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Biological Sciences

The Ecological and Economic Importance of Bat Pollination Services to Pitaya Production in Central Mexico

by

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Abstract

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Bats provide a range of ecosystem services, such as seed dispersal, pest suppression and pollination. Despite this, bats worldwide are commonly disregarded as pests and persecuted, necessitating research emphasising the benefits provided by bats to people. For example, bats are key pollinators in both natural and agricultural ecosystems, and pollinate many plants of socio-economic importance. This thesis aims to highlight the importance of ecosystem service provision by bats, by assessing the ecological and economic importance of bat pollination services to a major cash crop in central Mexico.

The role of bats as pollinators is largely undervalued. Data on the contribution of bats to food security and crop production is scarce, and there have been no assessments on the impact of bat pollination on crop quality. *Stenocereus queretaroensis* is a species of columnar cactus endemic to central Mexico that is cultivated commercially for its fleshy fruits, pitayas. I carried out exclusion experiments to assess the impact of bat pollination on fruit yield and quality relative to other pollinating taxa (i.e. birds and insects). I showed

that *Leptonycteris* bats are the principal pollinators of *S. queretaroensis*, enhancing both crop yield and quality. Fruit yield decreased by 35%, and fruits were 46% lighter, in the absence of bats (when pollination was carried out by birds and insects). I found that consideration of both crop yield and quality was essential therefore to fully understand the benefits of bat pollination, and that there would likely be severe socio-economic impacts on the pitaya production area if bat pollinator populations declined.

There have been no detailed assessments to date of the economic value of pollination services provided by bats to crops, and no disaggregated analysis of the distribution of these economic benefits between actors for pollination services of any kind. I conducted yield analysis to assess the market value of bat pollination services to pitaya production and showed that the gross annual income attributable to bats is approximately US$2,500 per hectare, through both enhanced fruit production and quality (size). I carried out value chain analysis to assess the distribution of benefits between actors in the pitaya commodity chain and showed that pitaya production provides a key seasonal income at a time of low agricultural activity, supporting livelihoods and household activities of the rural poor. However, profits are concentrated with privileged actors that have access to capital, land and markets. The high economic value of bat pollination services may be a powerful argument for conservation in the pitaya production area, but efforts at the community, government or NGO level are necessary for a fairer distribution of benefits among actors.

Finally, there is a lack of knowledge of the year-round diet and resource use of nectar-feeding bats in the pitaya production area, and previous studies of have relied on visual identification of pollen grains in faeces, limiting the taxonomic resolution of results. I used metabarcoding techniques to identify plant taxa present in the diet of *Leptonycteris yerbabuenae* in the pitaya production area and showed that *Stenocereus queretaroensis* is likely to be an important part of the diet during the flowering and fruiting season, highlighting the mutualistic relationship between crop and pollinator. I also found that a

diverse range of other plants found in tropical deciduous forest are consumed throughout the year, including when the availability of foraging resources within pitaya plantations was high. This indicates that the continued provision of bat pollination services to the pitaya crop necessitates landscape-scale conservation to maintain a high floristic diversity in the production area, for example by protecting remaining areas of tropical deciduous forest.

This thesis helps us to better understand the role that bats play in the pollination of a major crop, highlights the potential socio-economic consequences of declines in bat populations, and points to conservation actions to maintain pollination service provision.

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I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
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Chapter 1 General Introduction

1.1 Pollination

Pollination is the process of fertilisation whereby vectors such as wind, water and animals transfer pollen grains between the male and female parts of flowers (Potts et al. 2016b). Pollination is crucial for the production of fruits and seeds in flowering plants; though level of dependence varies between plants with different mating systems (Potts et al. 2016a). Nearly 90% of the world's 350,000 species of flowering plants are pollinated by animals, rising from an average of 78% of species in temperate regions to 94% in tropical communities (Ollerton et al. 2011).

Pollinators and animal-pollinated plants provide many benefits to humans. They play fundamental roles in ecosystems, maintaining diverse plant populations and underpinning ecosystem functioning (Potts et al. 2016). Pollinators and animal-pollinated plants also have a high cultural value, inspiring art, music, literature, religion, and design and technology; and provide aesthetic pleasure and recreational value (Hanley et al. 2015; Potts et al. 2016b). Many animal-pollinated plants are utilised for animal feed, building and other materials, and medicines (Potts et al. 2010), and form a crucial part of food security and crop production, the focus of much of the recent pollination services literature.

Nearly three-quarters of leading global crops show increases in size or quality of harvests when pollinated by animals (Klein et al. 2007). For example, crops such as coffee (Ricketts et al. 2004; Classen and Peters, 2014), strawberries (Klatt et al. 2014) and apples (Garratt et al. 2014) have all been shown to increase in quality when animal-pollinated, increasing in weight and showing fewer malformations. However, the degree of dependence of the harvest yield of animal-pollinated crops on pollinators varies from complete dependence – vanilla, for example, relies entirely on bees and hummingbirds for pollination and must be hand-pollinated in their absence – to a small reduction in reproductive success, such as is seen in tomatoes and chili peppers (Klein et al. 2007).

Though most of the world's staple crops are wind-pollinated — 60% by volume of the world's food production does not require animal pollination — the crops richest in micronutrients are often the most dependent on animals for pollination (Eilers et al. 2011). Vitamins A and C, calcium, fluoride and folic acid are all principally found in pollinator dependent crops, with the majority of fruits, seeds and nuts reliant on animals for pollination (Potts et al. 2016a). Therefore, declines in pollinators and animal-pollinated crops are likely to result in significant health issues worldwide (Smith et al. 2015).

In addition, many animal-pollinated crops have a high economic value, with many livelihoods globally dependent upon pollinators and their products. Pollinator-dependent crops have a production value per ton over four times higher than that of crops that are not reliant on animal-pollinators, with the economic value of pollination worldwide estimated to be €153 billion in 2005, 9.5% of the value of the total world food production (Gallai et al. 2009). Many cash crops grown almost exclusively in low- and middle-income countries, including important export goods such as coffee, oil palm, brazil nuts and cocoa, depend on animal pollinators, and provide employment and income for millions of people (Aizen et al. 2008; Potts et al. 2016a). Global agriculture has become increasingly dependent on pollinators in recent decades, with a disproportionate increase in the area of land cultivated with pollinator-dependent crops since 1961 compared to non-pollinator dependent ones (Aizen et al. 2008). Over 30% of cropland was given over to pollinator-dependent crops in 2006 (Aizen et al. 2008).

Insects, especially bees, are the most common animal pollinators, and the majority of literature to date has focussed on the ecological and economic importance of bee pollination (Potts et al. 2016b). However, vertebrates such as bats, birds, reptiles and rodents also play an important, though overlooked role, particularly in tropical and subtropical regions (Ratto et al. 2018). Bird pollination is more common than bat pollination, occurring in nearly 500 genera of plants, with at least six families of birds adapted for nectar-feeding (Fleming et al. 2009). Bats however pollinate a small but ecologically and economically important group of plants in tropical and desert habitats, and make an important contribution to ecosystem health, crop production and food security (Fleming et al. 2009). The benefits of vertebrate pollination can be substantial, particularly in environments where climatic conditions limit invertebrate activity, such as the ability of vertebrates to carry large loads of pollen long distances (Fleming et al. 2009). Vertebrate-pollinated plants show a strong dependence on their pollinators, with a reduced fruit and seed set of 63% on average when vertebrate pollinators are excluded from flowers, and bat-pollinated plants exhibit the highest dependence of all (Ratto et al. 2018).

1.2 Bat pollination

1.2.1 Chiropterophily

Chiropterophily is a pollination syndrome whereby plants are reliant on bats for pollination. Both plants and bats benefit from this mutualistic interaction, with plants providing a nutritional reward in return for pollen dispersal. Bats have been documented to be partly- or wholly- responsible for the pollination of around 528 species of plants worldwide, classified within 67 families of 28 orders (Fleming et al. 2009), but it is estimated that up to 1,000 species of plants in the New and Old World tropics are dependent on bats for pollination (Lobova et al. 2009).

Chiropterophilous flowers have a variety of forms, designed to optimise pollen transfer by bats. These include brush-like with multiple stamens that will cover a bat’s face with pollen, such as in the Bombacoidea subfamily of the Malvaceae family; or bowl-shaped, such as the flowers of the balsa tree (Malvaceae) (Tschapka and Dressler, 2002). Typical traits of bat-pollinated flowers include: nocturnal anthesis; wide flower openings; an accessible protruding, robust body; white or pale colouration; a musty odour; and the production of large amounts of pollen and a high volume of nectar at a high concentration of hexose (Von Helversen and Winter, 2003; Muchhala and Thomson, 2010). Pollen of flowers pollinated by bats has been found to contain a higher protein content than pollen from closely related flowers pollinated by other taxa, including high levels of amino acids particularly useful for maintaining collagenous tissues such as those found in the wing and tail membranes (Howell, 1974). It is more costly for plants to attract and reward bats as pollinators rather than insects, due to their larger size and greater energy requirements resulting from their endothermic metabolisms and energetically-costly hovering style of flight (Voigt and Speakman, 2007; Fleming et al. 2009). However, bats are highly effective pollinators (see Section 1.2.2) and the benefits of bat pollination are substantial despite the high costs (Fleming et al. 2009).

Around 53 species of bats worldwide are specialised nectar-feeders, with 38 found in the Neotropics and 15 in the Old World (Kunz et al. 2011). These bats are found within three families: the Pteropodidae in the Old World, the Phyllostomidae in the Neotropics and the Mystacinidae in New Zealand (Kunz et al. 2011). Specialised nectar-feeding bats have evolved adaptions to enable them to feed from flowers, such as elongated rostrums and long tongues, some covered in special filamentous papillae that become engorged with blood during feeding, increasing the surface area of the tongue and trapping nectar to carry it to the mouth (Tschapka and Dressler, 2002; Harper et al. 2013). Morphological adaptations of specialised nectar-feeding bats, such as smaller size and faster flight, aid the ability to find and exploit low density, scattered nectar resources (Tschapka, 2004). Glossophaginae bats (family: Phyllostomidae) have an excellent spatial memory, and use local spatial echo-location cues to identify and remember the placement of flowers with a profitable nectar content (Von Helversen and Winter, 2003). Glossophaginae bats are also able to use information acquired from other individuals to locate known floral resources and lessen foraging time, likely by eavesdropping auditory cues used by other bats as well as by visual observations (Rose et al. 2016).

Communities of nectar-feeding bats can vary seasonally, with additional species feeding on nectar in times of high resource availability (Tschapka & Dressler, 2002). Several species of frugivorous bats in the Phyllostomidae will feed seasonally on nectar, pollen, fruit and insects, depending on which resources are available at different times of the year (Lobova et al. 2009). Bat species from typically insectivorous families (e.g. Vespertilionidae) may also be opportunistic pollen and nectar feeders, such as the pallid bat *Antrozous pallidus* in Mexico, which more usually consumes large arthropods such as scorpions and crickets, but has been found to be a more effective pollinator of a columnar cactus than a specialised species of nectar-feeding bat (Frick et al. 2013).

1.2.2 Advantages of bat pollination

Bats can efficiently transfer large pollen loads in comparison to other pollinating taxa. Bat fur can take up and hold more pollen grains than feathers, which lose pollen grains easily (Muchhala and Thomson, 2010), while longer-distance insect pollinators typically deposit less pollen due to grooming behaviour and abrasion causing pollen loss between plants (Liu et al. 2015). The deposition of large amounts of pollen on stigmas is advantageous to the plant because it provides sufficient pollen to fertilise all the ovules of a flower, promoting competition amongst pollen for access to ovules (Fleming et al. 2009).

In additional to depositing large pollen loads on stigmas, bat pollination is advantageous because the foraging behaviour of bats promotes the deposition of pollen of multiple genotypes from multiple paternal plants, increasing the genetic diversity of progeny (Fleming et al. 2009). Bats can also carry pollen from other species, and different flower morphologies control the placement of pollen on specific parts of the bat to limit interspecific pollen transfer (Stewart and Dudash, 2016). This specialist pollination syndrome can result in a more efficient transferral of pollen between flowers, resulting in less wastage of nectar rewards and less risk of hybridisation with related species (Von Helversen and Winter, 2003).

