellifera* to enhance pollination services in *Citrullus lanatus* crops. *Apis mellifera* is capable of providing pollination services for a wide variety of agricultural species and is the most frequent single species of pollinator around natural systems and agrosystems (Calderone, 2012; Garibaldi et al., 2013; Hung et al., 2018). The use of *A. mellifera* as a pollinator is a process widely used in the study region, where farmers and beekeepers have contracts to use *A. mellifera* hives. The cost of *A. mellifera* hives for pollination supplementation, was around 16 dollars per hive during all flowering season of the crop, and once the season is over, the hives are moved away from the plantations.

A. mellifera appears to be a bad pollinator. For example, *A. mellifera* can fly long distances, but forager bees concentrate their foraging activity on spatially restricted nectar and pollen sources (Javorek et al., 2002), promoting the self-pollination and caused a quality limitation with genetically or physiologically poor fruits (Chacoff et al., 2008). Also, the activity of *A. mellifera* can occur when pollen is less viable, for example in *C. moschata* crops (Delgado-Carrillo et al., 2018). But maybe the great numbers of these bees

and complementary in pollination services with wild pollinators can be important to maintain the pollination process in the agroecosystem (Delgado-Carrillo et al., 2018; Hoehn et al., 2008; Winfree et al., 2018).

In *Citrullus lanatus* crops, in this study we observed *A. mellifera* as the principal visitor of *C. lanatus* flowers, this is consistent with other studies where honeybee is the main visitor, but pollination efficiency of *Apis* is lower for *C. lanatus* flowers. In this study, we found a small capacity of *A. mellifera* to transport pollen and none of the flowers with one visit of *A. mellifera* produced fruits. However, two possible scenarios can explain the pollination ability of *A. mellifera* to produce fruits of *C. lanatus* in our study: 1) the large populations of *A. mellifera* and high visitation frequency can turn this bee into an efficient pollinator because *A. mellifera* mean visitation rate is approximately 10 visits per flower per hour and each bee carries 200 pollen grains that are potentially deposited on each pistillate flower, 2) another scenario is that the pollination of *A. mellifera* bees is complemented by native bees reaching 23% of fruit set of open pollination treatment.

In our study site, open pollination of *C. lanatus* crops was low, only 23% of the flowers can be turned in fruits, and we doubled the successful fruits to 50% with the supplementation of manual pollination with viable pollen. The result of manual pollination is similar to the fruit set of 57.23% from open pollination of summer seedly *C. lanatus* in Egypt (Taha and Bayoumi, 2009), 48.6% from hermaphrodite flowers of seedy *C. lanatus* in Israel (Pisanty et al., 2015), and 80% in seedless *C. lanatus* in USA (Walters, 2005). Due to the low fruit set observed in open pollination treatment, it is possible that crops in the area of study need the presence of more pollinators.

Natives bees were observed in low numbers and we only can perform exclusion experiments with *Trigona fulviventris*, who did not produce fruits. However, native bees species can be efficient in *Citrullus lanatus* crops in other regions, as was demonstrated for *Lassioglossum* sp in Pakistan, and *Bombus impatiens* in USA (Ali et al., 2015; Stanghellini et al., 1998). In this study, we observed *Lassioglossum* bees in a low proportion of visits, but with a high pollen load. Maybe under good management, halictid bees can be good pollinators of *C. lanatus* crops.

Although there is a lot of information about *Citrullus lanatus* pollinators, little is known about *Physalis ixocarpa*, a species with enormous cultural and economic importance in Mexico. Their dependence on pollinators was not described, and we classify them as great dependence on pollinators according to Klein et al., 2007 category. Plants of *P. ixocarpa* are native to Mexico and flowers have been described as self-incompatible, (Mulato-Brito et al., 2007; Pandey, 1957), in this study, we did not observe the use of self-compatible mutants. The *P. ixocarpa* seeds used in the crops of the studied area come from greenhouses of companies dedicated to producing seeds, and the few farmers who tried to recover the seeds of their crops mentioned that the yield decreased in subsequent crops. In the treatment without visits, we obtained fruits, although the fruit set was statistically lower than the fruit set by open pollination. One explanation of the existence of fruits in auto-incompatible plants is the self-incompatibility system loses strength throughout the life of the flowers, being a similar system reported in other Solanaceae plants, such as *Nicotiana alata* and *Solanum Carolinense*, which has been reported that flowers become more self-compatible with floral age, and the strength of the system of self-incompatibility loses

strength at the end of the floral stages, indicating a plastic self-incompatibility system (Liao et al., 2016; Travers et al., 2004). Besides, we observed the life of the *Physalis ixocarpa* flowers for three days, in the first day we did not observe the dehiscent anthers, it was until the second and third day that the anthers become dehiscent, with the pollen visibly detached from the anther. This observation coincides with the observation of a weakening in the self-incompatibility system as the flower ages, so studies focused on evaluating this system in this plant of agricultural interest are required.

In *Physalis ixocarpa*, native bees can play an important role in the pollination of crops, but we also observed the presence of *Apis mellifera* in more than 90% of the visits, this is probably due to the season in which these crops bloomed. Similarly in a previous work with *Cucurbita moschata* crops, we found that *A. mellifera* dominates the visits in the dry season and, although *A. mellifera* is not efficient pollinating pumpkins, the pollination service is maintained due to the community of pollinators in the dry season, in contrast, during the rainy season, a single pollinator is able to carry out the pollination service (Delgado-Carrillo et al., 2018). This same phenomenon may be occurring for the case of *P. ixocarpa*, where wild *Physalis* species have been recorded blooming in the rainy season (Vargas-Ponce et al., 2016). Analogous to what occurs with *C. moschata* and the wild relative *C. argyrosperma sororia*, the wild *Physalis* blooms during the rainy season, so native pollinators of *Physalis* may be associated with this season. There are also specialist pollinators of *Physalis* analogous to the pumpkin pollinator, *Peponapis*, for example, in *Physalis viscosa*, the main pollinator is *Perdita halictoides* and this pollinator is oligolectic to plants of *Physalis* (Sullivan, 1984). So, an approach to the study of pollination of wild plants can be crucial to develop management programs for native pollinators of *Physalis*.

Management programs for pollinators should include remnants of natural and semi-natural habitats of surrounding crop areas. The structure of insects and floral plants community can be impacted by the vegetation structure, spatial location, and landscape surrounding fragments, and forest cover loss and habitat homogenization negatively affect the community of insects and can be a driver for the loss of species diversity (Kremen et al., 2004; Macedo-Reis et al., 2019; Novais et al., 2018; Papanikolaou et al., 2017). Also, the dependence of pollinators is greater in tropical forests due to the great variety of flowering plants, compared to temperate forests, where plant species that not required pollinators are found. It has been shown that, for social bee species, the distance of the forest is determinant in ensuring the richness and abundance of bees (Ricketts et al., 2008), therefore, crops close to tropical forests may benefit from a higher or stable pollination service. In *Physalis ixocarpa* crops, we observed a positive relationship between the abundance of pollinators and coverage of the forest for the year 2008, in addition to finding a positive relationship between the abundance of bees and the fruit set of each plot. Therefore, forest reserves and riparian corridors are key to the conservation of bee communities that contribute to pollination services in crops (Brito et al., 2017; Delgado-Carrillo et al., 2017). In some cultivars, distance to the forest is not necessarily to affect the crop production, but decline in pollination (number of pollen grains in stigmas) and pollinators (abundance and richness) is a clear tendency (Chacoff et al., 2008), for some crops, productivity can be high in crops near to forests. In Brazil, the orchards near or adjacent to the forests come to produce more coffee beans (*Coffea arabica*), reaching an average increase of 14.6% in productivity due to the pollination by insects (Marco and Coelho, 2004). In Costa Rica, forest fragments provide nearby coffee plantations with a

higher rate of bee visitation and pollen deposition than those coffee plantations furthest from forest fragments (Ricketts, 2004). The cultivation of the Indian nut (*Anacardium occidentale*) next to a more conserved fragment of forest, presents a greater diversity and abundance of floral visitors comparing it with the area next to the fragment more disturbed, this is reflected in the reproductive success of the crop (Flores et al., 2012). In macadamia (*Macadamia integrifolia*) and dragon's eye (*Dimocarpus longan*) orchards near a rainforest in Australia, it was shown that proximity to the tropical rain forest can improve pollination rates (Blanche et al., 2006). Undoubtedly, the pollination service is negatively affected in the absence of natural and semi-natural habitats, although it is not always expressed in terms of agricultural production. However, the conservation of pollinators will be crucial to maintain the pollination ecosystem service of both forests and agroforestry environments.

For *Citrullus lanatus* crops, we did not observe any correlation between the landscape and the abundances observed in the crops, nor for the results obtained by INVEST and the abundances observed. Perhaps this lack of correlation is due to the low number of sampled *C. lanatus* crops and to the fact that the farmers in the area supplement pollinators with hives of *Apis mellifera*. Supplementation with *A. mellifera* has been reported in the area since the 1990s, and practically all farmers dedicated to seedless *C. lanatus* crops hire beekeepers to rent hives of bees. In Mexico, the income from boxes of *A. mellifera* is a growing business and the northern states and crops for export are the main consumers of this type of pollinator supplementation (SAGARPA, 2008). We do not know the importance of wild populations of *A. mellifera* from forests in the supplementation of pollination services to crops in the area such as *C. lanatus*, so studies focused on studying this interaction are required to guarantee the yield of the pollinator-dependent crops. The

landscape is an important driver in the presence of insect pollinators, however, in our study, we do not find a clear tendency to the forest cover and distance of forest and pollination richness and abundance for years 2014 to 2017. This lack of association can be the effect of different factors. First, the supplementation with *Apis mellifera* in the region can affect the visits of the pollinator community in the studied crops, it will be necessary for future studies determined the role of managed and wild *A. mellifera* in the visits and pollination of crops and the role of landscape in wild populations of *A. mellifera* in the tropical dry forest. The second factor can be density-independent factors, such as natural disturbance events, for example, in the region studied, there is a study that shows the effects of the hurricane Patricia on the herbivore insect communities, where hurricane can change abruptly the conditions of tropical dry forest and positively affect the insect communities (Novais et al., 2018). We do not know the changes in pollination communities because of natural disturbance events, but changes in communities of bees can affect the presence of these bees in surveyed crops of years of 2015. We need to evaluate how this natural phenomenon can influence the community of pollinators and their interactions.

Finally, in the coastal region “Costa Alegre” of Jalisco, crops are economically important and rely on pollinators for food production, changes or lack of pollinators can compromise agricultural production and thus, the economy of farmers in the region. We do not know the changes of native pollinators and the effect in the fruit production of crops and we do not know the role of africanized and managed hives of *Apis mellifera* bees in the pollination services of forest and agroforestry environments.

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Figure 1. Percentage of visits per hour and per flower in crops of *Citrullus lanatus* (Pistillate and staminate flowers) and *Physalis ixocarpa* (hermaphrodite flower) by bees.