As well as depositing large amounts of pollen from multiple genotypes, bats can transfer pollen over considerable distances between plants. Bats are very mobile and can travel long distances, even over disturbed or open areas, unlike other pollinating taxa such as birds and insects (Avila-Cabadilla et al. 2012; Medellin et al. 2018). For example, lesser long-nosed bats *Leptonycteris yerbabuenae* fly up to 100 km a night to forage in the Sonoran desert (Fleming and Holland, 2018; Medellin et al. 2018), and the tailed tailless bat *Anoura caudifer*, which lives in tropical forests in South America, will fly up to 60 km a night (Von Helversen and Winter, 2003). Bats therefore are effective long-distance pollen dispersers, ensuring gene dispersal between genetically distinct individuals for the maintenance of genetic diversity at a population level and between isolated fragments (e.g. Horner et al. 1998; Law and Lean, 1999; Nassar et al. 2003; Ghazoul, 2005b; García-Morales et al. 2013; Aguiar et al. 2014; Bustamente et al. 2016). Genetic isolation of plants within habitat fragments and the subsequent loss of diversity through pollen limitation is a major threat to the long-term viability of plant populations (Law and Lean, 1999; Aguiar et al. 2014). Thus, long-distance pollen dispersers may be particularly important for plants which exist at low densities, naturally or as a result of anthropogenic disturbance. Many bat-pollinated plants occur at low densities, such as canopy trees in the Bombacoidae (Malvaceae), and columnar cacti (Cactaceae) and agaves (Agavaceae) in arid environments. In the genus *Durio* in south-east Asia, species in the subgenus *Boscia* are abundant and pollinated by insects, whereas those in the subgenus *Durio* are rare and pollinated by bats (Fleming et al. 2009).

Though data on pollination services to crops by nectarivorous bats remain scarce (Williams-Guillén et al. 2016), the role of bats as pollinators has been established for some tropical crops such as durian *Durio zibethinus*, bitter beans *Parkia* spp., and fleshy fruits of columnar (e.g. *Stenocereus* spp.) and vine (e.g. *Hylocereus* spp.) cacti (e.g. Ibarra-Cerdeña et al. 2005; Arias-Cóyotl et al. 2006; Valiente-Banuet et al. 2007; Bumrungsri et al. 2008, 2009). Durian is economically and culturally important throughout south-east Asia, with an export value of up to US$255 million in 2013 (Sheherazade et al. 2019). Durian is self-incompatible, with fruit bats and nectar bats shown to be the primary pollinators, enhancing fruit production (Bumrungsri et al. 2009; Aziz et al. 2017a; Sheherazade et al. 2019). However, despite literature demonstrating the importance of insect pollinators for crop quality and subsequent impact on economic value (Bartomeus et al. 2014; Garratt et al. 2014; Klatt et al. 2014), no studies thus far have examined the effect of bat pollination on the quality of any crop.

Bat pollination also plays a crucial role in maintaining genetic diversity in wild relatives of crops that are often propagated vegetatively, such as banana *Musa* spp. and *Agave* spp., which is critical for long-term food security (Hassan et al. 2005; Hopkins and Maxted, 2011; Williams-Guillén et al. 2016). Mezcal is a generic name for distilled alcoholic beverages which have been produced from over 39 species of agaves in Mexico for thousands of years (Zizumbo-Villarreal et al. 2013). Two of these *Agave* species are widely cultivated (*A. tequilana* and *A. angustifolia*), but the remainder are found in a variety of contexts, from wholly wild collection to mixed agroecosystems and are important to rural economies (Aguirre-Dugua and Eguiarte, 2013). Plants are harvested before they reproduce, leading to concerns of over-harvesting of wild populations and genetic bottlenecks. For the long-term sustainability of the industry, collection practices are recommended that ensure a proportion of plants are left to flower to be pollinated by bats, maintaining the genetic diversity of wild populations (Aguirre-Dugua and Eguiarte, 2013). Increased genetic heterogeneity within crops can decrease vulnerability to disease (Zhu et al. 2000).

1.3 Socio-economic consequences of losing bat pollination services

There has been much widespread concern in recent years about declining insect pollinator populations, which have been decreasing in abundance, occurrence and diversity at local and regional scales in Europe and North America (Potts et al. 2016b). Global assessments for vertebrate pollinators indicate that bird and mammal pollinator populations are also declining, with 16.5% of vertebrate pollinator species threatened with extinction (Potts et al. 2016a), and an average of 2.5 bird and mammal pollinator species per year moving one Red List category towards extinction in recent decades (Regan et al. 2015).

However, the population status of most bat species is much less well known than that of birds and other mammals. Over half of all bat species have unknown population trends, 18% are classed as Data Deficient, and 15% are considered threatened; meaning that 80% (988 species) require either conservation or research attention (Frick et al. 2019). Threats to bats include: deforestation and forest loss; agriculture (conversion of land and use of pesticides); urban development; energy production (e.g. collisions with wind turbines) and mining; climate change; invasive species such as the fatal fungal pathogen that causes white nose syndrome in North America; and hunting and disturbance (Frick et al. 2019).

Additionally, bats worldwide have historically been objects of superstition and fear, for many cultural, symbolic and religious reasons, and persecution is widespread (Kingston, 2016). Bats are intentionally killed for many reasons, including feared zoonotic disease transmission, conflict between bats and farmers, and to remove bats living in human structures (Frick et al. 2019). In Latin America, bat colonies and roosts are frequently destroyed to attempt to control sanguivorous vampire bats (*Desmodus rotundus*) which can cause substantial economic damage by transmitting bovine paralytic rabies (Williams-Guillén et al. 2016). Increased awareness of ecosystem service provision by bats could help conservation efforts, with the economic assessment of pollination services providing one such mechanism (see section 1.4).

A deterioration in pollination services would negatively impact human well-being. The resulting decline in yield and quality of pollinator-dependent crops would result in substantial revenue loss (Gallai et al. 2009) and poorer nutrition and health, particularly for populations already suffering from malnutrition (Chaplin-Kramer et al. 2014; Smith et al. 2015). It would also intensify demand for agricultural land, with increases in land cultivated for pollinator-dependent crops required to compensate for lower yields (Aizen et al. 2008; Garibaldi et al. 2011). Furthermore, loss of pollination services would negatively impact not only on food provision, but ultimately all ecosystem services that rely on plant diversity, such as nutrient cycling, and air and water purification (Ashworth et al. 2009).

Some social groups can be disproportionately affected by the loss of pollination services. Poor rural communities can be particularly reliant on pollination services for their livelihoods, and have a lower ability to cope with declines in pollination service provision (Hassan et al. 2005; TEEB, 2010). Subsistence or small-holder agriculture comprises 83% of the global agricultural population, predominantly located in lower income countries (Morton, 2007). These farming populations are the most vulnerable to declines in pollination services, lacking the ability to diversify if agricultural production fails due to social and economic barriers (Morton, 2007; Potts et al. 2016a). Despite this, smallholder agriculture has been largely neglected in pollinator research (Potts et al. 2016a).

Around 60% of cultivated plants experience pollen limitation, similar to the proportion of wild plants, indicating that many crops are vulnerable to declines in pollinator populations (Aizen et al. 2008). The term ‘pollen limitation’ describes a situation whereby either an inadequate amount of pollen (owing to too few visits by pollinating taxa, or too few pollen grains deposited per visit), or incompatible pollen (either interspecific, or from the same individual) is deposited on the stigma, resulting in decreased reproductive success (Ashman et al. 2004). Pollen limitation found in cultivated plants is most likely to occur in self-incompatible, animal-pollinated fruit crops (Bos et al. 2007).

Most wild plant species documented to have experienced reproductive decline due to loss of pollinators are vertebrate pollinated (Ghazoul, 2005). Plants with specialist pollination systems, especially those on islands or isolated ecosystems, depend on a reliable population of pollinators and are particularly vulnerable to pollinator decline (Fleming et al. 2001). Chiropterophilous plants are specialised in a greater degree compared to other zoophilous plants, with reproductive success 83% lower on average when bats are excluded from the flower for bat-pollinated plants relative to a decrease of 46% lower when birds are excluded from bird-pollinated plants (Ratto et al. 2018).

1.4 Economic value of bat pollination

1.4.1 Valuing pollination services

Economics is the study of the choices people make when faced with scarcity of resources or time, and the implications of these choices on society (Fisher et al. 2014). People constantly assess situations using some form of cost-benefit analysis, informal or formal, and make decisions accordingly, including those relating to the environment (Gómez-Baggethun et al. 2010). However, there is a lack of understanding of societal dependence upon biodiversity and associated ecosystem functions and services, perhaps a result of their sometimes intangible nature, which has led to environmental problems such as pollution, climate change and biodiversity loss (Vassallo et al. 2017).

There is a need to properly account for the true value to society of biodiversity and its associated ecosystem services, incorporating many dimensions such as ecological and socio-cultural as well as economic (De Groot et al. 2012). In the short term however, the expression of ecosystem service benefits in monetary terms can be an effective tool to communicate the value of ecosystem services and the benefits that we receive from nature to a diverse range of people that have decision-making powers (De Groot et al. 2010, 2012; Gómez-Baggethun et al. 2010; Breeze et al. 2016). This can be a useful way to raise awareness of the potential socio-economic impacts of declines in service provision, create economic incentives for conservation, and promote strategies to become more environmentally responsible; for example by leading to recommendations of sustainable agricultural practices or land-use policies (Breeze et al. 2016; Obst et al. 2018).

The monetisation of ecosystem services is a complex and challenging issue however, particularly where services are intangible and cannot be valued through existing markets, and raises issues of how humans relate to nature (Gómez-Baggethun et al. 2010; Adams, 2014). The oversimplification and commodification of ecosystem services that implicitly takes place within the monetisation of ecosystem services have been widely criticised, with concerns of detrimental effects in the long term for biodiversity conservation and equity of access to ecosystem service benefits (Gómez-Baggethun and Ruiz-Pérez, 2011). However, in some cases, there is already an economic value to ecosystem services that fits within existing markets, such as the direct contribution of pollinators to the production and quality of commercial crops (TEEB, 2010; Hanley et al. 2015).

The worth of a pollination service to a crop is dependent on the current value of the crop in the market, and on the level of dependency of the crop on pollinators (Hanley et al. 2015). There are many sources of difficulty and uncertainty within these parameters however (Melathopoulos et al. 2015; Potts et al. 2016):

i. estimating the actual dependency of crops on pollinator activity at different spatial scales, i.e. the increase in yield and quality when pollinators have access to the crop compared to when they are excluded, requires resource-intensive empirical field data collection, and so is often poorly understood;
ii. differences between cultivars are rarely examined despite sometimes exhibiting different mating systems;
iii. prices and markets fluctuate spatially and temporally, and are influenced by consumers, farmers, technical innovation and government policy;
iv. the proportion of pollinators that are wild rather than managed is often unknown.

Most existing economic valuations of pollination services have focussed on insect pollinators, primarily honeybees (Gallai et al. 2009; Winfree et al. 2011; Garratt et al. 2014; Klatt et al. 2014; Hanley et al. 2015). Additionally, most economic valuations of pollination services have been either global or based on case studies in higher income countries, with very few detailed studies in the global south (Potts et al. 2016a). However, many bat-pollinated plants in the tropics are extremely economically important, such as durian, wild bananas (*Musa spp*), agave and balsa (*Ochroma pyramidale*) (Kunz et al. 2011).

1.4.2 Methods for the valuation of pollination services

There are various possible methods to quantify the monetary value of pollination services, each with particular strengths and weaknesses (Hanley et al. 2015). The crop price method and managed pollinator prices method simply estimate the total market price of pollinator-dependent crops or managed pollination services respectively, with minimal data requirements but presenting the value of pollination services poorly, only reflecting the market price of crops or non-wild pollination services (Hanley et al. 2015) without accounting for all the parameters listed directly above.

The replacement cost method estimates the cost of replacing wild pollination services with managed pollinators or technology (Hanley et al. 2015). For example, declines in pollinators of apples (*Malus domestica*) in south-west China have forced orchard owners to recruit ‘human pollinators’, which is both difficult (apple blossoms must be pollinated within five days) and expensive (Partap and Ya, 2012). Some commercial durian farmers in south-east Asia are forced to hand-pollinate due to a lack of bat pollinators, a dangerous and time-consuming task owing to the large size of the trees (Aziz et al. 2017). However, the replacement cost method assumes that there are replacements available, and is not linked to benefits (Hanley et al. 2015).

Various complex modelling methods also exist to estimate the impact on both producer and consumer welfare in pollinator-loss scenarios, such as partial and generalised equilibrium models. However, these require a huge amount of detailed and accurate information on pollination benefits and are very difficult to estimate and analyse (Hanley et al. 2015).

Simplified production function approaches (that do not account for the impacts of other factors on crop production) measure the market price of additional crop production resulting from pollination services, either through yield analysis or dependence ratios, and are particularly useful for valuing services that support economic activity such as crop production (Potts et al. 2016). Yield analyses can directly capture impacts of pollinators on crop production and quality, and support more precise local decision-making; but are resource intensive, requiring detailed empirical data, are only appropriate at a local scale, and only estimate benefits to the producer (Hanley et al. 2015; Potts et al. 2016).

1.4.3 Distribution of benefits

Fair distribution of resources among groups and individuals is an important concept in economics (Farley, 2012). World economic inequality has increased in recent decades and poverty remains widespread in large swathes of the world, particularly in rural regions (Tirado von der Pahlen et al. 2018). Benefits from ecosystem services are not distributed evenly among individuals, social groups, or countries (Hassan et al. 2005; Daw et al. 2011; McDermott et al. 2013; Keane, 2016), and are dependent on many socio-economic factors, such as land rights, opportunity costs of labour and land, and access to markets (Shackleton et al. 2008). Economic analysis of pollination services commonly aggregate the value into one estimate for a total population or area. However, aggregating benefits received from ecosystem services disregards distributional inequality. Some groups may benefit from an ecosystem service while others do not, with dynamic access mechanisms determining 'winners' and 'losers', and the individual needs of each actor determining how this affects well-being (Daw et al. 2011). Secondly, such aggregated analyses can obscure informal, cash-based livelihoods (Daw et al. 2011). In the case of pollination services, especially in low-income areas, assessments of benefit distribution should include both equity of access to the service, as well as the distribution of benefits received.

1.4.4 Valuations of other ecosystem services provided by bats

While research on the valuation of bat pollination services remains scarce, there have been several studies in recent years that have assessed the value of crop pest suppression services provided by insectivorous bats to agricultural systems, which also benefit farmers by increasing crop yield and lessening expenditure on inputs such as pesticides (Taylor et al. 2018). Bats have been documented to consume pests of crops such as corn (Maine and Boyles, 2015; Whitby et al. 2020), grapevines (Baroja et al. 2019; Rodríguez-San Pedro et al. 2020), rice (Puig-Monserrat et al. 2015; Srilopan et al. 2018; Kemp et al. 2019), pecan nuts (Brown et al. 2015), cotton (Cleveland et al. 2006; Lopez-Hoffman et al. 2014; Kolkert et al. 2019), macadamia nuts (Taylor et al. 2018; Weier et al. 2019) and cacao (Maas et al. 2013). The value of crop pest suppression has been estimated in several ways, most commonly based on avoided cost models or exclusion experiments. Avoided-cost models use existing data (on bat diet and population, crop pest ecology, and crop market values), to estimate both the direct value of crop yield that would be lost to pests in the absence of bat predators as well as the indirect reduced expenditure on pesticides (e.g. Cleveland et al. 2006; Federico et al. 2008; Boyles et al. 2011; Lopez-Hoffman et al. 2014; Wanger et al. 2014; Puig-Monserrat et al. 2015; Taylor et al. 2018). Exclusion experiments measure the community-level impact of removing bat predators on crop yield and/or quality and use market prices to quantify the value of bat-mediated pest suppression (e.g. Maine and Boyles, 2015; Maas et al. 2013; Rodríguez-San Pedro et al. 2020). Estimates of the value of crop pest suppression services provided by bats range from US$0 per hectare (for cacao, Indonesia, and coffee, Costa Rica; Taylor et al. 2018) to US S$188-$248 per hectare (grapes, Chile; Rodríguez-San Pedro et al. 2020). The combined worth of bat- and bird-mediated pest suppression to Indonesian cacao agroforestry was found to be US$730 per hectare per year (Maas et al. 2013).

Bats also provide several other ecosystem services. Bats not only consume invertebrates that negatively impact crop production, but also those that threaten human health, such as mosquitoes (Wray et al. 2018; Puig-Monserrat et al. 2020). Insectivorous bat colonies can also produce large amounts of guano, which is rich in nitrogen and phosphates from the undigested remains of insects (Furey and Racey, 2016). Guano is an excellent fertiliser, and has a high economic value (Kunz et al. 2011). The sale of guano is commonplace in south-east Asia and is an important part of many local economies in the region (Furey and Racey, 2016). Frugivorous bats play a fundamental role in seed dispersal and recruitment, and forest regeneration processes (Kunz et al. 2011). Bats are also a source of bushmeat in some parts of the world, with a high protein content (Kunz et al. 2011). However, to my knowledge, there have thus far been no attempts to quantify the value of any of these services, except anecdotal reports of market prices of bushmeat and guano, or descriptions of the dispersal mechanisms of timber species (Hammond et al. 1996; Kunz et al. 2011).

1.5 Study system

Although around one-third of all angiosperm families have species that exhibit nocturnal pollination syndromes, night-blooming flowers are most often found in xerophytic families such as the Cactaceae, suggesting that it is often a response to a scarcity of water in the environment to avoid the increased water loss through evapotranspiration associated with daytime flowering (Borges et al. 2016). Mexico has the highest diversity of cacti in the world with around 850 species, of which at least 170 are columnar cacti (Casas and Barbera, 2002; Munguía-Rosas et al. 2009).

Columnar cacti play a keystone role to both animals and humans. They provide nutrients, water and structural resources for a diverse array of animal species (Rocha et al. 2006; Kunz et al. 2011; Frick et al. 2014), and fruits, fuels, materials and a strong cultural identity to humans (Casas et al. 1999). Columnar cacti (sub-family Cactoidae) are a dominant vegetation in tropical deciduous and thorn scrub forests in arid and semi-arid zones, as well as sub-humid tropics, which combined cover nearly two-thirds of Mexico (Casas and Barbera, 2002). Humans have lived in arid zones of Mexico for nearly 12,000 years, with over half of indigenous peoples inhabiting in these areas (Pérez-Negrón et al. 2014).

Nearly all species of Mexican columnar cacti are self-incompatible and rely on animal pollinators, with 72% of the 70 species found in Mexico displaying a chiropterophilous syndrome (Valiente-Banuet et al. 1996). Bats have been shown to be the primary pollinators of many species of columnar cacti throughout Latin America (Fleming et al.

1996; Valiente-Banuet et al. 1996, 1997; Nassar et al. 1997; Ibarra-Cerdeña et al. 2005). There are 12 species of bats that feed on columnar cacti in Mexico (tribe: Glossophagini), mostly associated with tropical and subtropical dry areas (Arita & Santos-del-Prado, 1999). The distribution of species diversity of nectarivorous bats shows a clear correlation with that of columnar cacti in Mexico, with both occurring in the highest numbers in south-central Mexico (Valiente-Banuet et al. 1996).

L. yerbabuenae is a migratory species of nectar-feeding bat distributed from the south-west U.S.A to Honduras and El Salvador at the southerly extreme (Cole and Wilson, 1996). Migratory populations follow ‘nectar corridors’ from central Mexico to the southern U.S.A: one up the western coast, arriving in the spring to south-western Arizona and coastal Sonora; and the other following the Sierra Madre foothills, arriving later in the summer to south-eastern Arizona and New Mexico (Cole and Wilson, 1996; Frick et al. 2014). There are however also non-migratory populations, with year-round resident populations of *L. yerbabuenae* present in south-central Mexico and coastal Jalisco (Valiente-Banuet, 2002). The species is documented to be an important pollinator and seed disperser of many species of Cactaceae and Agavaceae throughout its range (Arizmendi et al. 2002; Stoner et al. 2003; Rojas-Martínez et al. 2012; Frick et al. 2013), including members of the *Stenocereus* genus (e.g. Ibarra-Cerdeña et al. 2005; Arias-Cóyotl et al. 2006).

The 22 species of arborescent columnar cacti in the *Stenocereus* genus have been used by local Mexican communities for thousands of years, as animal fodder, medicines, rubber, soap, living fences, firewood, and fleshy fruits (Bárcenas and Jiménez, 2010). Several species have been domesticated and are now cultivated, the most important of which is *Stenocereus queretaroensis*, a species endemic to west-central Mexico (Pimienta-Barrios and Nobel, 1994). Wild populations grow on shallow, rocky soils at elevations of 1300 –

1600 m, while cultivated populations are found at slightly lower altitudes (Pimienta-Barrios and Nobel, 1994).

Home garden cultivation of *S. queretaroensis* has occurred since the late 1800s, with the cactus brought under systematic commercial cultivation towards the end of the 19th century (Pimienta-Barrios and Nobel, 1994), for the production of its fleshy fruits ‘pitayas’. The most important pitaya production area is the Sayula Basin, Jalisco, approximately 80 km south-west of Guadalajara (Pimienta-Barrios and Nobel, 1994). The pitaya is an economically attractive crop, requiring a low input of water, fertilisers or pesticides, and with cactus plants producing fruit for over 100 years (Pimienta-Barrios and Nobel, 1994; Pimienta-Barrios, 1999b). Additionally, the tolerance of *S. queretaroensis* to drought and poor soils makes it a sustainable crop in the arid production area (Pimienta-Barrios and Nobel, 1994). There are various recognised commercial cultivars of *S. queretaroensis,* mostly classified for the colour of the fruit pulp (Pimienta-Barrios and Nobel, 1994). Domesticated cacti have been selected for the production of bigger, sweeter fruits; cv. Mamey is the most popular, accounting for up to 80% of fruit production (Pimienta-Barrios and Nobel, 1994).

I carried out my research in the municipality of Techaluta de Montenegro, Jalisco, Mexico, located in the semiarid Sayula Basin (Fig. 1.1). The pitaya is the most valuable crop grown in Techaluta de Montenegro, generating approximately Mx$19,200 per tonne, and registered pitaya production is increasing yearly (SIAP, 2018). The pitaya generates a high profit margin, due to low production costs (Pimienta-Barrios and Nobel, 1994), and provides one of the primary employment opportunities in an area with a high emigration rate resulting from a lack of jobs and low returns on other agricultural products (‘Plan de desarrollo municipal’, 2018).

1.6 Diet and bat conservation

The seasonal ecology of nectar-feeding bat populations is closely linked with the availability of foraging resources, which are heavily impacted by changes in climate and land-use (Burke et al. 2019, Frick et al. 2018, Frick et al. 2019). Climate change is projected to substantially reduce areas of suitable habitat for food plants of nectar-feeding bats in Mexico (Gomez-Ruiz and Lacher, 2019), while agricultural activities put increasing pressure on remaining habitat (Mendoza-Ponce et al. 2019).

The diet of *L. yerbabuenae* bats differs throughout their range, based on temporal and spatial availability of food plants. Northern populations specialise in feeding from cacti and agaves, while more southerly populations rely more heavily on non-succulent plant taxa such as bat-pollinated trees found in tropical deciduous forest (Fleming and da Silva, 1993; Stoner et al. 2003; Ober et al. 2005; Edwards et al. 2018). Understanding ecological differences of animals across their range, such as resource use, is a crucial part of effective conservation strategies (Frick et al. 2018), to inform landscape and habitat management. Though bats are highly mobile, they are constrained by available roosting sites (Ober et al. 2005). *L. yerbabuenae* bats have been found to have a similar home-range and habitat use between years of differing food availability, suggesting that reductions in foraging habitat will increase the energy demands for bats that then have to spend longer foraging for fewer floral resources (Ober et al. 2005). Protection of cave roosts is a priority conservation measure for *L. yerbabuenae* but it is also necessary to protect foraging resources within range of cave roosts (Medellín, 2016).

1.7 Thesis structure and research objectives

The primary goal of this thesis is to conduct research to highlight the importance of ecosystem service provision by bats, historically disregarded as pests in many parts of the world, and thereby promote efforts to conserve them. To do this, I assess the ecological and economic importance of bat pollination services to a major cash crop in central Mexico, and derive conservation recommendations for the principal species of pollinating bat. A framework is presented to outline the issue, aims, objectives and main analytical methods used (Table 1.1). This Ph.D. thesis consists of three research chapters, presented as three independent research articles, to address the following knowledge gaps:

First, though bats are important pollinators of many economically important plants in the tropics, data on their contribution to crop yield is scarce, and there have been no assessments on the impact of bat pollination on crop quality. Fleshy fruits (pitayas) from the columnar cactus *S. queretaroensis* are an important cash crop in central Mexico. In Chapter 2, I quantify the impact of bat pollination on pitaya crop yield and quality in one of the most important pitaya production areas, the Sayula Basin, Jalisco (Fig. 1.1). I carried out exclusion experiments to collect empirical data on the effect of different pollinator taxa (bats, birds and insects) on pitaya yield (fruit set) and quality (fruit weight and seed set). Camera traps were used to determine principal pollinating species and visitation rates of bats and birds to pitaya flowers. Mixed effect models were used to estimate the change in pitaya yield and quality in the absence of bat pollinators.