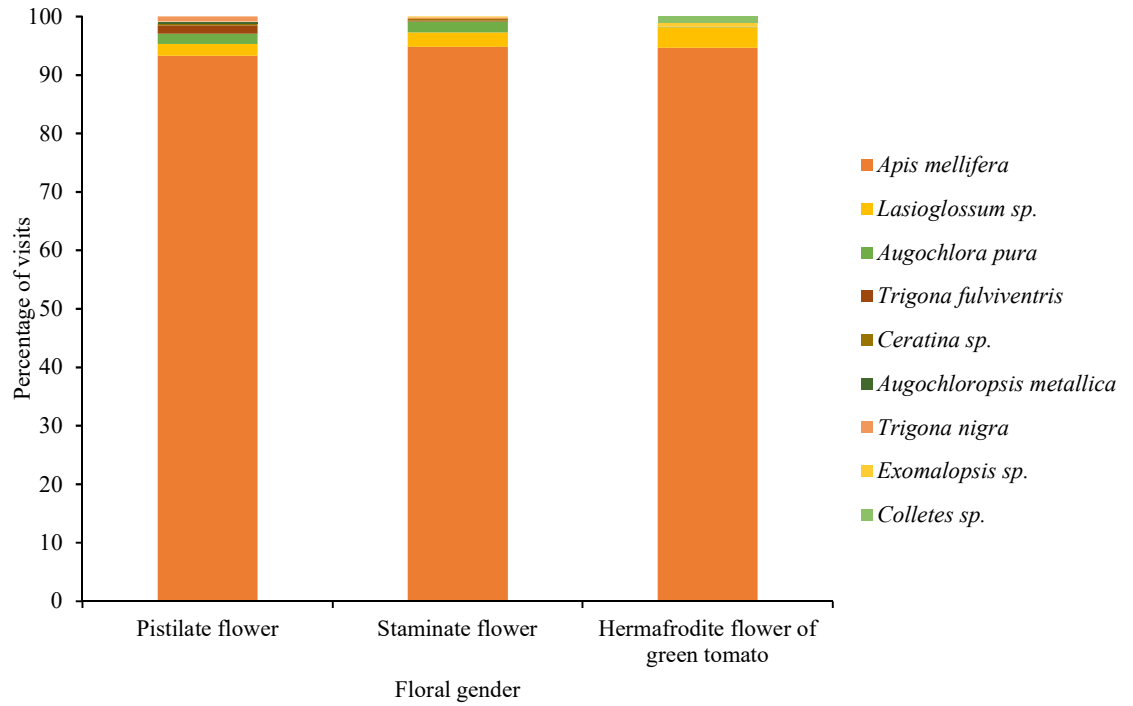


Figure 2. Mean (\pm SE) number of pollinator visits flower⁻¹ h⁻¹ to pistillate and staminate flowers through floral anthesis. a) Mean pollinator visitation rate in *Citrullus lanatus* flowers and b) *Physalis ixocarpa* flowers.

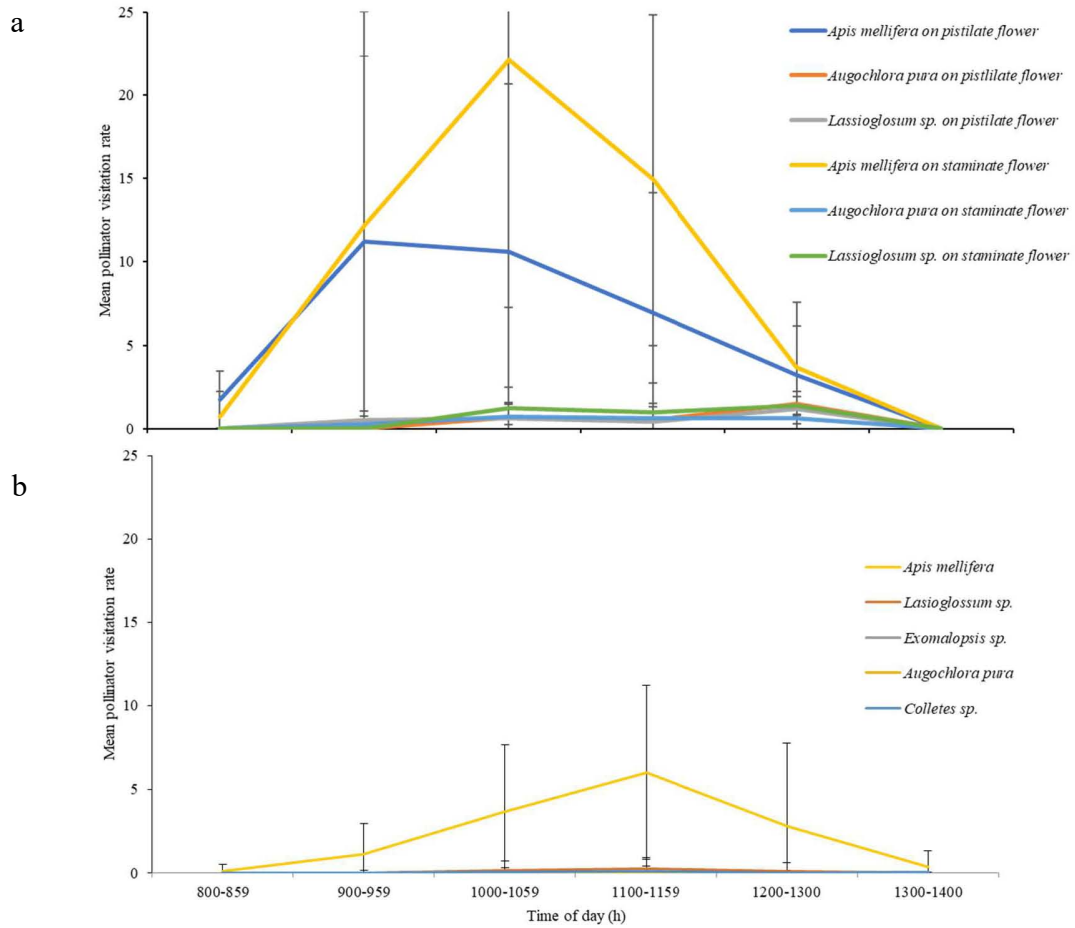


Figure 3. Mean (\pm SE) number of pollinator visits flower⁻¹ h⁻¹ by pollinator taxa in *Citrullus lanatus* crops. Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

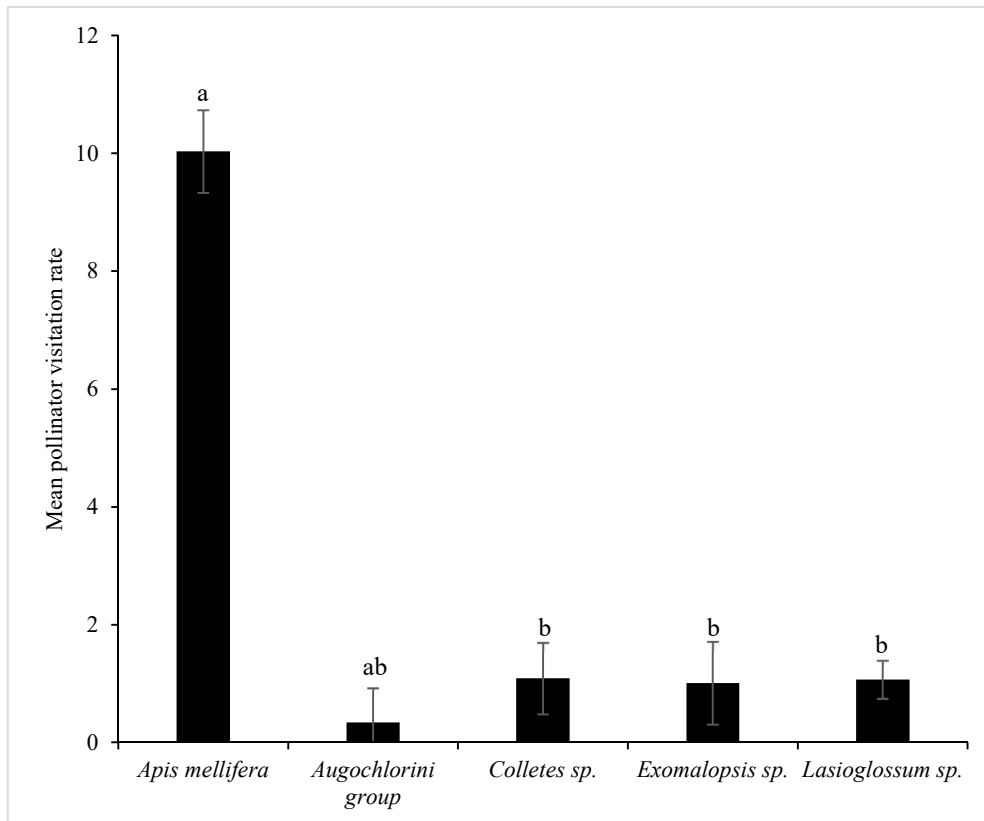


Figure 4. Mean (\pm SE) number of *Citrullus lanatus* pollen grains on the bodies of each pollinator taxa in watermelon crops. Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

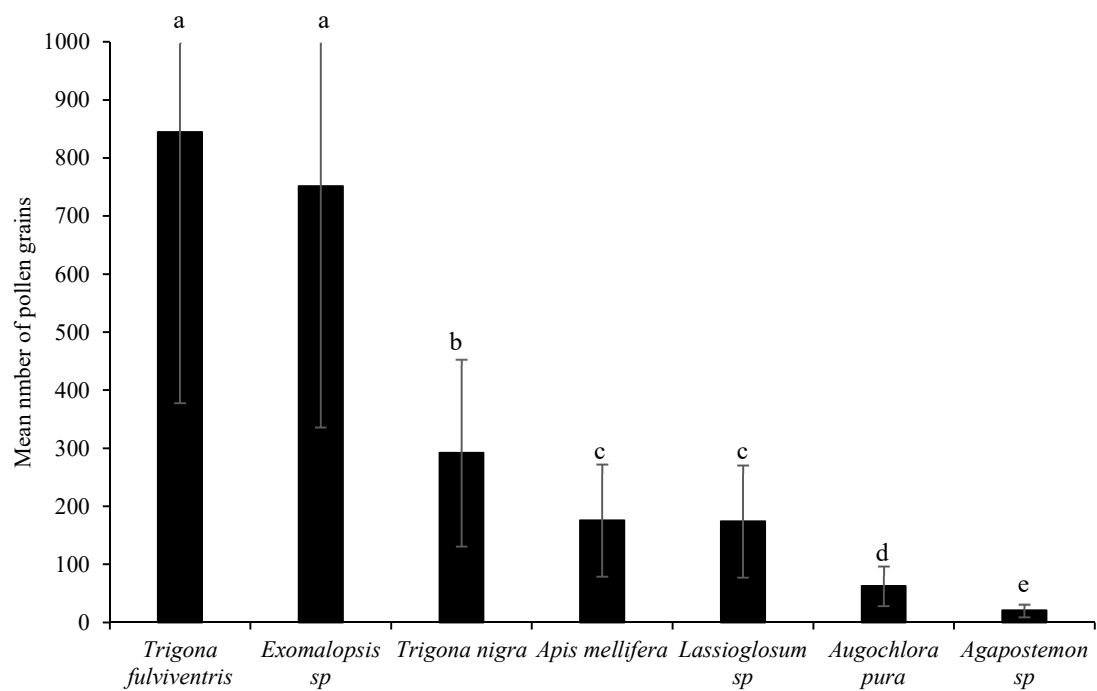


Figure 5. Mean (\pm SE) number of *Physalis ixocarpa* pollen grains on the bodies of each pollinator taxa in watermelon crops. Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