Second, there have been no detailed assessments to date of the economic value of pollination services provided by bats to crops, and no disaggregated analysis of the distribution of these economic benefits between actors for pollination services of any kind. In Chapter 3, I quantify the economic value of bat pollination services to pitaya production, and assessed how these benefits were distributed between actors. I used yield analysis to estimate the market value of increased fruit yield and quality with bat pollination, combining empirical data on changes in fruit set and quality collected in Chapter 2, with pitaya production and marketing data collected through interviews with pitaya farmers. I used value chain analysis to assess how the economic benefits received from bat pollination were distributed between actor groups, using data on income and profits collected through interviews with representatives from all actor groups in the pitaya value chain.

Finally, there is a lack of knowledge of the year-round diet and resource use of nectar-feeding bats in the Sayula Basin, and previous studies of nectarivorous bat diet in Mexico have relied on visual identification of pollen grains in faeces, limiting the taxonomic resolution of results. In Chapter 4, I identified a roost in the pitaya production area of the principal pollinator, *L. yerbabuenae*, and collected samples of faeces and pollen found on the fur across one full year (Fig 1.1). I used metabarcoding to identify plant taxa present in the pollen and faecal samples, and calculated occurrence-based metrics to quantify the importance of plant taxa in the diet. Knowledge of food plants used by *L. yerbabuenae* bats during the months that *S. queretaroensis* (i.e. the major cash crop) is not in flower informs the identification of priority habitats for their conservation.

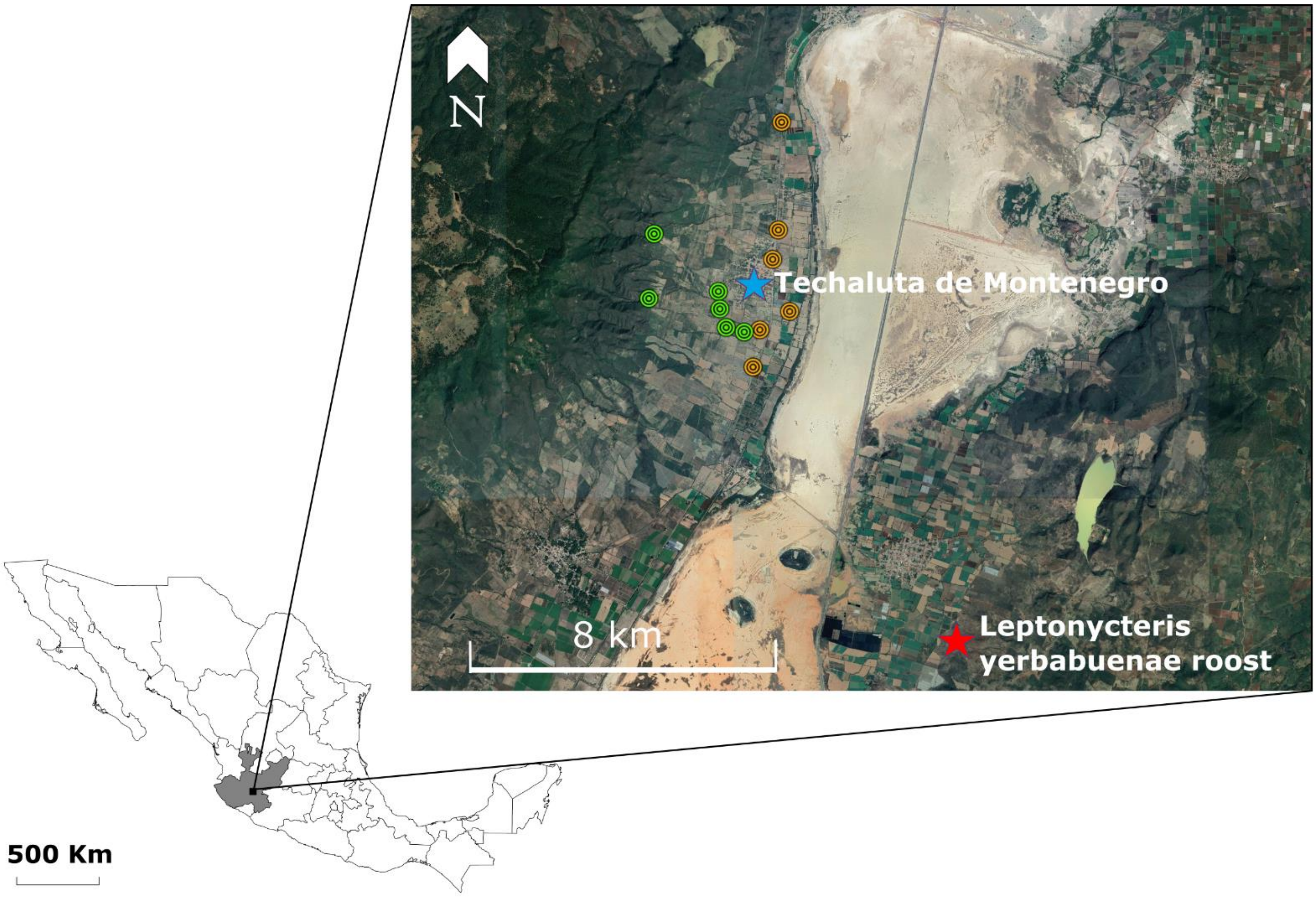

Figure 1.1. Map of the study site in the Sayula Basin, Jalisco, central Mexico. Sites used in Chapter 2 are shown in the town of Techaluta de Montenegro, marked by a blue star, an important production centre for the pitaya (*Stenocereus queretaroensis*): wild sites are marked as green circles and pitaya plantations are orange. The *L. yerbabuenae* roost studied in Chapter 4 is marked with a red star, approximately 8km south-east of the town. Image made using Google Earth imagery.

Table 1.1. Logical framework summarising the thesis aims, objectives and main analytical methods

Issue and Aim	Objective	Data collection	Results	Analytical methods
Issue Human activities and environmental changes have a negative impact on bat populations; and there is a lack of awareness of the benefits provided by bats	**Chapter 2 -** Quantify the dependence of the pitaya crop, both wild individuals and cultivars, on bat pollinators for fruit yield and quality	Exclusion experiments	Quantitative data on the fruit set, fruit weight and seed set of fruits produced from pollination by bats, birds and insects	Mixed effect models
		Camera trapping	Visitation rates of vertebrate pollinators and impact on fruit quality	Linear regression
Aim Investigate the ecological and economic importance of bat pollination services to a major cash crop in central Mexico	**Chapter 3 -** Investigate the socio-economic benefits of bat pollination to the local community	Structured interviews to collect pitaya production and marketing data from pitaya farmers	Monetary valuation (through market prices) of increased fruit yield and size from bat pollination	Yield analysis
		Structured interviews to collect income and profit data from all actor groups	Analysis of the distribution of economic benefits between actors	Value chain analysis
	Chapter 4 – Explore the year-round diet of the principal pollinator of the pitaya crop, the nectar-feeding bat *L. yerbabuenae*	Bats captured at one colony across one year to collect samples of pollen from the fur of the bat, and faeces	Occurrence data of plant species found in pollen and faecal samples	Metabarcoding bioinformatics to sequence samples and identify plant taxa present Occurrence-based metrics

Chapter 2 Pollination by bats enhances both quality and yield of a major cash crop in Mexico

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Abstract

1. Bats pollinate many plants of high socio-economic value, including the majority of columnar cacti (Cactaceae) in Mexico, which have been used by humans for food and materials for thousands of years. However, the importance of bats as pollinators has been overlooked, with a consequent lack of knowledge of the reliance of crops on bats for harvest yield and quality.
2. Exclusion experiments were used to determine the effect of different pollinator taxa on the yield and quality of pitayas (fruit of *Stenocereus queretaroensis* (F.A.C. Weber) Buxbaum), a major crop in central Mexico. We studied the three most economically important cultivars and wild individuals in the principal region for pitaya production. For each pollinator taxon we recorded fruit set and measured three key parameters of fruit quality: weight, sucrose concentration and seed set. We placed camera traps to determine pollinator identity and the effect of visitation rate on fruit quality.
3. We found the primary pollinators of pitayas to be nectarivorous bats in the genus *Leptonycteris*. When bats were excluded from flowers and flowers were pollinated by other taxa (i.e. diurnal birds and insects), pitaya yield decreased by 35%, though pollination dependence varied between cultivars. Fruit quality decreased significantly in the absence of bat pollination across all cultivars, with fruits 46% lighter and 13% less sweet when pollinated by other taxa; reducing economic value, as size determines market price. Additionally, seed set (an indicator of effective pollination) was significantly lower in the absence of bat pollinators. Visitation rate had no effect on fruit quality.
4. *Synthesis and applications.* Our study shows that bats provide a vital ecosystem service by pollinating a crop of major socio-economic importance, and that consideration of both crop quality and yield are essential to fully understanding the

benefits of bat pollination. A reduction of this service would result in a decrease in both the size and quality of the harvest, causing substantial loss of income for rural communities. Bats worldwide face many threats, and management efforts targeted to the enhancement of wild bat pollinator populations would preserve the sustainability of both bat-pollinated crops and wild plants.

Keywords: pitayas, exclusion experiments, crop yield, columnar cacti, ecosystem services, crop quality, bats, pollination

Resumen

1. Los murciélagos polinizan un gran número de plantas de gran valor socioeconómico, incluyendo la mayoría de los cactus columnares (Cactaceae) en México, los cuales han sido utilizados por los humanos durante miles de años para obtener alimento y materiales. Sin embargo, se ha pasado por alto la importancia de los murciélagos como polinizadores, con la consiguiente falta de conocimiento de la dependencia de los cultivos a la polinización por los murciélagos para el rendimiento y calidad de la cosecha.
2. Utilizamos experimentos de exclusión para determinar el efecto de diferentes taxones polinizadores en el rendimiento y la calidad de las pitayas (fruto de *Stenocereus queretaroensis* (F.A.C. Weber) Buxbaum), un cultivo importante en el centro de México. Estudiamos los tres cultivares más importantes económicamente e individuos silvestres, en una de las regiones más importantes para la producción de pitaya. Para cada taxón polinizador registramos la producción de frutas y medimos tres parámetros clave de la calidad del fruto: peso, concentración de sacarosa y cantidad de semillas. Colocamos cámaras trampa para determinar la identidad de los polinizadores y el efecto de la tasa de visitas en la calidad de la fruta.

3. Encontramos que los principales polinizadores de las pitayas son los murciélagos nectarívoros del género *Leptonycteris*. Cuando los murciélagos fueron excluidos de las flores y éstas fueron polinizadas por otros taxones (es decir, aves e insectos diurnos), el rendimiento de las pitayas disminuyó en un 35%, aunque esta dependencia de polinización varió entre los cultivares. La calidad de la fruta disminuyó significativamente en ausencia de la polinización de murciélagos en todos los cultivares, con frutas 46% más livianas y 13% menos dulces cuando se polinizaron por otros taxones, reduciendo así el valor económico ya que su tamaño determina el su precio en el mercado. Adicionalmente, el número de semillas (un indicador de polinización efectiva) fue significativamente menor en ausencia de los murciélagos polinizadores. La tasa de visitas no tuvo efecto sobre la calidad del fruto.
4. *Síntesis y aplicaciones:* Este estudio muestra que los murciélagos proporcionan un servicio ecosistémico vital al polinizar un cultivo de gran importancia socioeconómica, y que la consideración de la calidad y el rendimiento del cultivo son esenciales para comprender completamente los beneficios de la polinización de los murciélagos. Una reducción de este servicio resultaría en una disminución tanto en el tamaño como en la calidad de la cosecha, causando una pérdida sustancial de ingresos para las comunidades rurales. Los murciélagos en todo el mundo enfrentan muchas amenazas y los esfuerzos de manejo dirigidos a la mejora de las poblaciones de murciélagos polinizadores apoyarían a la conservación tanto de los cultivos polinizados por murciélagos como de las plantas silvestres.

Palabras clave: pitayas, experimentos de exclusión, rendimiento del cultivo, cactus columnar, servicios ecosistémicos, calidad del cultivo, murciélagos, polinización

2.1 Introduction

The majority of the world’s 350,000 species of flowering plants rely on animal pollinators for reproduction (Ollerton et al. 2011). Animal-pollinated plants play fundamental roles in ecosystems, underpinning biodiversity and the provision of ecosystem services, such as the supply of building materials, biofuels, medicines, and food (Potts et al. 2016a). Three quarters of leading global crops show increases in yield or quality when pollinated by animals (Klein et al. 2007). Furthermore, the crops richest in micronutrients are often the most dependent on animals for pollination (Eilers et al. 2011).

Vertebrates such as bats, birds and reptiles play an important, though often overlooked, role (Ratto et al. 2018). Bats may be the main pollinators for up to 1,000 species of plants across the tropics, including many of socio-economic importance such as durian and mango (Lobova et al. 2009; Kunz et al. 2011). Chiropterophilous plants are specialised in a greater degree compared to other zoophilous plants, with lower reproductive success when bats are excluded from the flower for bat-pollinated plants relative to when birds or reptiles are excluded from plants pollinated by those taxa (Ratto et al. 2018). Through depositing large amounts of pollen from a variety of genotypes, frequently over long distances, bats enhance reproductive output as insufficient or closely related pollen deposition can limit seed production (Fleming et al. 2009; Aizen and Harder, 2007; Muchhala and Thomson, 2010).

Bat populations are severely threatened in many parts of the world however, with 80% of bat species requiring research or conservation attention (Frick et al. 2019), and there is an urgent need for research demonstrating the ecosystem services provided by bats. The role of bats as pollinators of tropical crops has been established for species such as durian, bitter beans, jackfruit and fleshy fruits of columnar cacti (e.g. Ibarra-Cerdeña et al. 2005; Bumrungsri et al. 2008, 2009; Aziz et al. 2017; Lim et al. 2018); however, despite literature demonstrating the importance of insect pollinators for crop quality and subsequent market value (e.g. Garratt et al. 2014; Klatt et al. 2014), no studies thus far have examined the effect of bat pollinators on crop quality as well as yield. Moreover, previous studies have been unable to directly isolate the impact of bat pollination on crop yield from that of other vertebrate and/or invertebrate pollinators. This lack of information severely hinders our ability to assess the full range of benefits of bat pollination to crop production and the impact of potential declines in bat pollinator populations, and consequent efforts to justify conservation actions (Melathopoulos et al. 2015).

Additionally, previous studies on the importance of pollinators to world crop production (e.g. Klein et al. 2007) have overlooked small-scale but regionally important crops, despite the vulnerability of subsistence and small-scale farmers (who account for 83% of agricultural production) to declines in pollinator populations, as social and economic barriers reduce their ability to diversify if agricultural production fails (Morton, 2007; Potts et al. 2016a).

In this study, we aim to elucidate the importance of bat pollination to the production of the pitaya fruit, harvested from an arborescent columnar cactus (*S. queretaroensis*), endemic to semi-arid habitats in western central Mexico (Ibarra-Cerdeña et al. 2005). *S. queretaroensis* has a high cultural value in Mexico and has been important for the subsistence of local communities since pre-Hispanic times (Pimienta-Barrios and Nobel, 1994). *S. queretaroensis* is a sustainable crop in arid regions; able to tolerate drought and rocky, infertile soils, and producing its fruits in the dry season when few other crops are available (Pimienta-Barrios, 1999b). The flowers provide important nutrition to animals such as bats, birds, rodents and insects when other sources of food are scarce (Pimienta-Barrios, 1999b). Pitaya fruits are now commercially cultivated, providing significant local income (Pimienta-Barrios and Nobel, 1994). We carried out exclusion experiments to assess the effect of different pollinating taxa on yield and quality (here, fruit size and sweetness, and seed set) across both wild plants and three cultivars; and used camera traps to identify pollinating taxa.

2.2 Materials and methods

2.2.1 Study area and species

We conducted this research in the municipality of Techaluta de Montenegro, Jalisco, Mexico (20.074°, -103.550°), one of the most important areas for pitaya production (Pimienta-Barrios and Nobel 1994), during 2016 and 2017. The pitaya is the most valuable crop grown in Techaluta de Montenegro, generating approximately Mx$19,200 per ton, with production increasing yearly (SIAP, 2018). In the wider state of Jalisco, the pitaya is the thirteenth most valuable crop of 110 grown (SIAP, 2018). Though some other areas continue to collect pitayas from wild cacti, pitaya production in Techaluta de Montenegro is dominated almost entirely by small commercial plantations, with an average size of 2.6 has ± 2.8 (Tremlett et al. unpublished data; Pimienta-Barrios, 1999b). The average density of wild *S. queretaroensis* individuals in Techaluta de Montenegro is 25 per ha, while a commercial plantation has approximately 1000 cacti per ha (Pimienta-Barrios, 1999a). Fruits from popularly cultivated varieties have a higher market value, owing mainly to their larger size (see Appendix A.2).

S. queretaroensis is self-incompatible and is primarily bat-pollinated in the wild, like other members of the *Stenocereus* genus (Ibarra-Cerdeña et al. 2005; see Appendix A.1). However, the dependence of cultivated populations of *S. queretaroensis* on bat pollination for crop yield is unknown; as well as the impact of bat pollination on parameters of pitaya quality. We studied wild individuals of *S. queretaroensis* (cacti of 50+ years grown naturally) as well as three cultivars (Blanco, Mamey and Tenamaxtle) chosen for their economic importance, accounting for the majority of fruit production in the area (see

Appendix A.2). The study was carried out in six plantations, each containing all three cultivars; and six ranches with wild cacti (Fig. 2.1).

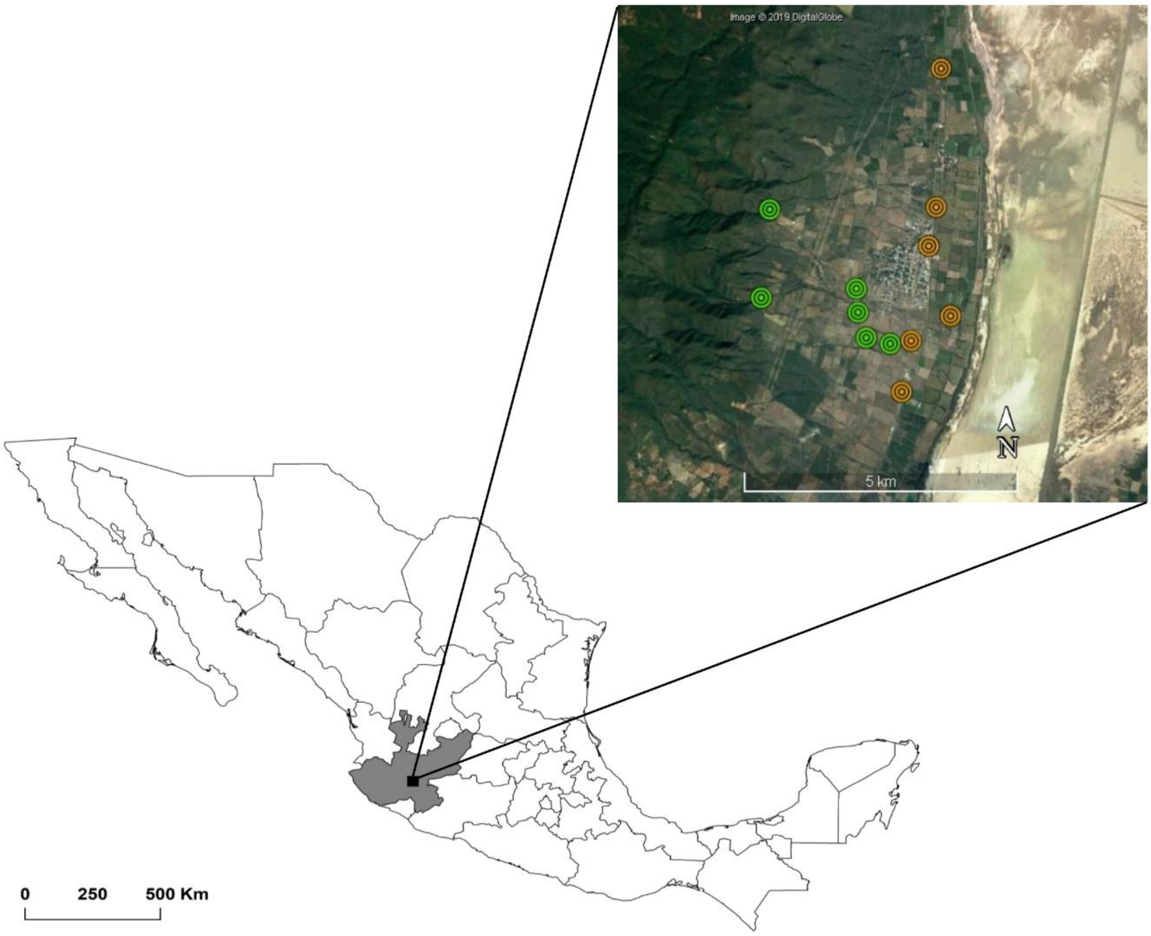

Figure 2.1. A map of the study area located in the municipality of Techaluta de Montenegro, located 80km south-west of Guadalajara in the state of Jalisco, Mexico. Locations of the six plantations used as cultivated sites (orange) and the six ranches used as wild sites (green) are shown. The seasonally dry RAMSAR lagoon is visible to the east of the town, with mountainous dry tropical forest to the west (Google Earth 2019).

2.2.2 Pollination dependency of *Stenocereus queretaroensis*

We carried out exclusion experiments to determine the efficiency of different pollinators, using six pollination treatments to differentiate between both nocturnal and diurnal pollinators, as well as invertebrate and vertebrate pollinators. To exclude certain pollinators, bags of different mesh sizes were placed on flowers either during the day or at night. Bags made from a very fine mesh prevented all pollinators from visiting the flower, and bags made from 2 cm^2 mesh allowed only insects to pollinate flowers (i.e. excluded vertebrate pollinators).

We randomly selected five cacti of each cultivar in each plantation, and five wild cacti at each ranch. Six different treatments were carried out on each cactus, with each treatment on a separate flower: nocturnal pollinators only (NP: fine mesh bag during the day and unbagged at night), nocturnal insects only (NI: fine mesh bag during the day and large mesh bag at night), diurnal pollinators only (DP: unbagged during the day and fine mesh bag at night), diurnal insects only (DI: large mesh bag during the day and fine mesh bag at night), open pollinated control (OC: unbagged during the day and at night), and closed control (CC: fine mesh bag during the day and at night). Bags were changed at 06:00 and 18:00, with experiments lasting 24 hours. We placed all treatments on flowers opening on the same night where possible and on consecutive nights if not. We used randomised stratification to ensure a range of flower heights for each pollination treatment and recorded flower height.

To assess the impact of treatment on pitaya yield and quality we monitored experimental flowers to record mature fruit set (success or failure). We collected successful fruits to measure six different variables of interest: fruit length, fruit width, pulp weight, fruit weight, sucrose content and seed set. We used the ripening times of the first fruits to mature to establish standardised collection times of 52, 57, 54 and 52 days for Blanco, Mamey, Tenamaxtle and wild fruits respectively. We excluded fruits that were damaged by insects or by local people.

We weighed each fruit without spines, and measured the length and width. We peeled the fruits and weighed the fruit pulp. We chose fruit weight as the final indicator of fruit size, as it showed the strongest correlation with the other size parameters (see Appendix A.3). Sucrose content in one quarter of the fruit pulp (by wet weight) was measured using a handheld refractometer. We calculated seed set for each fruit by dividing the total seed number (estimated from counting the seeds in one quarter of the fruit by wet weight, and multiplying by four) by the average number of ovules counted in fifteen extra flowers from each cultivar type and wild individuals (collected from cacti not used in exclusion experiments, but from the same sites; see Appendix A.4).