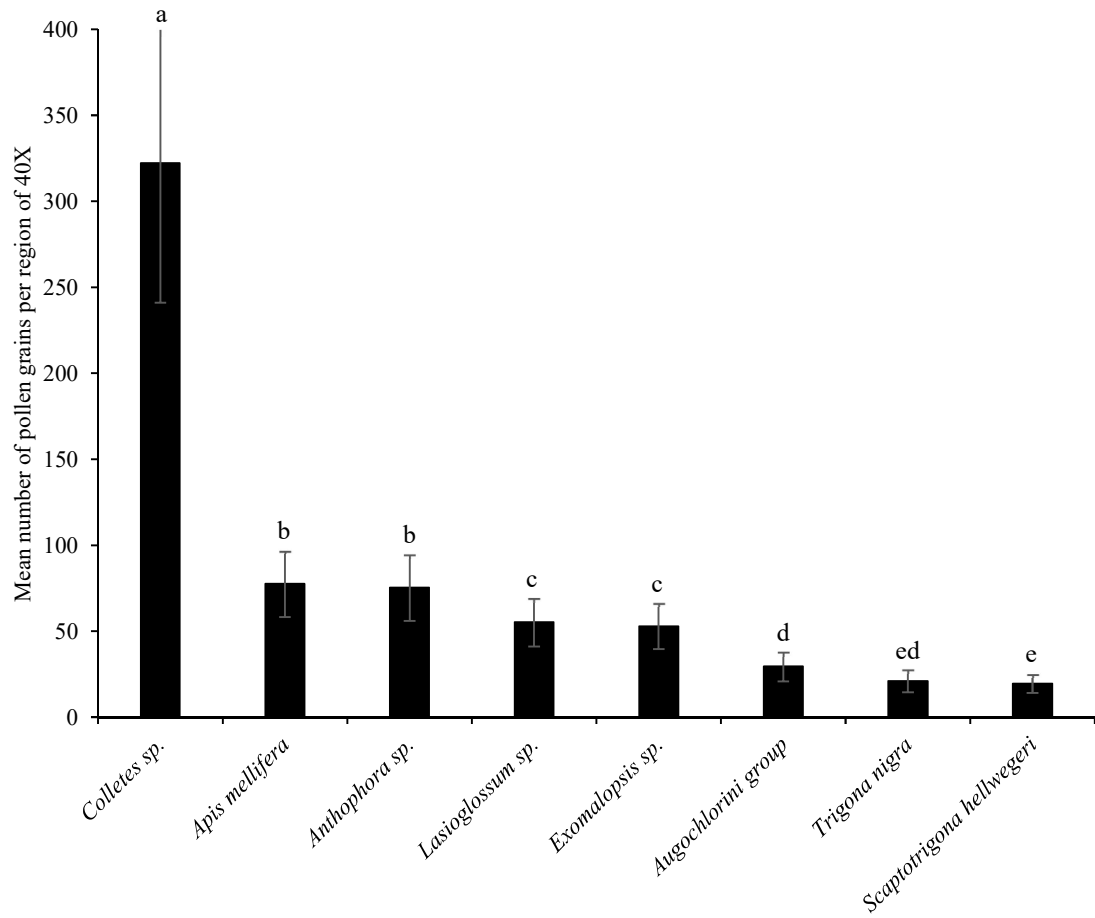


Figure 6. Mean (\pm SE) fruit set of pollinator efficiency treatments on *Citrullus lanatus* plants. Different letters indicate significant differences between treatments ($p < 0.05$) with Tukey ad hoc test. Treatments with one visit of *Apis mellifera* and *Trigona fulviventris* and with pollinator exclusion did not produce fruits.

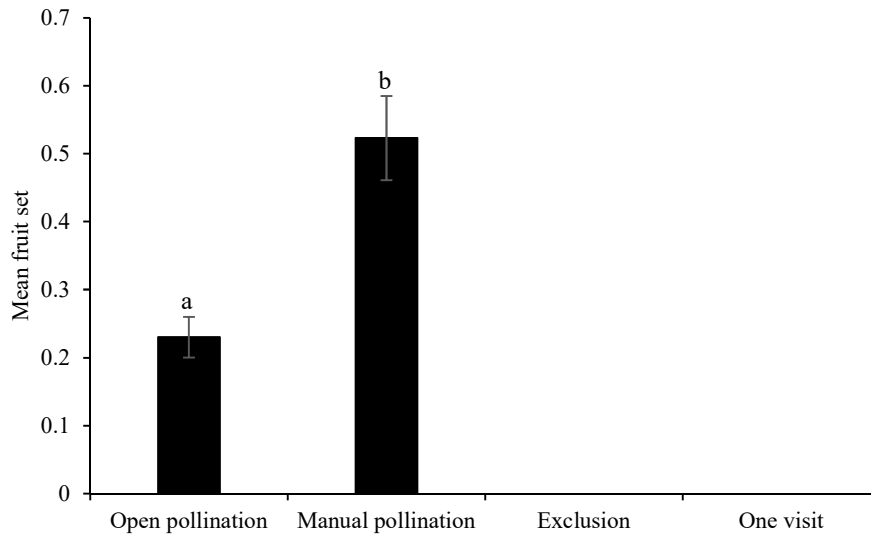
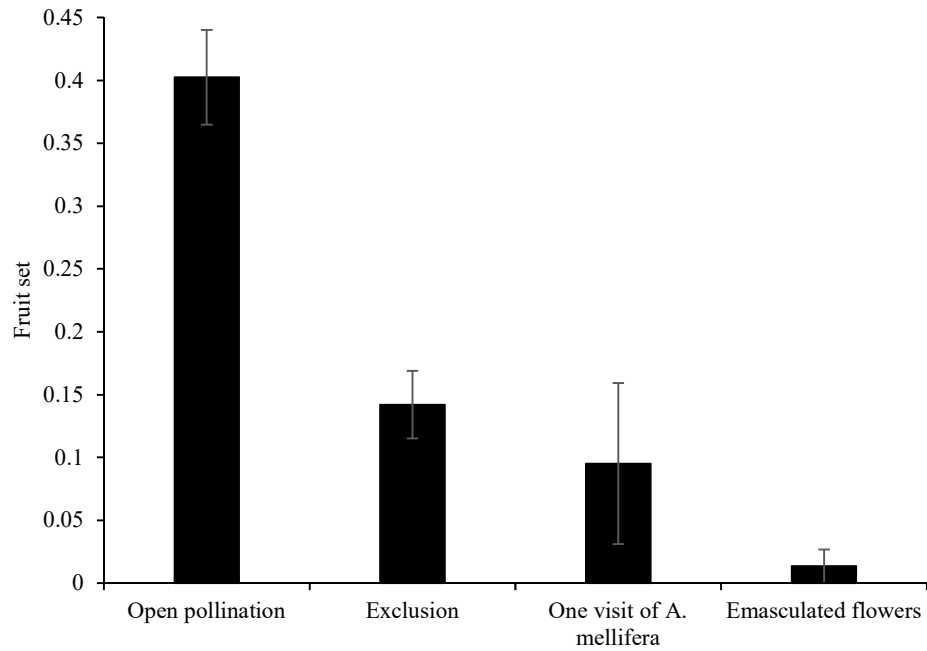



Figure 7. a) Mean (\pm SE) fruit set of pollinator efficiency treatments on *Physalis ixocarpa* plants. Different letters indicate significant differences between treatments ($p < 0.05$) with Tukey ad hoc test.





Temporal variation in pollination services to *Cucurbita moschata* is determined by bee gender and diversity

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Abstract. It has been proposed that species-rich insect communities and species turnover across landscapes enhance the pollination efficiency of crops through complementarity, where both the dominant and less abundant species contribute to reaching a yield threshold from pollination. Alternatively, fluctuations in the most abundant pollinator species, rather than changes in species richness, may drive temporal variation in pollination services. In this study, we used *Cucurbita moschata* as a model to investigate temporal variation in pollinator communities in a Mexican tropical dry forest region. We sampled floral visitors in the coastal region of Jalisco during the wet and dry seasons and determined the pollination efficiency of all floral visitors. Our results showed that there was temporal variation in the pollinator community and in the pollination efficiency of the main pollinators of *Cucurbita moschata* crops. In the wet season, native bees of the genus *Peponapis* were the most frequent and effective pollinators of *C. moschata*, whereas in the dry season, *Peponapis* bees were scarce and *Apis mellifera* became the most frequent floral visitor. *Apis mellifera* transfers smaller pollen loads than *Peponapis*, but it provides an effective pollination service in conjunction with other native bees during the dry season. There was also an interaction between flower gender and pollinator species, where *A. mellifera* had higher visitation rate to female *C. moschata* flowers, and *Peponapis* bees to staminate flowers. Mean visitation rate by *Peponapis* female bees was 17 times higher than visitation rate by male bees. This is the first report of a vis-à-vis relationship of pollinator gender with respect to plant gender in which plants of the genus *Cucurbita* that produce unisexual staminate and pistillate flowers are differentially visited by *Peponapis* male and female bees, where females are the main pollinators. Understanding the temporal variation in pollinator communities and the contribution of the different species of pollinators to the reproductive success of different crop species and varieties can be crucial to maintaining pollination services under the current global pollination crisis.

Key words: *Apis mellifera*; bees; crop pollination; fruit set; native pollinators; *Peponapis*; plant reproductive success; pollination service; pollinator gender; seed production; squash; tropical dry forest.

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INTRODUCTION

Recent evidence of the decline of introduced and wild pollinators in North America and Europe has caused great concern worldwide given its potentially catastrophic consequences on food security (Potts et al. 2010a, b). The global decline in pollinator abundance has caused a reduction in pollination services and crop yields (Millennium Ecosystem Assessment 2005). Under this scenario, the conservation of native pollinators may be crucial to ensure crop pollination success (Winfree et al. 2007, Garibaldi et al. 2013), since the contribution of native pollinators to fruit and seed production is often higher than the contribution of introduced pollinators (Canto-Aguilar and Parra-Tabla 2000, Gibbs et al. 2016). This is possibly the result of a shared evolutionary history between plants and native pollinators that has often resulted in specialized plant–pollinator interactions (Ashworth et al. 2009, Gibbs et al. 2016). Studies investigating pollination services of native pollinators to crop species are increasingly important to ensure global food security, crop productivity, and yield stability.

Despite the importance of native pollinators for crop productivity, only a few studies have documented the performance of native pollinator species and the impact of seasonal changes of the pollinator community on crop productivity. Diverse pollinator communities provide more stable and efficient pollination services (Hoehn et al. 2008, Klein et al. 2009, Rogers et al. 2014, Winfree et al. 2018), and temporal changes of pollinator assemblages through the year may contribute to the diversity of pollinator communities and to the stability of crop production. Alternatively, seasonal variation in primary pollinators or the lack of efficient pollinators in one season may result in a seasonal reduction in crop production. Temporal changes in pollinators have been documented for *Citrullus lanatus* and *Vaccinium angustifolium* in North America (Kremen et al. 2002, Bushmann and Drummond 2015); however, little is known about temporal variation in pollinator communities and pollinator efficiency in tropical regions. To propose viable strategies for the management and conservation of pollinator services for tropical crops, it is necessary to understand how changes in pollinator communities impact crop production across different seasons.

Temporal variation in pollinator services in tropical regions is relevant to annual and perennial crops with long or inducible flowering seasons. Most crop species are annual and have a single flowering season per year. However, irrigation allows cultivation at different times of the year, enabling farmers to harvest more than once a year, especially in areas near rivers and wetlands (Armillas 1949). This is the case of crops that are cultivated in Neotropical dry forest regions during the dry and wet seasons, such as the squash, *Cucurbita moschata*. Given that plant flowering time and pollinator activity should be evolutionarily tuned to maximize both plant and pollinator fitness, native pollinators should be present or active during the natural flowering season of a native crop species. Moreover, the native pollinators of a crop species should be more effective than introduced pollinators. However, if native pollinators are absent when native crops are cultivated out of their season, then introduced pollinators may provide alternative pollination services.