2.2.3 Pollinators of *Stenocereus queretaroensis*

To determine pollinator taxa and visitation rates, we placed camera traps (Spypoint Force 11D, trigger speed 0.07 seconds) to take photos of vertebrate flower visitors, of which any with a pollinating animal (i.e. bats and birds) in the frame was considered as a visit. We placed cameras at a total of 38 flowers across the flowering season (Blanco = 9, Mamey = 11, Tenamaxtle = 10, wild = 8). We used randomised stratification to ensure a range of heights (between 0.9 and 3.2 m). Camera traps were placed at 20:00 and collected the following day after 24 hours. To determine the effectiveness of pollination visits, we monitored flowers for fruit set, and successful fruits were collected and processed as above (exclusion experiments). We also placed a Bushnell camera Trophy Cam Aggressor HD (Low-glow) at 16 different flowers to take video footage for analysis of bat feeding behaviour, set to record 60 seconds of footage followed by an interval of four minutes, from 20:00 to 08:00. We classified a flower visit as one where the tongue or snout of the bat was inserted into the flower.

To determine the visit frequency of each vertebrate pollinator taxon (e.g. birds, bats), we counted their occurrence in camera trap photos. We analysed feeding behaviour by watching video footage and recording the taxa of flower visitors and visit duration (to 0.1 seconds). Each filmed visit was classified into four categories based on feeding style (tongue, tip, most, or all of the snout/face in the flower).

2.2.4 Statistical analysis

We evaluated differences in fruit set among pollination treatments using a binomial generalised linear mixed effect model (GLMM); the effect of pollination treatment on fruit weight [log transformed] and sucrose concentration [cube transformed] using linear mixed effects models (LMM); and on seed set using a weighted LMM (using R package ‘lme4’ for all models; Bates et al. 2015). In all models, cactus nested within site were random effects, and cultivar type (including wild) and pollination treatment were fixed effects. Models were calibrated to the treatment of nocturnal pollinators and to wild cacti, and as such parameter estimates are interpreted in relation to these factors.

We arrived at minimum adequate models by first running a full model complete with all fixed effects (pollination treatment, cultivar type, and flower height) and interactions between them, then removing them one by one from the model based on significance and AIC values, choosing models with lower AIC values. Maximum likelihood was used to compare models due to the nested random effects and the differing number of fixed effects. We could not include closed control fruits or fruits pollinated by nocturnal insects in analyses of fruit weight, seed set or sucrose concentration, as no fruits were successfully produced under these treatments.

We calculated the effect of each pollination treatment on fruit weight, sucrose concentration, and the likelihood of flowers developing into fruits, using the parameter estimates produced by the mixed effect models described above (using R package ‘sjPlot’; Lüdecke 2019). The parameter estimates for nocturnal pollinators were subtracted from the estimates produced for diurnal pollinators and the result was divided by the latter, accounting for original data and model transformations. We used estimated marginal means to estimate overall yield change between pollinators across cultivars and wild cacti (using R package ‘emmeans’; Lenth 2019). We reported mean seed set values instead of the percentage changes based on parameter estimates as these are more biologically meaningful.

To reveal significant differences (averaged across cultivar type) in fruit set, fruit weight, seed set and sucrose concentration between nocturnally pollinated flowers and those under the other pollination treatments, we carried out pairwise post-hoc analysis using Tukey’s HSD test for the GLMM, and via Wald-statistics approximation (treating t as Wald z) for the LMMs, setting nocturnal pollination as the reference to which the other pollination treatments were compared (using R packages ‘emmeans’ and ‘sjPlot’; Lenth 2019; Lüdecke 2019). To determine if the difference in fruit set between nocturnally pollinated and diurnally pollinated flowers was significant for each cultivar type, we generated P-values by setting each cultivar type as the reference level and rerunning the GLMM.

To determine whether there was a correlation between fruit weight and seed set, we ran a linear regression on log transformed fruit weight explained by seed set. To investigate the effect of the number of bat visits to a flower on fruit quality, we ran a linear regression on log transformed fruit weight, and a generalised linear model on seed set [using a quasibinomial distribution to account for seed set values of 1], explained by number of pollinator visits. To investigate whether there was a difference in number of bat visits between cultivars and wild individuals, we ran a linear regression on log transformed visit number explained by cultivar type (including wild).

Visual inspection of residual plots of all final models showed no obvious deviations from homoscedasticity or normality, except for heteroscedastic residuals from the seed set LMM which were therefore weighted by 1/fitted value^2 to ensure homoscedasticity. Statistical analysis was done using R version 3.5.3 (R Core Team, 2019).

2.3 Results

2.3.1 Pollination dependency of *Stenocereus queretaroensis* on bats

Exclusion experiments were placed on a total of 109 *S. queretaroensis* cacti (30 wild, 22 Blanco, 30 Mamey and 27 Tenamaxtle plants: 8 Blanco and 3 Tenamaxtle were mis-identified and therefore were not included). A total of 178 fruits were collected from the exclusion experiments out of a total of 654 flowers bagged. 453 flowers did not produce fruits and 23 fruits were accidentally picked or damaged by local harvesters so we could not collect them. From the 178 fruits collected, 165 fruits (93%) were included in subsequent analyses; 13 were damaged by insects and not included. Natural pollination conditions (open control) resulted in a fruit set of 77% in Blanco, 53% in Mamey, 85% in Tenamaxtle, and 67% in wild individuals (Fig. 2.2). No fruits were produced by the self-pollination treatment (closed control) or nocturnal insect pollination (Fig. 2.2).

Fruit set depended on pollination treatment (GLMM: $\chi^2 = 286.7$, $P < 0.0001$; Table 2.1). Averaged across cultivars and wild type, flowers pollinated by nocturnal animals (i.e. bats) were 35% more likely to develop into mature fruits compared to when pollinated by diurnal animals. However, the dependence on bats for fruit set differed between cultivars and wild plants (Fig. 2.2). Pollination by bats (NP) relative to diurnal pollinators (DP) resulted in a significantly higher probability of fruit set of 27% for Mamey individuals (GLMM: $P < 0.001$) and 35% for wild individuals (GLMM: $P = 0.002$). There was no difference in the probability of fruit set for Blanco (GLMM: $P = 0.60$) and Tenamaxtle (GLMM: $P = 0.65$) individuals when pollinated by NP relative to DP.

Table 2.1. Outputs from Linear Mixed Models (LMMs) and Generalised Linear Mixed Model (GLMM) investigating the effect of pollination treatment on fruit set, fruit weight, seed set and sucrose concentration across cultivars and wild S. queretaroensis.

	Fruit set			**Fruit weight**			**Seed set**			**Sucrose concentration**		
	(GLMM: χ^2 = 286.7, *df = 5, P* < 0.0001)			*(LMM:* χ^2 *= 51.5, df = 3, P < 0.0001)*			*(LMM:* χ^2 *=93.5, df =3, P < 0.0001)*			*(LMM:* χ^2 *=9.88, df =3, P = 0.0196)*		
Predictors	Estimates	95% CI	P	Estimates	95% CI	P	Estimates	95% CI	P	Estimates	95% CI	P
NP	0.51	-0.08 – 1.09	na	3.65	3.44 – 3.86	na	0.62	0.51 – 0.73	na	2.03	1.93 – 2.12	na
OC	0.84	0.24 – 1.45	0.890	-0.09	-0.24 – 0.05	0.206	-0.05	-0.16 – 0.06	0.385	-0.01	-0.07 – 0.05	0.716
DI	-2.04	-2.76 – -1.33	**<0.001**	-0.55	-0.24 – 0.05	**<0.001**	-0.55	-0.66 – -0.43	**<0.001**	-0.10	-0.19 – -0.01	**0.036**
DP	-0.95	-1.55 – -0.35	**<0.001**	-0.61	-0.79 – -0.43	**<0.001**	-0.54	-0.64 – -0.44	**<0.001**	-0.09	-0.16 – -0.02	**0.010**
Blanco	0.78	0.05 – 1.50	**0.035**	0.23	-0.05 – 0.52	0.110	0.15	0.02 – 0.27	**0.021**	0.09	-0.05 – 0.22	0.201
Mamey	-0.85	-1.54 – -0.17	**0.015**	0.98	-0.68 – 1.28	**<0.001**	0.07	-0.07 – 0.20	0.319	0.14	-0.00 – 0.28	**0.045**
Tenamaxtle	0.81	0.12 – 1.49	**0.021**	0.76	-0.48 – 1.03	**<0.001**	0.29	0.16 – 0.41	**<0.001**	0.01	-0.12 – 0.14	0.917
Random effects	τ00 cactus(site)		0.24	τ00 cactus(site)		0.06	τ00 cactus(site)		0.02	τ00 cactus(site)		0.02
	τ00 site		0.02	τ00 site		0.02	τ00 site		0.00	τ00 site		0.01
	residual		0.00	residual		0.13	residual		0.17	residual		0.02

Pollination treatments: NP = nocturnal pollinators; OC = open control; DI = diurnal insects; DP = diurnal pollinators. Results are shown with wild cacti as the intercept. Effect sizes are relative to nocturnal pollination for LMMs on fruit weight, seed set and sucrose concentration; while logit values are shown for each treatment for fruit set, with effect sizes of cultivars relative to wild cacti (see Appendix A.6 for estimates for each cultivar). Variance is provided for random terms. Significant ($P < 0.05$) differences between NP and other pollination treatments, averaged across cultivar type, are displayed in bold: p values computed via Wald-statistics approximation (treating t as Wald z) using sjPlot package in R for LMMs, and via Tukey method using emmeans package in R for GLMM.

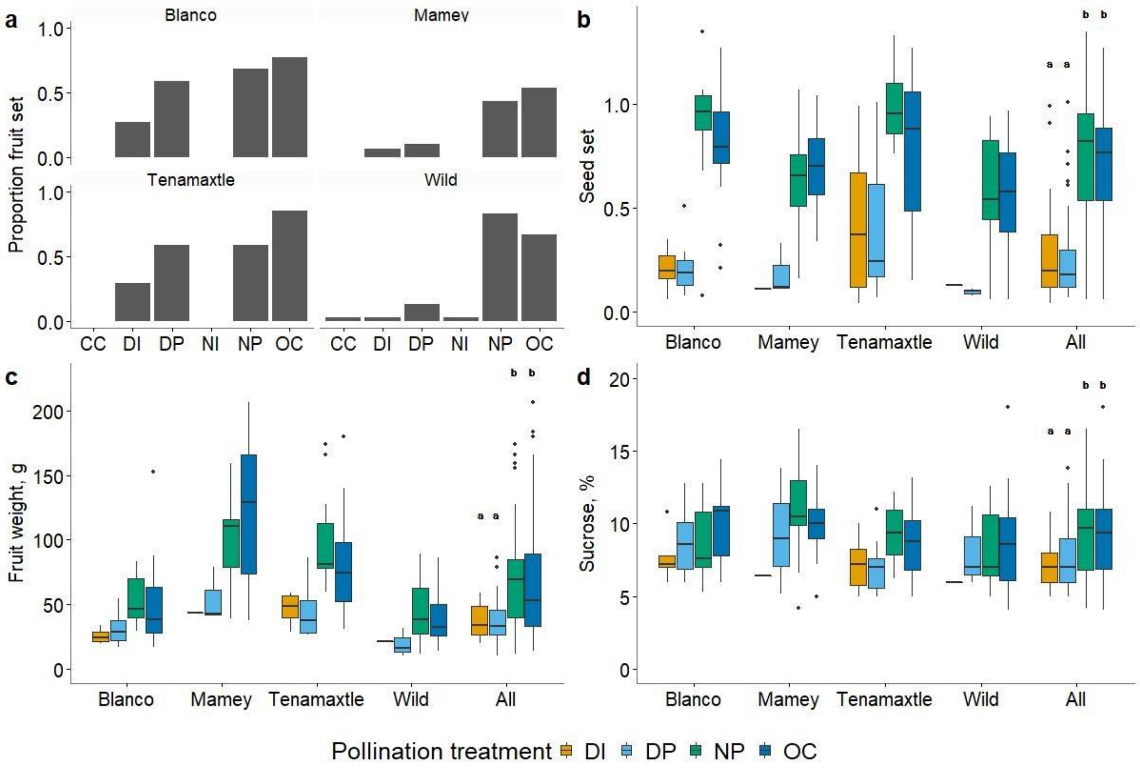

Figure 2.2. Effect of pollination treatments on: **a)** fruit set (Blanco: n = 22, Mamey: n = 30, Tenamaxtle: n = 27 and wild individuals: n = 30) under pollination treatments (CC = closed control, DI = diurnal insects, DP = diurnal pollinators, NI = nocturnal insects, NP = nocturnal pollinators, OC = open control); **b)** seed set, **c)** fruit weight and **d)** sucrose concentration. Mixed effects models showed pollination treatment had a significant effect on seed set, fruit weight and sucrose concentration; different letters above bars represent significant differences between treatments of pooled data (Blanco: N = 39 (diurnal: insects only (n) = 5, diurnal (n) = 1, nocturnal (n) = 9, open (n) = 14); Mamey: N = 26 (diurnal: insects only (n) = 1, diurnal (n) = 3, nocturnal (n) = 10, open (n) = 12); Tenamaxtle: N = 57 (diurnal: insects only (n) = 8, diurnal (n) = 16, nocturnal (n) = 14, open (n) = 19); wild individuals: N = 42 (diurnal: insects only (n) = 1, diurnal (n) = 3, nocturnal (n) = 19, open (n) = 19). Figure produced using R package ‘ggplot2’ (Wickham, 2016).