In agricultural fields of the tropical dry forest regions of Mesoamerica, farmers take advantage of natural rainwater in the wet season to grow crops like maize, backyard squash (*Cucurbita moschata*) and crops for livestock food. Crops of *C. moschata* are mainly cultivated for self-consumption. In the dry season, agriculture is more technified because farmers need to irrigate their crops from nearby water sources. Farmers generally use the alluvial soils of riverbanks that flood during the wet season but remain arable land during the dry season. This practice eliminates the risk of flooding and loss of crops that often occurs in the wet season. Furthermore, because the populations of many adult insect herbivores are reduced during the dry season, there should be lower herbivory (Dirzo and Domínguez 2002, Cuevas-Reyes et al. 2006), which is expected to increase crop yields. In Mexico, some crops are grown in both the dry and wet seasons, and include various species of squashes, such as *C. moschata* and *C. pepo*. These species are ideal to evaluate temporal and spatial variation in pollinator assemblages and pollination services for native crop species.

The genus *Cucurbita* is a group of monoecious, self-compatible plants, pollinated mainly by bees, and cultivated worldwide (Hurd et al. 1971, Lira-Saade 1995). There are about 27

species, of which five species have been domesticated: *C. pepo*, *C. ficifolia*, *C. moschata*, *C. maxima*, and *C. argyrosperma* (Whitaker 1974, Lira-Saade 1995). Mexico is considered an important center of genetic diversity of the genus *Cucurbita* (Lira-Saade and Montes 1992, Lira-Saade 1995); since pre-Hispanic times, four of the five species have been domesticated in the country, and 11 wild-type taxa have been recorded (Lira-Saade 1995). Bees of the genus *Peponapis* have been documented as the main pollinators of *Cucurbita* (Hurd et al. 1971, 1974, Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000), although some studies report other bee genera as frequent floral visitors (Artz et al. 2011, Enriquez et al. 2015). *Peponapis* is exclusive to the American continent, and in particular, *Peponapis pruinosa* and *P. crassidentata* share a close evolutionary history with the distribution and domestication of *Cucurbita* (López-Urbe et al. 2016).

We evaluated the pollination services to *Cucurbita moschata* in the coastal region of Jalisco, Mexico, during the wet and dry seasons. *Cucurbita moschata* is an ideal study species because it is highly dependent on pollinators for seed production (Free 1993, Klein et al. 2007); in North America, native and introduced bees perform most of the pollination (Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000, Artz et al. 2011), and in tropical dry forest habitats, crops are cultivated during both dry and rainy seasons. We studied the pollinator community composition, foraging behavior, and efficiency of pollinators for fruit and seed production of *C. moschata* across two seasons. *Peponapis* and *Xenoglossa* bee species are pollen specialists on *Cucurbita*, and have active nests during the wet season, coinciding with the natural flowering time of *Cucurbita* (Rozen and Ayala 1987, Ayala 2004, Delgado-Carrillo et al. 2017). Therefore, we predict that during the wet season, the pollinator community of *C. moschata* crops will be dominated mainly by native bees with high pollination efficiency. In the dry season, the pollinator community should change to more generalized and less efficient pollinators.

MATERIALS AND METHODS

Study plant

Cucurbita moschata is an annual herbaceous, climbing, and monoecious plant. Full development

of plants occurs within one to three months, and flowering lasts approximately two to three months (Lira-Saade and Montes 1992). *Cucurbita moschata* has staminate and pistillate yellow-orange flowers on the same individual plant. Floral anthesis lasts approximately six hours, with flowers opening just before dawn and closing at noon. Both types of flowers produce abundant nectar. Staminate flowers have a long pedicel, measuring between 16 and 18 cm. Pistillate flowers have shorter pedicels, 3–8 cm long, three-lobed stigmas, and an ovary that contains approximately 700 ovules (Canto-Aguilar and Parra-Tabla 2000). The pollen grains are large and sticky; thus, their transfer requires a biotic vector (Free 1993). Pollinators of *C. moschata* are essential for fruit and seed production (Klein et al. 2007).

Study site

We conducted the study in the municipalities of Cihuatlan and La Huerta, located in the southwest coast of Jalisco, Mexico. The altitude in this region ranges from 0 to 1300 m.a.s.l. The climate is predominantly warm subhumid with summer rains, with a mean annual temperature of 20–28°C, and a mean annual precipitation of 600–2000 mm. Agriculture occupies 21–25% of the area of the municipalities (INEGI 2009), while tropical dry forest occupies 56.1% of the coastal area of Jalisco (Sánchez-Azofeifa et al. 2009).

Crops of *Cucurbita moschata* are cultivated during the wet and the dry seasons. The dry season in this tropical dry forest begins in November and lasts to the end of May. This season coincides with the cultivation of other pollinator-dependent crops such as watermelon (*Citrullus lanatus*), tomatillo (*Physalis ixocarpa*), chili (*Capsicum annum*), cucumber (*Cucumis sativus*), chayote (*Sechium edule*), and tomato (*Solanum lycopersicum*). The wet season begins in June and lasts until October–November. Cultivation of other pollinator-dependent crops (e.g., tomatillo, *Physalis ixocarpa*; and watermelon, *Citrullus lanatus*) in this season is rare because farmers avoid cultivating these crops to prevent the risk of losing crop production to flooding or pests. We conducted our sampling at five *Cucurbita moschata* cultivated fields, two field plots during the wet season of 2015 (mid-September–October) and three field plots during the dry season of 2016

(January–March). Specific coordinate locations are provided in the supplementary material (Appendix S1: Fig. S1).

Pollination community and foraging behavior

We evaluated differences in the composition of the pollinator community and in visitation patterns between seasons, flower genders, and pollinator species. We conducted 190 h of video recording, focusing on staminate and pistillate flowers from 7:00 to 11:30 hour, in both the dry and the wet seasons. In the wet season, we filmed 10 staminate flowers and ten pistillate flowers on 20 individual plants (i.e., one flower observed per plant) in two different plots, plot one with 8 flowers and plot two with 12 flowers (Appendix S1: Fig. S1). In the dry season, we filmed 15 staminate flowers and 17 pistillate flowers on 32 individual plants (i.e., one flower observed per plant) in three different plots, plot three with 13 flowers, plot four with 9 flowers, and plot five with 10 flowers (Appendix S1: Fig. S1). For each floral visit, we recorded the pollinator species (to the lowest possible taxonomic level), time of arrival at the flower, and duration of the visit; we also quantified pollination events by registering visitors' contact with the reproductive organs of the flower. For identification, we collected individuals of each floral visitor species and used the bee species guide and world checklist (Asher and Pickering 2017). We collected two species of *Peponapis*, but due to the difficulty of identifying species in video recordings, we report visitation by bees in the genus *Peponapis*, rather than by species. We were able to separate *Peponapis* by sexual gender using the sexual characters antennae length (longer in male bees) and presence of scopae in female bees (Canto-Aguilar and Parra-Tabla 2000, Cane et al. 2011).

We calculated visitation rates (visits per flower per hour) for each pollinator species and observation period (i.e., for each flower filmed). We evaluated the foraging behavior of insect species that contacted the reproductive organs of the flower by assessing the duration in minutes of each pollination event.

Pollen loads on pollinators' bodies

To determine the capacity of different floral visitors to carry pollen of *C. moschata*, we captured visitors at staminate flowers (six species of

visitors, 3–15 individuals of each species) and examined pollen loads. Captures were performed between 8:00 and 10:00 hours. Each individual was captured on a separate killing vial. We removed pollen from each animal body dabbing one piece of fuchsin gel over four different parts of the pollinator body, maintaining the samples separated: back, head, ventral abdomen, and ventral torso. We did not remove pollen from specialized structures for pollen transport (i.e., corbiculae, scopae). We deposited each pollen sample on a slide, and counted the number of pollen grains of *C. moschata* with a stereoscopic microscope and the Zen program V 1.1.2 (Zen 2012). For statistical analyses of pollen samples, we considered *Peponapis crassidentata* and *P. utahensis* as a single taxon to be consistent with other analyses because we could not discriminate among these species on video recordings. We analyzed pollen loads separately for male and female *Peponapis* because they visit flowers at different rates and carry different amounts of pollen.

Pollinator efficiency experiments

To evaluate pollinator efficiency and the relative contribution of the different species to the pollination of *C. moschata*, we performed exclusion experiments at the five field plots during the flowering season of the crop. These experiments were conducted only for *Peponapis* sp., *Apis mellifera*, and *Trigona fulviventris*, because sample sizes were too low for the remaining species. In each plot, we examined all plants on the day of sampling and covered with a mesh bag five to ten large flower buds. The following day, upon opening, flowers were exposed to natural floral visitation and assigned to one of the following six treatments according to the number of visits received by particular insect species: (1) one visit by female *Peponapis* spp. ($n = 16$ plants), (2) one visit by *Trigona fulviventris* ($n = 13$ plants), (3) one visit by *Apis mellifera* ($n = 28$ plants), (4) three visits by *A. mellifera* ($n = 15$ plants), (5) all visitors excluded, a control for autonomous self-pollination ($n = 15$ plants), (6) open pollination control in wet season ($n = 28$ plants), and (7) open pollination in dry season ($n = 42$ plants). We only conducted treatment number 5 (pollinator exclusion) during the wet season. We did not repeat this treatment in the following dry season because the flowers that received the exclusion treatment in

the wet season did not produce fruit. After floral visitation, flowers were labeled and re-bagged for the rest of the day, removing the bag the following day upon flower senescence. In the case of open-pollinated flowers, the bag was removed before anthesis and the flower was marked. A week later, we counted the flowers that initiated fruit development, and a month later, we collected mature fruits and counted all viable seeds in each fruit. Variation in sample sizes among treatments was the result of different visitation frequencies of the different bee species.

Statistical analyses

To determine whether pollinator visitation rates and duration of pollinator visits differed between pollinator species, seasons, and flower gender, we used the GLIMMIX procedure in SAS version 9.4 (SAS Institute Inc 2014). We consider pollinator visitation rate as the number of visits by each pollinator to each flower per hour and the duration of pollinator visits as the time in minutes that each pollinator made to each flower. We conducted generalized linear mixed models to analyze: (1) the effect of season (dry or wet), pollinator species and their interaction (fixed effects) on pollinator visitation rates, and duration of individual pollinator visits (response variables); (2) the effect of flower gender, pollinator species, and their interaction on the same response variables. In both analyses, field plot was included as a random effect in the model. We specified a Poisson distribution and a log link function for both response variables. We considered male and female *Peponapis* as separate entities of the variable pollinator species to determine the contribution of each gender to pollination. In all analyses, we specified the ILINK option of the LS-MEANS statement to obtain back-transformed least square means and a Tukey adjustment for multiple comparisons.

To evaluate the capacity of different floral visitors to carry pollen of *C. moschata*, we performed a generalized linear mixed model with GLIMMIX procedure in SAS version 9.4 (SAS Institute 2014). The model included pollinator species as a fixed effect and pollen count as response variable. This analysis used a Poisson distribution and a log link function, the ILINK option of the LS-MEANS statement was used to obtain back-transformed least square means, and field plot

was included as a random effect in the model. All bees contacted stigmas of pistillate flowers of *C. moschata* with the lower part of their body; thus, for analyses, we used the sum of pollen loads from ventral abdomen and ventral torso. Because we only captured three species in both seasons (*Apis mellifera*, *Agapostemon* sp., *Augochloropsis metallica*), we considered bees collected in the dry and wet seasons as separate entities in this statistical analysis; for example, we considered *Apis mellifera* in wet season different from *A. mellifera* in dry season. To compare the relative contribution of the different pollinator species to fruit set and seed set, we performed generalized linear models with GENMOD procedure in SAS version 9.4 (SAS 2014). The model used pollination treatment as the independent variable with the proportion of flowers that developed into fruit and number of seeds produced per fruit as response variables. The analysis used a binomial distribution and a logit link function for fruit set and a normal distribution for seed set. We used the Tukey-adjusted *P*-values for multiple comparisons.

RESULTS

Pollinator community and foraging behavior

The most frequent floral visitors in the wet season were bees of the genus *Peponapis*, accounting for 92% and 95% of the total visits to staminate and pistillate flowers, respectively. Peak visitation occurred between 07:30 and 08:30 hours, with female *Peponapis* leading the visits to both staminate and pistillate flowers. In contrast, in the dry season, the most frequent visitor was *Apis mellifera*, accounting for 77% and 70% of the total visits to staminate and pistillate flowers, respectively; peak visitation in this season occurred between 09:00 and 10:00 hours (Fig. 1).

During the dry season, we observed 15 species of floral visitors; five of these were excluded from statistical analyses because they were all recorded a single time the same sampling day. In the wet season, we recorded a subgroup of six pollinator species. We did not find significant differences in pollinator visitation rates between seasons ($F_{(1,3)} = 2.33$, $P = 0.2$), but we found significant differences in pollinator visitation rates between pollinator species ($F_{(9,3)} = 13.08$, $P = 0.02$) and a significant interaction between season and

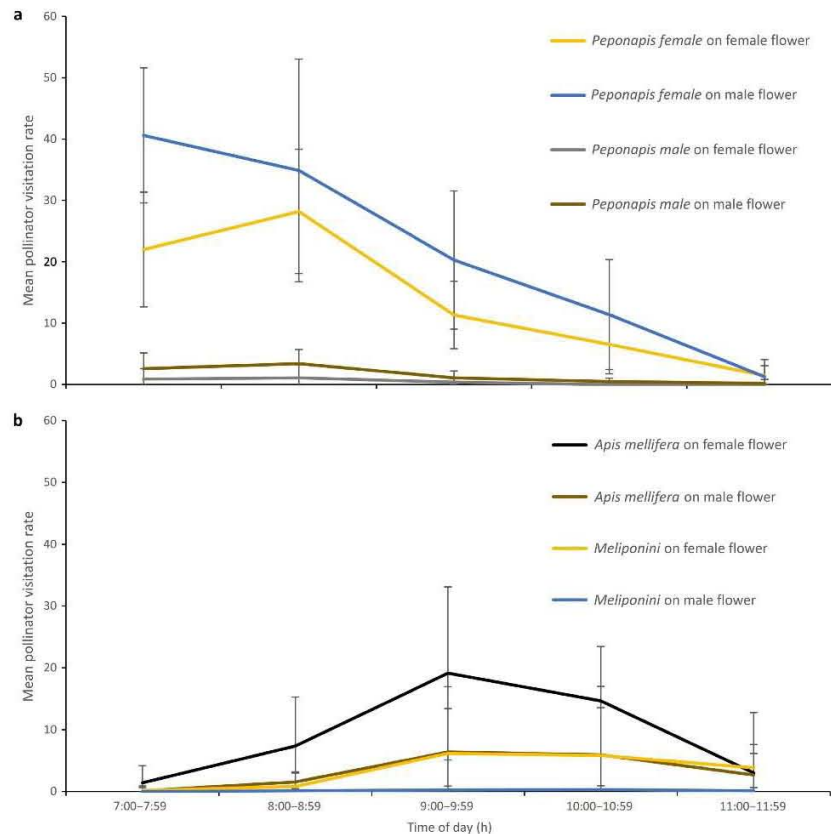


Fig. 1. Mean (\pm SE) number of pollinator visits-flower⁻¹·h⁻¹ to female and male flowers of *Cucurbita moschata* throughout floral anthesis in (a) wet season and (b) dry season. Bee species not included in (a) (*Apis mellifera*, meliponini and halictid bees) and (b) (*Peponapis* and halictid bees) had less than one visit-flower⁻¹·h⁻¹. Meliponini bees include *Scaptotrigona mexicana*, *Trigona fulviventris*, and *T. nigra*.

pollinator species ($F_{(5,3)} = 13.32$, $P = 0.02$), with *Peponapis* as the main floral visitor in the wet season, and *Apis mellifera* as the main visitor in the dry season; pollinators from nine other species were only present in the dry season (Fig. 2).

We did not find significant differences in pollinator visitation rates by flower gender ($F_{(1,3)} = 2.04$, $P = 0.2$), but we found significant

differences between pollinator species ($F_{(9,3)} = 29.39$, $P = 0.009$) and a significant interaction between flower gender and pollinator species ($F_{(9,3)} = 12.6$, $P = 0.03$), where we observed a strong preference for staminate flowers by *Peponapis* and by pistillate flowers in *Apis mellifera*, *Trigona fulviventris*, and *Scaptotrigona mexicana*, and for staminate flowers in *Peponapis* female bees (Fig. 3).

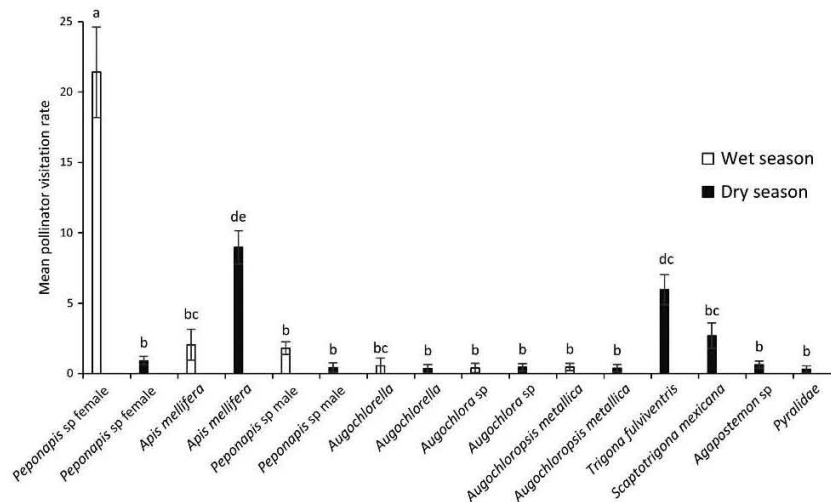


Fig. 2. Mean (\pm SE) number of pollinator visits-flower⁻¹·h⁻¹ by pollinator taxa in the wet season (white bars) and dry season (black bars). Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

We did not find significant differences in the duration of visits to flowers by season ($F_{(1,3)} = 1.57$, $P = 0.29$), but we found significant differences between pollinator species ($F_{(9,3)} = 42.8$, $P = 0.005$) and in the interaction between season and pollinator species ($F_{(5,3)} = 10.45$, $P = 0.04$; Fig. 4). Both *Apis mellifera* and *Peponapis* made significantly longer visits to flowers in the wet season than in the dry season (Fig. 4). We did not find significant differences in the duration of flower visits by flower gender ($F_{(1,3)} = 6.42$, $P = 0.08$), nor in the interaction between flower gender and pollinator species ($F_{(8,3)} = 6.22$, $P = 0.08$), but we found significant differences in the duration of flower visits by pollinator species ($F_{(9,3)} = 73.34$, $P = 0.002$). The duration of flower visits by *A. mellifera* was at least three times higher than the duration of other pollinator species.

Pollen load on pollinators' body

We collected 93 individuals from six bee species. All the bees captured in staminate flowers had pollen in some part of their body. Since we found

flowering individuals of *C. argyrosperma* in the wet season and it was difficult to differentiate the pollen of each *Cucurbita* species, we report pollen counts for *Cucurbita* spp. We found significant differences among pollinator species ($F_{(9,80)} = 674.56$, $P < 0.0001$; Fig. 5). In the dry season, *A. mellifera* carried pollen loads that were four times greater than the loads carried by this species in the wet season; other bees did not change their efficiency or were simply present in one season. The pollen load of *Peponapis* female bees was similar to the pollen load of other pollinator species that carry large amounts of pollen in their bodies.

Pollinator efficiency experiments

Due to the changes in pollinator assemblages between seasons, we only performed efficiency experiments with *Peponapis* spp in the wet season and with *Apis mellifera* and *Trigona fulviventris* in the dry season. We found significant differences in fruit set between pollination treatments ($\chi^2 = 64.2$, $df = 6$, $P < 0.0001$). Flowers under complete pollinator exclusion and flowers

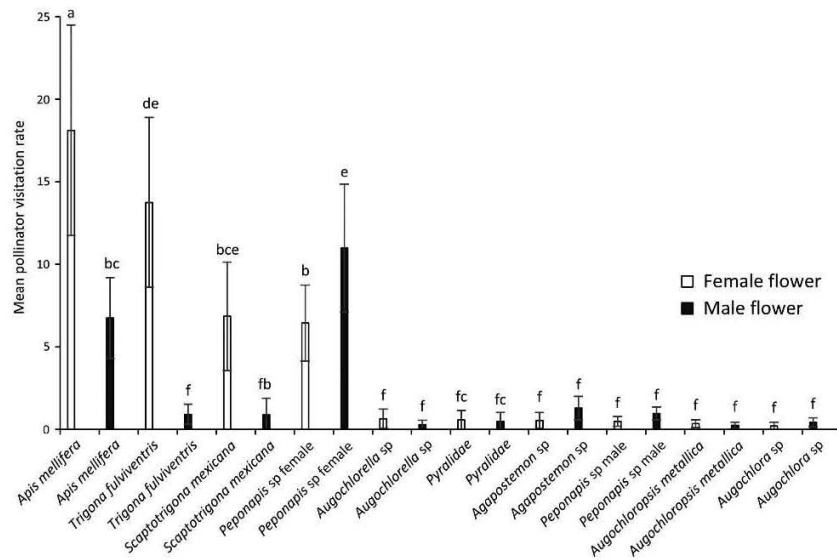


Fig. 3. Mean (\pm SE) number of pollinator visits-flower⁻¹·h⁻¹ by pollinator taxa in female (white bars) and male flowers (black bars). Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

exposed to a single visit of *T. fulviventris* did not produce any fruit. We did not find significant differences in fruit set after one visit of *Peponapis* female bees and open pollination, and these two treatments produced the highest fruit set. Fruit set after one and three visits of *A. mellifera* was significantly lower than the fruit set of open-pollinated flowers and the fruit set of one *Peponapis* visit treatment (Fig. 6). For seed production, we collected a total of 56 fruits of *C. moschata*; on average, fruits from open-pollinated flowers produced 538 ± 32.4 seeds ($n = 22$) in the wet season and 510 ± 29.8 seeds ($n = 26$) in the dry season, fruits from the single female *Peponapis* visit produced 446 ± 61.9 ($n = 6$) seeds, and fruits developed from flowers that received three *A. mellifera* visits produced $384 (\pm 69.3, n = 2)$ seeds. There were no differences in seed number per fruit between the single *Peponapis* visit treatment and the open pollination treatment in wet and dry seasons ($\chi^2 = 1.81, df = 2, P = 0.4$). The

three *A. mellifera* visits treatment was excluded from the statistical analysis due to the small sample size ($n = 2$ fruits).