When pollination was carried out by birds and diurnal insects only, resulting fruits were significantly lighter (46% and 42% lighter for fruits pollinated by birds and diurnal insects respectively; LMM: $\chi^2 = 51.5$, $P < 0.0001$; Fig. 2.2), and significantly less sweet (13% and 14% lower sucrose concentration respectively; LMM: $\chi^2 = 9.88$, $P = 0.0196$; Fig. 2.2), than those pollinated by bats. Seed set was also significantly lower (LMM: $\chi^2 = 93.5$, $P < 0.0001$). Mean proportion of seed set was 0.77 (± 0.04 SE) for bat-pollinated fruits relative to 0.28 (± 0.04 SE) for diurnal pollinators and 0.32 (± 0.08 SE) for diurnal insects (Fig. 2.2; Table A.4). There was no difference between bat-pollinated flowers and flowers under natural pollination conditions (open control) in terms of fruit set, weight, sucrose concentration or seed set (Table 2.1; Fig. 2.2). Fruit weight was positively correlated with seed set (LM: $F_{1,162} = 79.2$, $r^2 = 0.32$, $P < 0.0001$).

2.3.2 Visits to flowers

We placed camera traps at 38 flowers for one night and day consecutively and recorded a total of 1156 visits by vertebrates. Of these, 99% were made by bats (1142 visits) and 1% by diurnal birds (14 visits). We did not record invertebrate pollinators and no vertebrate nocturnal visitors other than bats were recorded. The majority (78%) of the 311 observations of bats feeding in the video footage could be attributed to *Leptonycteris* bats (it is not possible to differentiate between *Leptonycteris* species from footage as forearm length is a key distinguishing feature). Visits to flowers lasted between 0.1 and 2.8 seconds. In 88% of flower visits, the bat inserted its whole head into the flower. Video footage of 12 bird visits to flowers showed that nectar-feeding birds with long beaks such as hummingbirds (n = 8) inserted the whole head into the flower to feed in 75% of cases, likely making contact with the anthers and stigma; while insectivorous birds looking for insects (n = 4) inserted just the tip of the beak, and did not appear to make contact with the reproductive parts of the flower. Bat visitation rate per flower was erratic, ranging from 0 to 127 visits, but did not differ significantly between cultivars and wild individuals (LM: $F_{3,42} = 0.23$, $r^2 = -0.05$, $p = 0.88$). The number of visits did not influence fruit weight (LM: $F_{1,30} = 0.79$, $r^2 = -0.01$, $p = 0.38$) or seed set (GLM: $F_{1,30} = 0.79$, $p = 0.50$).

The species of birds recorded by camera traps visiting the flowers were: *Amazilia violiceps* (4 visits); *Icterus cucullatus* (2 visits); *I. parisorum* (3 visits); *Icterus* sp. (2 visits); Trochilidae sp. (2 visits); *Setophaga coronata* (1 visit). Nectarivorous hummingbirds are therefore likely to be the main diurnal vertebrate pollinators of pitayas, though other species searching for insects may also contribute to pollination.

2.4 Discussion

This is the first study to fully quantify the impact of bat pollination on both the quality and yield of a crop of high socio-economic importance, across both wild plants and multiple cultivars, with important implications for ecosystem management. We find that in the absence of pollination by nectarivorous bats, yield and quality (i.e. fruit weight, as size determines market value) of *S. queretaroensis* decreased significantly by 35% and 46% respectively. Hence, nectarivorous bats contribute substantially to the economic welfare of the rural production region. Sustainable agricultural practices (such as reduced pesticide use) are therefore essential to ensure the continued provision of pollination services by nectarivorous bats in plantations; along with conservation efforts to protect wild bat pollinator populations at roost sites and along migration routes.

2.4.1 Benefits of bat pollination

Bats are the most effective pollinators of *S. queretaroensis*, enhancing both fruit yield and quality. Here, fruits pollinated by bats had a higher seed set than those pollinated by diurnal animals, indicating pollination by bats is more effective even where fruit set is maintained by both bats and birds. Visitation rate did not affect either fruit or seed set, with one visit by bats to the flower enough for effective pollination. The feeding style of bats resulted in an apparently higher likelihood of contact with the reproductive parts of the *S. queretaroensis* flower than that of birds, we found a much higher visitation rate by bats than birds, and bats then have a higher capacity to take up and hold pollen on their fur compared to avian pollinators on feathers (Muchhala and Thomson, 2010).

Unlike in crops such as some citrus fruits, an increased seed content of pitayas does not reduce market value, as the seeds are small, easily digested and high in protein (Pimienta-Barrios, 1999b). Developing seeds produce phytohormones which promote cell expansion in the surrounding fruit tissue, thereby increasing fruit size and weight (Gillaspy et al. 1993). Additionally, these hormones limit the expression of expansins, proteins that soften fruit and reduce shelf-life (Klatt et al. 2014), which may be beneficial for pitaya producers, as the perishability of pitayas is a challenge to market growth (Pimienta-Barrios and Nobel, 1994). In the later stage of development, cells accumulate carbohydrates, which are then metabolised into sugars on ripening (Gillaspy et al. 1993; Gray et al. 1992). The increased seed set associated with bat pollination likely therefore causes both the higher fruit weight and higher sucrose concentration found in bat-pollinated fruits relative to fruits pollinated by birds or insects.

Deposition of unsuitable pollen on stigmas, from closely related or the self-same plants, reduces fruit and seed production due to the sharing of self-incompatibility alleles or the disabling of pollen tubes with self-pollen (Aizen and Harder, 2007). Growers of *S. queretaroensis* rely mainly on vegetative propagation with few plants grown from seed, resulting in plantations containing large numbers of clonal individuals and high genetic differentiation between plantations (Pimienta-Barrios, 1999a; Ruán-Tejeda et al. 2014). Hence, outcrossing from pollen arriving from outside the plantation is extremely important, and *Leptonycteris yerbabuenae* bats have been found to travel up to 100 km per night to forage in arid landscapes, visiting flowers from multiple plants (Medellin et al.

2018). Bat pollinators are therefore less likely to deposit unsuitable pollen on stigmas than other pollen vectors, like insects or birds, which disperse pollen locally (Aizen and Harder, 2007; Fleming et al. 2009).

2.4.2 Pollination system of *Stenocereus queretaroensis*

Fruit set in cultivars Blanco and Tenamaxtle was not dependent on bats, if birds were present; whereas cv. Mamey and the wild cacti were highly dependent on bats. This may reflect the spatial and genetic composition of cacti in plantations and ranches. Commercial plantations in Techaluta de Montenegro are dominated by cv. Mamey, propagated clonally and therefore sharing self-incompatibility (SI) alleles; with a smaller number of the other cultivars present (see Appendix A.2). Consequently, we expect that pollen vectors dispersing pollen locally (i.e. birds and insects) will deposit mostly Mamey pollen to all the cultivar types in our study plantations. The absence of bats would therefore not affect fruit production on the minority cultivars, but Mamey individuals would be more reliant on longer distance pollen transfer, enabled by bats that are more likely to bring pollen from a plant outside the plantation (not a clone). For wild cacti, the lower densities of plants and the larger number of flowers blooming at one time per plant may also result in little movement between individuals for bird and insect pollinators; hence wild cacti are also likely to rely on bats for successful pollination.

The role of bats as long-distance pollen dispersers may be particularly important in disturbed areas and agrosystems. The density of wild *S. queretaroensis* plants is lower in our study area than in other locations (e.g. Pimienta-Barrios, 1999a) due to agricultural activities, reducing the probability of inter-individual pollen transfer, with implications for the long-term viability of wild *S. queretaroensis* populations.

Our study has demonstrated the critical importance of considering both crop quality and yield for a full understanding of the potential impacts of declines in pollinator abundance on crops. While dependence on bat pollination varied with cultivar, all cultivars and wild types experienced a large reduction in fruit quality in the absence of bats, reducing market value. The benefits of bat pollination therefore are more significant than suggested by previous studies that considered yield only (e.g. Bumrungsri et al. 2008, 2009).

2.4.3 Potential socio-economic consequences of losing bat pollination services

In Latin America, bat colonies and roosting sites are frequently destroyed to kill vampire bats (*D. rotundus*, which can cause economic damage to livestock by transmitting bovine paralytic rabies; Williams-Guillén et al. 2016), also threatening other species of cave-dwelling bats, such as members of the genus *Leptonycteris*. *L. yerbabuenae* and *L. nivalis* are important pollinators of many species of wild Cactaceae in the Neotropics, which play keystone ecological roles by providing nutrients, water and structural resources for many animal species (Fleming and Valiente-Banuet, 2002; Kunz et al. 2011; Frick et al. 2014). A decline in bat populations, with a corresponding decline in *S. queretaroensis* and other columnar cacti, would have catastrophic cascading effects. The high reliance of *S. queretaroensis* on bat pollinators indicates specialisation and increased vulnerability to pollinator loss, unlike in regions where less seasonally reliable bat populations result in more generalised pollination syndromes (Molina-Freaner et al. 2004).

The most valuable cultivar, Mamey (accounting for nearly 60% of fruit production in the study area; unpublished data), and wild cacti were highly reliant on bats for fruit production. The pitaya is the most valuable crop grown in Techaluta de Montenegro, and is a chief source of employment in an area lacking in economic opportunity and where nearly half the population (49%) already have an income insufficient to provide wellbeing (CONEVAL, 2016; SIAP, 2018). In other areas, pitaya production is based entirely on the collection of wild fruits (Pimienta-Barrios, 1999b). Loss of bat pollination services may therefore result in a substantial loss of income from the reduction of both fruit yield and quality, both in an agricultural and a wild context.

2.4.4 Implications for ecosystem management

Protection of cave roosts will help to safeguard the continued provision of ecosystem services provided by cave-dwelling bats (including pest-control and guano production from insectivorous bats). Loss of suitable foraging habitat is a key driver of declines in pollination services worldwide (Potts et al. 2016a), and populations of *Leptonycteris spp.* bats are migratory, following ‘nectar corridors’ that run from south-west USA to central and southern Mexico (Frick et al. 2014). Conservation management actions are therefore vital throughout the migration route to enhance bat pollinator populations by maintaining a high species richness of food plants, especially in tropical dry forests (Burke et al. 2019). Pitaya plantations in the study area likely represent important feeding grounds for nectarivorous bats, as *S. queretaroensis* flowers when few other species are flowering (Pimienta-Barrios and Nobel, 1994). Additionally, the conservation of wild populations negatively impacted by agricultural activities and cattle grazing (Pimienta-Barrios, 1999b) is key to both provide floral resources for nectarivorous bats, and to maintain a reservoir of genetic diversity in the species. Increased genetic heterogeneity within crops decreases vulnerability to disease, which may become increasingly important as pitaya production increases (Zhu et al. 2000).

Currently, pitaya production is largely organic, with fruits sold at local markets (Pimienta-Barrios and Nobel, 1994), but there are now efforts to begin international exportation of this crop. Such increased demand and commercialisation should not result in an increased use of pesticides and other chemicals, as commonly seen when agricultural systems are industrialised (Pingali and Rosegrant, 1995). Intensity of pesticide use overall in Mexico has shown rapid growth in recent decades (Schreinemachers and Tipraqsa, 2012). Bats can consume pesticides when feeding from flowers or fruits that are treated with chemicals, with negative consequences such as reduced reproductive output, immunosuppression, and increased mortality (Williams-Guillén et al. 2016). Additionally, biodiversity-friendly farming practices would also benefit insectivorous bats that likely have positive impacts on crop production through pest control (Maas et al. 2015).