DISCUSSION

Few studies have shown that species-rich insect communities enhance the pollination efficiency of native plants and crops through complementarity, where both the dominant and less abundant pollinator species contribute to reaching a yield threshold (Hoehn et al. 2008, Winfree et al. 2018). Moreover, species turnover across landscapes can also be important to attain this threshold (Winfree et al. 2018). However, in some agricultural systems, fluctuations in the frequency of the most abundant pollinator species, rather than changes in species richness, may drive temporal variation in pollination services (Genung et al. 2017). This study showed that temporal variation in the pollinator community

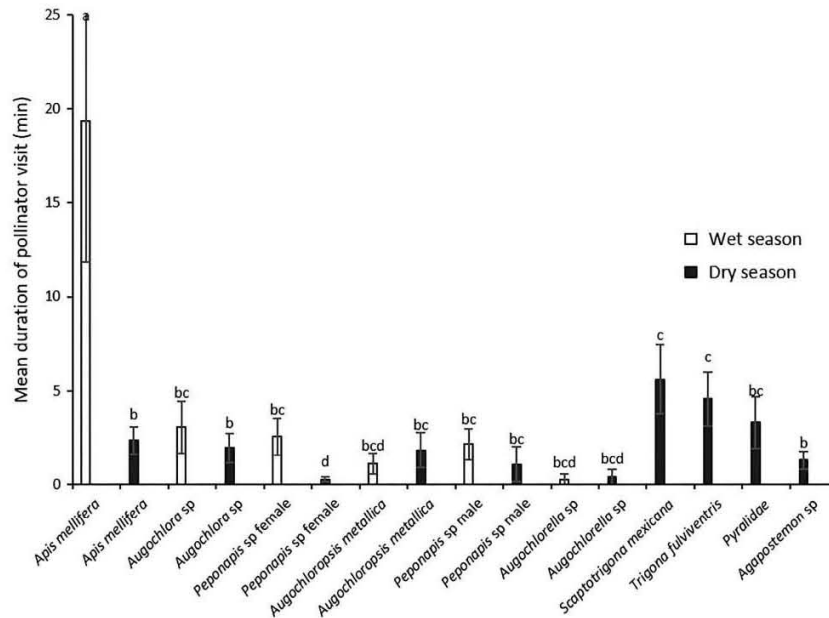


Fig. 4. Mean (\pm SE) duration (min) of pollinator visits-flower⁻¹.h⁻¹ by pollinator taxa in wet (white bars) and dry season (black bars). Different letters indicate significant differences between groups ($P < 0.05$) with Tukey's ad hoc test.

significantly affected the pollination efficiency of *Cucurbita moschata* crops between wet and dry seasons in cultivated areas surrounded by tropical dry forest. In the wet season, native bees of the genus *Peponapis* were the most frequent and effective pollinators of *C. moschata*, with little contribution from other bee species. In contrast, in the dry season, *Peponapis* bees were scarce and *Apis mellifera* became the main floral visitor of *C. moschata* crops. The pollination efficiency results of our study indicate that three visits of *A. mellifera* did not increase fruit set over one visit of *A. mellifera*, but the fruit set of open pollination was similar in both seasons; therefore, in the dry season, complementarity by other bee species contributes to fruit production in *C. moschata* crops. Similarly, complementarity by native bees in conjunction with *A. mellifera*

contributes to pollination services in other crops (Greenleaf and Kremen 2006, Brittain et al. 2013).

Temporal variation in pollinator visitation and efficiency determined the reproductive success of *C. moschata*. In the wet season, *Peponapis* bees visit *C. moschata* flowers early in the morning before other species arrive at flowers, removing and depositing more viable pollen than other pollinator species; furthermore, *Peponapis* bees had four times greater pollen loads and visitation rates to pistillate flowers than *A. mellifera*. These results suggest that when *Peponapis* bees are present, they are the most effective pollinators of *C. moschata*. However, the importance of *Peponapis* bees in the pollination of *Cucurbita* species is not consistent across the geographic distribution of these crops. In the Yucatan Peninsula in Mexico and in western United States, the genus

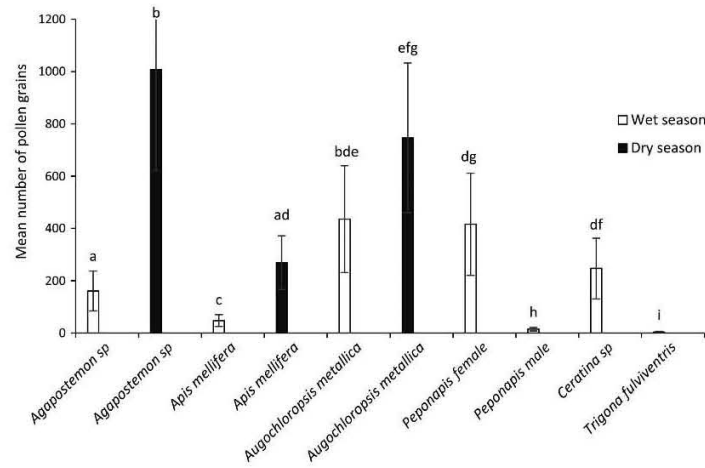


Fig. 5. Mean (\pm SE) number of *Cucurbita* pollen grains on the bodies of each pollinator taxon. White bars represent the pollinators in the wet season and black bars pollinators in the dry season. Different letters indicate significant differences between groups ($P < 0.05$) with Tukey ad hoc test.

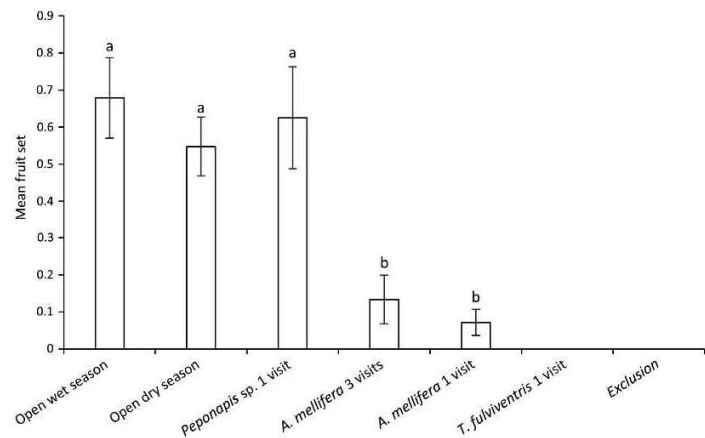


Fig. 6. Mean fruit set (\pm SE) of pollinator efficiency treatments. Different letters indicate significant differences between treatments ($P < 0.05$) with Tukey ad hoc test. The treatments pollinator exclusion and one visit of *Trigona fulviventris* did not produce fruits.

Peponapis is reported as one of the main pollinators of squash crops (Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000); however, at other sites in North America, Guatemala, and Brazil, this group of bees is apparently poorly represented or not observed in squash crops (Serra and Campos 2010, Petersen et al. 2013, Enríquez et al. 2015, Phillips and Gardiner 2015). One possible explanation for the apparent incongruence in the importance of *Peponapis* bees for squash crops from different geographic regions is that most studies have not considered temporal variation in floral visitation to these crops.

The phenology of *Peponapis* bees in seasonal environments is probably an important determinant of the temporal variation in pollinator assemblages documented in this study. As observed by Delgado-Carrillo et al. (2017), the life cycle of *Peponapis* bees in the Central Pacific coast of Mexico is synchronized with the natural blooming period of squash crops in the wet season. Species of *Peponapis* are reported as annuals; for example, *Peponapis pruinosa* in North Eastern United States overwinters as a prepupa in the soil, pupation begins in mid-June and adult activity ceases in early September (Mathewson 1968, Julier and Roulston 2009). Active nests of *Peponapis crassidentata* and *P. utahensis* have been described in the late wet season in Mexican dry forests (Rozen and Ayala 1987, Delgado-Carrillo et al. 2017), and the few *Peponapis* individuals observed during the dry season in this study were probably the last adults of the previous year. Because both species of *Peponapis* at the study site spend the majority of the dry season as a prepupa, they are not important pollinators of *C. moschata* crops during this season. *Peponapis* bees are fully dependent on the resources provided by flowers of the genus *Cucurbita* for nutrition of both larvae and adults (Hurd et al. 1971, Delgado-Carrillo et al. 2017). Furthermore, the phenology of *Peponapis* is synchronized with the flowering phenology of the wild gourd *C. argyrosperma*—a species that occurs along riverbanks and seasonal wetlands along the Pacific coast of Mexico (Mariano and Dirzo 2002, Balvino-Olvera et al. 2017). Similar synchronicity has been found for bat pollinators and the flowering phenology of Bombacaceous trees in Neotropical dry forests (Lobo et al. 2003). Therefore, the association between *Peponapis* and *Cucurbita* reflects a long

shared evolutionary history between plants and bees that has probably led to the observed synchrony in their reproductive phenologies.

Bees of the genus *Peponapis* and squash crops of the genus *Cucurbita* have their center of origin in Mexico and naturally occur throughout Mesoamerica (Hurd et al. 1971, 1974, Lira-Saade 1995, Ayala and Griswold 2012). Cultivated species of *Cucurbita* have been used by humans for thousands of years, and the distributions of some *Peponapis* species have been associated with human dispersal of squash crops (Nee 1990, Lira-Saade 1995, López-Uribe et al. 2016, Balvino-Olvera et al. 2017). At present, these crops are dynamically cultivated in small orchards and fields, according to peasants' necessities and market prices in Mexico; thus, cultivated squashes are not always available to *Peponapis* bees. Nevertheless, the strong interaction between *Peponapis* and *Cucurbita* species (both wild and cultivated) has resulted in high pollination efficiency, as was demonstrated in this and other studies between native bees and native crops (Tepedino 1981, Canto-Aguilar and Parra-Tabla 2000, Isaacs and Kirk 2010, Frier et al. 2016, Gibbs et al. 2016). Therefore, native species are expected to be the most efficient pollinators of native crops.

As stated before, *Cucurbita moschata* crops are cultivated using artificial irrigation during the dry season, when annual species of *Cucurbita* and *Peponapis* bees do not naturally occur. The absence of native effective pollinators would suggest a reduction in the fruit set of *Cucurbita moschata* crops in the dry season. However, we did not find significant differences in natural fruit set or seed production between the two seasons. Our result suggests that introduced and native bee species maintain pollination services for this crop during the dry season. Specifically, *Apis mellifera* is a less efficient pollinator than *Peponapis* at single flower visits, but low pollination efficiency is compensated by its high visitation rate and by the pollination effected by native halictid and stingless bees during the dry season. Africanized *Apis mellifera* colonized Mexico in 1986 and gradually expanded its prolific populations to most agroecosystems, natural areas, and even urban regions of Mexico (Lobo et al. 1989, Lobo 1995, Quezada-Euán 2007). In our study region, we did not find managed hives near

Cucurbita crops; therefore, honey bees pollinating *Cucurbita* flowers most likely come from feral Africanized bee populations. A previous study in the same region indicates that generalist pollinators, such as *Apis mellifera*, are common in areas under ecological succession and these abundant bees are also capable of providing pollination services to the plants in the surrounding tropical dry forest (Lopezaraiza-Mikel et al. 2013). This study shows that naturalized Africanized bees that inhabit natural forests in conjunction with native bees allow offseason pollination of squash crops when *Peponapis* bees are absent or in low numbers.

Another group of potential pollinators are the native halictid bees, *Ceratina* bees, and stingless bees. *Augochloropsis metallica* and *Agapostemon* sp. visit squash flowers in both seasons, and they carry large loads of *Cucurbita* pollen on their bodies. Although the pollination efficiency of these bees was not measured in this study, halictid bees have been described in other studies as frequent visitors and efficient squash pollinators (Ali et al. 2015). However, visitation rates of halictid bees to *Cucurbita moschata* are low for both flower genders compared to *Peponapis*. *Ceratina* bees carried pollen loads comparable to *Peponapis*, but their visitation rates were low, only 10 individuals were captured in the wet season, and no *Ceratina* bees were observed in video recordings. One species of stingless bee is apparently an important pollinator of *Cucurbita pepo* in a cloud forest in Guatemala, but the pollination efficiency of *Peponapis* was not measured at that site (Enriquez et al. 2015). In Chamela during the dry season, we observed stingless bees taking nectar from pistillate flowers and a few individuals visiting staminate flowers with low pollen loads on their bodies; however, the *Trigona fulviventris* one-visit treatment did not produce any fruits, suggesting that more than one visit is required to develop a fruit, or that stingless bees act as nectar and pollen robbers in squash flowers. Halictid and *Ceratina* bees carry similar pollen loads as female *Peponapis* bees; however, they have little impact on the pollination service provided to *Cucurbita moschata* due to their low abundance.

Our study represents the first report of a vis-à-vis relationship of pollinator gender with respect to plant gender. Monoecious plants of the genus

Cucurbita produce unisexual staminate and pistillate flowers, which are differentially visited by *Peponapis* male and female bees; female bees visited staminate flowers nearly twice as often as pistillate flowers, while male bees visited both flower genders at a low frequency. This result is possibly explained by the high dependence of female *Peponapis* bees to the pollen provided by staminate flowers, which they use to feed their larvae (Hurd et al. 1971, Delgado-Carrillo et al. 2017). There was also a strong gender bias in *Peponapis* visitation rates to *Cucurbita* staminate flowers, where mean visitation rate by female bees was 17 times higher than the visitation rate by male bees (Fig. 3). This result is in apparent contradiction with a study that showed higher visitation rates of *P. pruinosa* males than females to flowers of *C. pepo* (Cane et al. 2011); however, the latter study did not differentiate visitation to pistillate and staminate flowers and did not analyze the contribution of male and female bees to the plant's reproductive success. Female *Peponapis* bees also visit pistillate *Cucurbita* flowers in search of high-energy sources, because pistillate flowers of some *Cucurbita* species like *C. pepo* tend to produce higher quantities of nectar with greater sucrose concentration (Nepi et al. 2001). Therefore, *Peponapis* female bees are the major contributors to the pollination service and fruit production of squash crops during the wet season. Differential use of floral resources by male and female pollinators of the same species has been associated with differences in energetic requirements or feeding behaviors related to the pollinator gender (e.g., Hymenoptera, Ne'eman et al. 2006, Lepidoptera, Alarcón et al. 2010, Diptera, de Jager and Ellis 2012, hummingbirds, Temeles et al. 2009). Likewise, in plant species with unisexual flowers, pollinators may prefer a particular flower gender due to differential production of floral rewards in pistillate and staminate flowers of the same plant species and this pollinator selectivity can influence the evolution of floral sexual dimorphism (e.g., Begonia, Le Corff et al. 1998, Ashman 2000). However, the reciprocal impact that male and female pollinators of the same species have on male and female function of a particular plant species has been little studied and deserves further attention.

Although fruit set and seed production are similar in open pollination treatments in both

seasons, other studies have shown that pollen competition affects seed quality and progeny viability due to differences in the quality and quantity of *Cucurbita* pollen (Quesada et al. 1991, 1993, Winsor et al. 2000). Flowers of *Cucurbita* species are ephemeral, and their pollen loses viability within a few hours (Nepi and Pacini 1993, Agbagwa et al. 2007, Franchi et al. 2014). *Peponapis* bees, which arrive in great numbers at flowers early in the morning when pollen is more abundant and viable, might carry higher loads of viable pollen, which may in turn result in better quality progeny. In contrast, *Apis mellifera* and the other native bees in dry season arrive at flowers later in the day, a behavior that might be associated with decreased progeny quality. This hypothesis, which deserves experimental testing, predicts changes in the seed quality and progeny viability of fruits produced at different time during the day, and at different seasons by different types of pollinators. Furthermore, knowledge of pollen viability and seed quality in association with progeny performance are key for the management of native and introduced pollinators to obtain better seeds and fruits of economically important crops.

Finally, natural protected areas provide an important pollination ecosystem service to crops because they provide nesting sites and floral resources for squash pollinators. Riparian habitats and forest reserves can maintain native and feral pollinators of crops (Brito et al. 2017), and a good example was shown for the provisioning of floral resources and nesting sites of *Peponapis crassidentata* in the study area (Delgado-Carrillo et al. 2017). Currently, only 4% of this study area is riparian habitat; thus it is crucial to protect this environment for providing resources for bees and other pollinator species (Sánchez-Azofeifa et al. 2009).

In Mexico, 85% of the crop species depend to some degree on pollinators (Ashworth et al. 2009); therefore, it is necessary to study spatial and temporal variation of pollinator communities and the contribution of the different species of pollinators to the reproductive success of crop species. A better understanding of these ecological factors will allow improved management and conservation actions in agroecosystem landscapes that ensure the presence of insect pollinators throughout the year.

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SUPPORTING INFORMATION

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A scientific note on the first record of nesting sites of *Peponapis crassidentata* (Hymenoptera: Apidae)

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solitary bees / nesting behavior / *Peponapis* nests

Peponapis is a genus of solitary bees that breeds once a year, nests in the ground, and is distributed exclusively in the Americas. Mexico is considered its center of origin, holding all 15 currently known species of *Peponapis* (Hurd et al. 1971; Ayala and Griswold 2012). *Peponapis* spp. are closely associated with the plant genus *Cucurbita* (Hurd et al. 1971; Hurd et al. 1974), whose center of origin is also Mexico (Lira-Saade 1995). *Peponapis pruïnosa* (Say, 1837) in North America and *Peponapis limitaris* (Cockerell, 1906) in Mexico are the most effective pollinators of *Cucurbita* spp. (e.g., Canto-Aguilar and Parra-Tabla 2000; Minter and Bessin 2014). The pollen of *Cucurbita* also seems to be the main food source of some *Peponapis* sp. larvae (Hurd et al. 1974). Little is known about the natural history of most *Peponapis* species, and only nest descriptions of *P. pruïnosa* (Mathewson 1968; Hurd et al. 1974), *P. fervens* (Smith, 1879) (Michener and Lange 1958; Krug et al. 2010), and *P. utahensis* (Cockerell, 1905) (Rozen and Ayala 1987) are known under natural conditions. Most studies of *Peponapis* are taxonomic descriptions and observations of *Cucurbita* flower visitation by these bees (Hurd and Linsley 1966;

Wille 1985; Ayala and Griswold 2012). Here, we describe the nesting sites and the structure of nests of *Peponapis crassidentata* (Cockerell, 1949).

P. crassidentata (Figure 1a) is distributed from southern Texas (USA) to Costa Rica (Wille 1985; Giannini et al. 2011), and it has been observed foraging on *Cucurbita* flowers (Wille 1985). No other information has been reported for this species. We searched for nesting sites of *P. crassidentata* near *Cucurbita moschata* Lam. crops in the coastal region of Jalisco state, Mexico. In November and December 2014, we found two nesting sites (sites 1 and 2), and in January 2016, a third nesting site (site 3, Table I). These sites were found along temporary streams with riparian vegetation surrounded by tropical dry forest. We registered 188 nests (Table I). Nesting activity occurred within the flowering season of nearby cultivated *C. moschata* (Jul Dec 2014 and Nov 2015 Mar 2016) and wild *Cucurbita argyrosperma* Huber (Jun Dec 2014 and 2016). From February onwards, we did not observe bees provisioning nests or visiting flowers at any site. In January 2016, there was no activity of adult bees at the nests found in 2014.

Nest descriptions We found nests in bare vertical walls of sandy soil and mud in river banks shaded by vegetation, making nest entrances inconspicuous. All nests formed a tunnel, and their entrances

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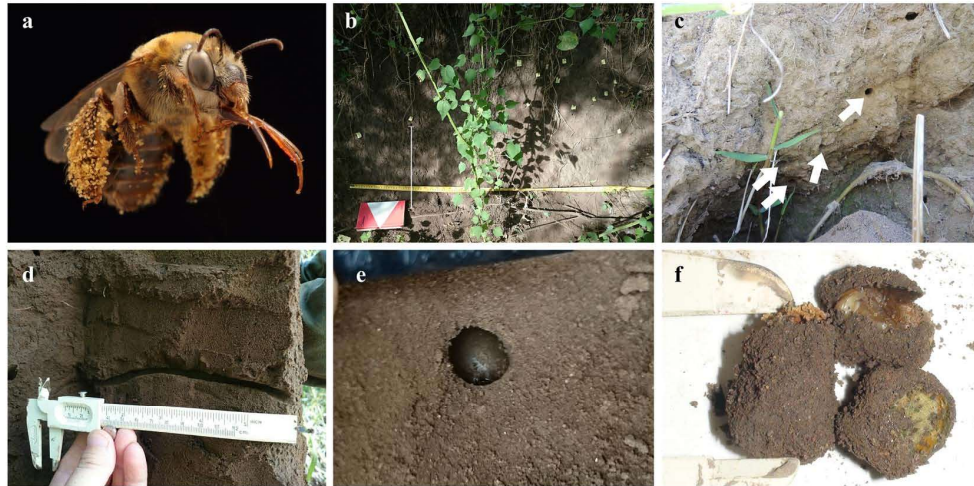


Figure 1. **a** *Peponapis crassidentata* female. **b** Nests on a river wall. Each nest is marked with a yellow piece of paper. **c** Nests on the roof of a cavity at a river wall; *white arrows* show the active nests. **d** Cross section of active nests. **e** Chamber with a waterproof and shiny lining. **f** Cells containing larvae of *P. crassidentata*.

were located horizontally on the walls (Figure 1b) and vertically on the roof of a cavity (Figure 1c). Beneath the entrance of active nests, we found loose soil and *Cucurbita* pollen. We excavated two nests in 2014, and no antechamber was observed. Tunnels wound and descended horizontally (Figure 1d); the tunnel substrate was of homogeneous, sandy, moist soil, and in the brood cells, the floor was also moist but more clayey. The tunnel walls were smooth. The brood cells were at 35–95 cm from the entrance. The cells were located vertically adjacent to the tunnel floor but separated by soil from the main

tunnel. The first nest was 120 cm long and contained 14 brood cells. The second nest was 95 cm long and contained only one cell. The interior walls of the brood cells were smooth and dark, with a waterproof and shiny lining (Figure 1e), similar to the cell walls of *P. pruinosa* (Mathewson 1968), *P. fervens* (Michener and Lange 1958), and *P. utahensis* (Rozen and Ayala 1987). The brood cells were ovoid chambers, each containing one larva with its respective pollen supply (Figure 1f) or one pupa. Due to a large number of nests, it was not possible to determine if all 14

Table I. Description of the three nesting sites of *Peponapis crassidentata*. Values for nest traits are mean \pm sd

Site	Geographical coordinates	Number of nests	Wall length \times height (m)	Distance (cm) from the nest entrance to the horizontal plane of the floor (white bar in 1b)	Nest diameter (cm)	Distance from the nearest crop (m)
1	19° 36' 11" N 105° 6' 15.1" W	164	4.2 \times 1.8	112.8 \pm 38.2	0.67 \pm 0.05	100
2	19° 35' 15" N 105° 6' 7.8" W	16	5 \times 0.9	46.1 \pm 11.5	0.68 \pm 0.04	20
3	19° 23' 19.2" N 104° 58' 15.4" W	8	9.5 \times 1.9	82.5 \pm 0.75	0.65 \pm 0.04	55

Nesting sites of *Peponapis crassidentata*

cells belonged to the same nest that was originally dug up. The cells of the first excavated nest contained an exarate pupa, six pupae, and seven larvae, the cell of the second nest contained one larva. We sampled pollen from excavated cells and confirmed under a microscope that all samples contained only *Cucurbita* pollen. This coincides with the observation, in the same period, of individuals of *P. crassidentata* foraging on *C. moschata* and *C. argyrosperma* in the area.

Discussion Previous reports on the nesting biology of *Peponapis* spp. contrast with some patterns observed here for *P. crassidentata*. While *P. pruinosa*, *P. utahensis*, and *P. fervens* nest on flat ground with vertical entrances located in proximity to their pollen sources (Mathewson 1968; Hurd et al. 1974; Rozen and Ayala 1987; Krug et al. 2010), *P. crassidentata* nests on vertical river banks, mainly with horizontal nest entrances. Another notable difference is that *P. pruinosa* and *P. fervens* nest in exposed and semi-exposed sites (Michener and Lange 1958; Mathewson 1968; Krug et al. 2010), whereas *P. utahensis* and *P. crassidentata* prefer shaded sites near riparian habitats. Such feature is possibly associated with temperature regulation of nests during the day. A shared characteristic by all *Peponapis* spp. studied is their gregarious nesting habit during the flowering period of nearby *Cucurbita* flowers.

By nesting on the walls of river banks, away from main crops, *P. crassidentata* may be less susceptible to destruction by plowing. In contrast, because *P. pruinosa* nests on flat ground, agricultural practices can affect population density by delaying the emergence of offspring (Ullmann et al. 2016; Shuler et al. 2005). Other disturbances such as fire, agrochemicals, and sand and gravel extraction are important threats to all *Peponapis* species, including *P. crassidentata*. Such knowledge must be conveyed to farmers, who are often unaware of the identity and biology of *Peponapis* species that are the most efficient pollinators of *Cucurbita* cultivars. This study will allow identification, protection, and perhaps creation of potential nesting sites of *P. crassidentata*.

Our study suggests that riparian habitats are essential for *P. crassidentata* nesting. Riparian habitats cover only 4% of the study area but provide resources for the maintenance of many other animal species, particularly during the dry season (Sanchez-Azofeifa et al. 2009). These areas are key for maintaining the biodiversity and ecosystem services of tropical dry forests and deserve special attention to ensure the integrity of pollination services, such as the important evolutionary interaction

of *Peponapis* spp. with squashes in Mesoamerica (Hurd et al. 1971).

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Note scientifique sur la première observation de sites de nidification de *Peponapis crassidentata* (Hymenoptera: Apidae)

Eine wissenschaftliche Anmerkung über die erste Beschreibung des Nistplatzes von *Peponapis crassidentata* (Hymenoptera: Apidae)

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9 Discusiones y conclusiones generales

El estudio de los servicios de polinización ha cobrado una gran importancia debido a una amenaza en las poblaciones de las abejas a nivel mundial y una crisis potencial de los servicios de polinización en cultivos. Los cultivos dependientes de polinizadores son los más susceptibles ante una disminución de polinizadores. En mi área de estudio documenté a 10 especies vegetales usadas en cultivos que dependen de polinizadores en algún grado. De estos cultivos, usé como modelo de estudio por su relevancia económica, cultural y biológica a tres especies; La sandía (*Citrullus lanatus*), el tomate de cascara (*Physalis ixocarpa*) y la calabaza de castilla (*Cucurbita moschata*). Estos tres cultivos son dependientes de polinizadores y la disminución de sus polinizadores se traduciría en una disminución de la productividad y en pérdidas económicas para los agricultores de la región, por lo que es de vital importancia conocer los procesos involucrados en el mantenimiento de los servicios de polinización. Entre estos procesos destaca el manejo y conservación de los bosques, como el bosque tropical seco (BTS), el cual cubre una importante superficie en México y ocurren una gran cantidad de interacciones biológicas, como la polinización.

El BTS es un ecosistema estacional, donde se distinguen dos grandes temporadas, época de lluvias y época seca. Cada época es diferente entre sí, e influye en la presencia de ciertas especies de polinizadores. Las abejas *Apis mellifera* son las principales visitantes de los cultivos durante la temporada seca, conformando la mayor parte de las visitas en las tres especies de cultivos muestreados. Sin embargo, en los experimentos de eficiencia mostré que *A. mellifera* no es un polinizador tan efectivo en los tres cultivos, y es posible que en conjunto con la comunidad de polinizadores se este manteniendo el servicio de polinización brindado a los cultivos dependientes de polinizadores durante la temporada seca. En contraste, durante la temporada lluviosa documente una interacción entre un polinizador especialista y una planta nativa del BTS, el polinizador especialista fue capaz de ser efectivo y de sostener el servicio de polinización durante esa temporada del BTS. Las abejas especialistas son pertenecientes al género *Peponapis* y son las principales responsables de la polinización en cultivos de calabaza de castilla (*Cucurbita moschata*) y la interacción posiblemente vaya más allá de la producción de frutos. Es posible que

Peponapis al presentar una actividad al inicio de la floración, logre mover granos de polen con una mayor viabilidad a comparación de las abejas que presentan su actividad horas más tarde. Se ha documentado que el polen de *Cucurbita* pierde viabilidad conforme la flor se vuelve senescente (Nepi and Pacini 1993; Agbagwa et al. 2007; Franchi et al. 2014), por lo que posiblemente el polen con una mayor viabilidad influya en una mejora en la progenie de las calabazas y un mayor rendimiento en la productividad de éstas. Procesos similares al de la interacción de *Peponapis* con *Cucurbita* posiblemente se encuentren ocurriendo en otros tipos de cultivos en los que las versiones silvestres florecen dentro de ciertas temporadas. Un ejemplo de este caso puede estar ocurriendo en los cultivos de tomate de cascara, en el cual se encuentran especies silvestres floreciendo durante la temporada lluviosa en el BTS y, además, se han documentado especies de abejas especialistas en *Physalis* (Sullivan 1984). Posiblemente las semillas obtenidas en la temporada lluviosa y visitadas por polinizadores asociados a *Physalis* sean de mejor calidad, y aun más, en nuestras observaciones en campo notamos que los agricultores evitan la siembra de semillas de *Physalis* provenientes de sus cultivos, debido a que el rendimiento de su parcela sería menor. Un estudio detallado de cada cultivo nos podrá proporcionar información de las mejores temporadas para sembrar ciertos cultivos en base a la presencia del polinizador ideal.

En el caso de sandía, este cultivo es originario del oriente de África, por lo que los polinizadores ideales se encuentran en dicha zona, sin embargo, otros polinizadores asociados a las especies silvestres del oriente pueden estar funcionando como polinizadores ideales (Stanghellini et al. 1998; Ali et al. 2015). En este estudio observé varias abejas nativas pertenecientes a la familia halictidae, abejas que también se han registrado como eficientes en ciertos estudios con sandía dentro de su área natural de distribución. En mi estudio no fue posible evaluar la influencia de estos polinizadores en la polinización de sandía debido a la baja densidad de abejas nativas en los cultivos muestreados. Posiblemente, bajo un manejo adecuado estas especies puedan aumentar sus visitas en las parcelas y logren contribuir de manera significativa a los servicios de polinización a comparación de las visitas de *Apis mellifera*, quien fue el principal visitante en los cultivos muestreados. Además, los cultivos de sandía en la región de estudio se encuentran ante un escenario de falta de polinización, debido a que el tratamiento de suplementación de polen

incremento el doble la probabilidad de obtener frutos a comparación de la probabilidad obtenida en el tratamiento de polinización abierta. Esto nos indica que la renta de colmenas de *A. mellifera* es insuficiente para cubrir la totalidad de los servicios de polinización, aunque los agricultores obtienen un rendimiento redituable, es posible incrementar este rendimiento bajo un esquema adecuado de uso y conservación de polinizadores.

La africanización de las abejas *Apis mellifera* permitió su establecimiento dentro de los agroecosistemas y bosques en México, además, *A. mellifera* es una especie introducida y generalista común en áreas bajo sucesión ecológica y estas abejas al ser abundantes son capaces de proveer de los servicios de polinización a las plantas alrededor del bosque tropical seco (Lobo et al. 1989; Quezada-Euán 2007; Lopezaraiza-Mikel et al. 2013). La baja eficiencia de esta abeja observada para los tres cultivos nos habla sobre un polinizador poco eficiente en escenarios de bajas visitas, sin embargo, su baja eficiencia es compensada con la gran abundancia durante la temporada seca, por lo que las abejas africanizadas dentro del bosque pueden estar llevando un papel importante en brindar el servicio de polinización a los cultivos dependientes de polinizadores dentro de la región de estudio, por lo que es importante el llevar a cabo un estudio para evaluar la contribución de las abejas africanizadas a los cultivos y el papel que juegan los diferentes tipos de hábitats en el establecimiento y persistencia de las poblaciones de abejas africanizadas.

En el análisis paisajístico de la temporada seca observé una correlación entre las abejas visitantes de *Physalis ixocarpa* y la cobertura al bosque para el año 2008, también observé que los nidos de *Peponapis crassidentata* se encuentran muy asociadas al bosque ripario del bosque tropical seco, por lo que estas zonas conservadas pueden estar funcionando como reservorios de las abejas *Peponapis*. Aunque las abejas *Peponapis* también anidan en sitios con perturbación, al ser abejas temporales, las mismas actividades humanas pueden comprometer los nidos donde se encuentran las generaciones de la siguiente temporada, por lo que bosques riparios conservados pueden estar funcionando como reservorios de polinizadores. Las abejas africanizadas también se encuentran beneficiándose de este tipo de bosques, y posiblemente esto ayude a su fuerte abundancia en los cultivos de la temporada seca. El Programa INVEST predijo que las áreas cercanas a la reserva de la biosfera Chamela-Cuixmala se presentan una mayor probabilidad de

anidación y servicio de polinización para los cultivos. Por lo que los bosques tropicales secos pueden estar funcionando como fuentes de polinizadores, al tener importantes zonas de anidamiento y recursos florales para muchas especies, incluyendo a la abeja introducida *Apis mellifera*.

La conservación de los bosques tropicales secos debe ser considerados dentro de las actividades agropecuarias, así como también la biología de las especies que actúan como polinizadores de cultivos, para así asegurar las poblaciones de polinizadores que contribuyen a la productividad de los cultivos. El correcto manejo y conservación de los bosques contribuirán en la preservación de los servicios de polinización a largo plazo, servicio no solo útil para los cultivos, sino que también útil para el mismo mantenimiento de los bosques.

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