2.5 Conclusions

Despite the huge economic value of some bat-pollinated crops such as durian (Bumrungsri et al. 2009; Aziz et al. 2017), the importance of bats as pollinators is often overlooked. Additionally, studies focusing on globally important products have omitted small-scale crops, such as pitayas, that are important to local communities. This study provides evidence that *L. yerbabuenae* (the lesser long-nosed bat) and other nectarivorous bat pollinators are crucial for the production of a local crop of high socio-economic importance in Mexico. Recognition of the ecosystem services provided by bats provides an alternative narrative for inhabitants of the production area that may currently associate bats with ecosystem disservices such as disease transmission and crop raiding. Management actions targeted to the enhancement of bat pollinator populations are crucial both in the production area and along the whole migration route in order to sustain rural livelihoods and wellbeing.

2.6 Data availability statement

Data available via the Dryad Digital Repository at https://doi.org/10.5061/dryad.dr7sqv9v2 (Tremlett et al. 2019).

2.7 Authors' contributions

VZG and KSHP conceived the research, CJT and VZG designed the experiments and collected the data, CJT did the analyses with input from MM, MAC provided expertise that greatly aided the research, and CJT wrote the paper with inputs from all co-authors. All authors gave final approval for publication.

Chapter 3 Value and benefit distribution of pollination services provided by bats in the production of cactus fruits

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Highlights

- Bats are vital pollinators of the pitaya, an important cash fruit crop in Mexico
- Bat pollination of the pitaya crop is worth approximately US$2,500 per ha
- Economic benefit supports rural livelihoods and is retained as cash income
- The commercialisation of the pitaya has concentrated profits with some actors
- Loss of bat pollinator populations would have severe socio-economic consequences

Abstract

Despite providing important ecosystem services in both natural and agricultural systems in the tropics, bats are often disregarded as pests; and research quantifying their importance as pollinators is scarce. We quantified the value and benefit distribution of bat pollination in the production of a major fruit crop in Mexico. We used yield analysis to assess the market value of pollination services provided by nectar-feeding bats to the production of pitayas, combined with value chain analysis to assess the distribution of these economic benefits among actors. Our results show that bat pollination services to pitaya production are worth approximately US$2,500 per ha through increases in both fruit yield and size, with bats contributing around 40% of gross income across producers sampled. Participation in the pitaya value chain provides a key seasonal source of cash income at a time of low agricultural activity, supporting livelihoods and household activities of the rural poor. However, the commercialisation of the pitaya has concentrated economic benefits with privileged groups who have access to land and markets. Our research highlights the potential socio-economic consequences of losing bat pollinators, and the need to improve equity of access to bat pollination service benefits across actors.

Keywords: cash crop; columnar cactus; economic valuation; ecosystem services; *Leptonycteris;* value chain; *Stenocereus queretaroensis*

3.1 Introduction

Pollinators provide many benefits to humans, improving food production and security, and underpinning biodiversity and crucial ecosystem functions (Potts et al. 2016a). Nearly 90% of flowering plants are reliant on animals for pollination; with three quarters of leading global crops, particularly those that are richest in micronutrients, showing increases in production or quality when pollinated by animals (Eilers et al. 2011; Klein et al. 2007; Ollerton et al. 2011; Potts et al. 2016b). Bats pollinate many plants of high socio-economic value across the tropics (Kunz et al. 2011). However, bat populations are threatened in many parts of the world, with 80% of bat species requiring research or conservation attention (Frick et al. 2019), and the value of bats to the maintenance of ecosystems and human wellbeing is largely underestimated (Kingston, 2016).

The quantification of ecosystem service benefits in monetary terms is frequently used to support biodiversity and ecosystem conservation, though it is a complex and challenging issue, particularly where services are intangible and cannot be valued through existing markets (Adams, 2014; Hanley et al. 2015; Breeze et al. 2016). However, the economic valuation of pollination services, such as the direct contribution of pollinators to commercial crop production and quality, can be a useful mechanism to alert decision-makers to the consequences of losing pollinators (Hanley et al. 2015). Existing assessments of pollination services have either focussed on the economic importance of insect pollinators, primarily honeybees (Gallai et al. 2009; Winfree et al. 2011; Hanley et al. 2015) or have established the role of bats as pollinators of tropical crop species, such as durian and fleshy fruits of columnar cacti (e.g. Ibarra-Cerdeña et al. 2005; Bumrungsri et al. 2009; Aziz et al. 2017a). To our knowledge, none have directly valued the effects of bat pollinators on yield and quality of a commercial crop in economic terms (though see Sheherazade et al. 2019 for a rough estimation of the value of bat pollination to durian production in Indonesia).

One important issue is that, worldwide, ecosystem service benefits – including those of pollination services – are not distributed equitably between different social groups (Hassan et al. 2005). Rural and traditional populations in poor areas are often more dependent on ecosystem services for their livelihoods and will be disproportionately affected by declines in pollinator populations (Hassan et al. 2005; Kumar, 2012). Subsistence or smallholder farmers are less likely to have the economic power to switch to different crops if production fails, or to replace free wild pollinator mediated services with bought services (Morton, 2007). At the same time, the ecosystem service benefits to different stakeholders depend on many socio-economic factors, such as market accessibility, land rights, and opportunity costs of labour and land (Shackleton et al. 2008). While access to ecosystem services can have an equalising impact on rural households, where there are constraints to access, some groups may be further marginalised (Kamanga et al. 2009). There is a considerable gap in the literature concerning the distribution of ecosystem service benefits across different stakeholders, particularly in Latin America; and a subsequent need for disaggregated analysis to identify constraints and improve access (Carpenter et al. 2006; Daw et al. 2011; Breeze et al. 2016; Laterra et al. 2019).

This paper uses the pollination by bats of an important cash crop in Mexico, the pitaya (*Stenocereus queretaroensis*) as a case study. Bats in the *Leptonycteris* genus are the principal pollinators of *S. queretaroensis,* enhancing both yield and quality of the pitaya crop (Tremlett et al. 2019). *Leptonycteris yerbabuenae*, the lesser long-nosed bat, and *L. nivalis,* the greater long-nosed bat, are species of nectar-feeding migratory bats distributed from Central America to the southern U.S.A. (Cole & Wilson, 1996). They are important pollinators of columnar cacti and agaves throughout their range, which play keystone ecological roles in arid ecosystems by providing structural resources, nutrients and water for a variety of animals (Frick et al. 2014).

The two main goals of this study are to a) quantify the value of pollination services to the pitaya sector in the most important production centre, and b) assess how these economic benefits are distributed between different actors throughout the pitaya commodity chain. Increased awareness of the economic importance of the contribution of bat pollination services may enable local communities and decision makers to take appropriate actions to ensure the protection of bat pollination services. A greater understanding of how these benefits are distributed intends to inform how future policies can enable more equitable access to, and participation in, the pitaya chain.

We use a direct yield analysis approach to estimate changes in both crop yield and quality between open pollinated and pollinator-excluded pitaya crops, and use current market prices to value these changes. Yield analysis is particularly useful for assessing benefits of pollination services at a local level, directly capturing the benefits of pollination services to a crop and differences between cultivars (Breeze et al. 2016; Potts et al. 2016b). However, only benefits accruing directly to the producer are measured using this method. We therefore use value chain analysis to assess how the economic benefits are distributed among different actor groups, affecting livelihoods and wellbeing more widely (Bolwig et al. 2010; Schaafsma et al. 2014).

A value chain describes the system and processes that occur along the chain of the production of a commodity and is often used to identify inequalities and constraints in the chain, particularly from the perspective of weaker actors (Kaplinsky and Morris, 2001; M4P, 2008; Meaton et al. 2015). Assessment of profits earned is a useful mechanism to identify barriers in the chain, as greater barriers to particular roles result in higher profits (Kaplinsky and Morris, 2001). However, it is also important to evaluate the returns to labour earned by different actors in the value chain. The poor must often work long hours to meet household needs, indicating ‘time poverty’ even where daily income is sufficient to provide wellbeing (Bardasi and Wodon, 2010). In this paper, we use survey and interview data to assess how income is distributed among actors using distribution of profits and hourly wages as indicators of inequality. We then assess the constraints faced to access more profitable roles and suggest potential mechanisms to encourage fairer participation in the chain by actor groups.

3.2 Study system

3.2.1 Study site

In Mexico, 85% of all cultivated plant species are at least partly dependent on animal pollinators; this, combined with high poverty levels and population densities, means that pollination services are crucially important to a large component of the population (Ashworth et al. 2009). Most columnar cacti (Cactaceae) are highly dependent on bats for pollination, including all 22 members of the *Stenocereus* genus, which have been widely utilised for fruit production in Mexico since pre-Hispanic times (Casas et al. 1999; Kunz et al. 2011). However, pollinating bat species continue to be threatened in Mexico by land use and climate change, mining, and disturbance at roost sites (Zamora-Gutierrez et al. 2018; Frick et al. 2019).

Techaluta de Montenegro is one of the most important areas for the commercial production of the pitaya, the fruit of *S. queretaroensis,* a species of arborescent columnar cactus endemic to central-western Mexico (Ibarra-Cerdeña et al. 2005; Pimienta-Barrios and Nobel, 1994). Home garden cultivation of *S. queretaroensis* has occurred since the late 1800s, while intensive commercial production of pitayas began in the 1970s (Pimienta-Barrios, 1999b). Low input requirements of water, fertilisers and pesticides result in a substantial financial return (Pimienta-Barrios, 1999b). Additionally, the tolerance of *S.*

queretaroensis to drought and poor soils, as well as the production of fruit in the dry season when other crops are scarce, make it a sustainable crop in the arid production area (Pimienta-Barrios and Nobel, 1994).

The municipality of Techaluta de Montenegro has an area of 79 km^2 (Mejía Rodríguez, 2012), nearly 40% of which is used for agriculture (INEGI, 2009). The main crops by registered volume (tonne) produced in Techaluta de Montenegro are alfalfa (13726 t), hay/pasture (4496 t), maize (3173 t), pitaya (719 t), avocado (700 t), sorghum (484 t) and squash (329 t) (SIAP, 2018). The pitaya generates the highest price per tonne of any crop grown in Techaluta de Montenegro, generating approximately Mex$19,200 / US$998 per tonne (SIAP, 2018). Registered pitaya production is expanding yearly, increasing by 71% from 420 t in 2003 to 719 t in 2018 (SIAP, 2018). This growth is driven by an increase in area under production (56 ha registered in 2003 to 86 ha in 2017; SIAP, 2018). Figures for both pitaya production and value are underestimates however, as much production is not officially registered with the government.

The main income streams for inhabitants of Techaluta de Montenegro are agriculture and remittances sent from relatives working abroad, who are prompted to leave the area by a lack of employment opportunities and low prices received for most agricultural commodities (Plan de Desarrollo Municipal, 2018). Despite the high prices received for pitayas, they can only provide income for a few months of the year. Nearly half the inhabitants of Techaluta de Montenegro are classed as living in poverty, with 49% having an income insufficient to provide wellbeing, and 80% with a lack of access to social security (CONEVAL, 2010; Appendix B.9). In 2015, the average daily wage in the wider state of Jalisco was Mx$267, across all employment types (CONASAMI, 2015).

3.2.2 Pitaya value chain

The key stages in pitaya production are cultivation, processing (harvesting, peeling fruits, making products), marketing, and consumption. Pitaya production in Techaluta de Montenegro is dominated almost entirely by small commercial plantations and home gardens (Pimienta-Barrios, 1999a). The value chain is short, due to the high perishability of the fruit (fruits must be eaten within one to two days of harvest) and subsequent localised market (Pimienta-Barrios, 1999b). Most fruits are sold fresh, but a small but increasing proportion is used to make products. Producers largely sell fruits directly to the consumer, either at the roadside or at a market. Actors commonly have multiple functions in the value chain, and the use of intermediaries (defined here as an agent that buys fruit from producers to sell to vendors) is rare (see Appendix B.1 for a more detailed overview of the stages in the pitaya chain).

3.3 Methods and data collection

We conducted our fieldwork in Techaluta de Montenegro (20.074°, -103.550°) during 2016 and 2017. The exclusion experiments we carried out to generate empirical data on changes in yield and fruit size between openly pollinated and pollinator-excluded pitaya crops are detailed in Chapter 2. Next, we collected quantitative production and marketing data from 61 pitaya producers (Section 3.3.1). We combined these data to estimate the economic value of bat pollination to the pitaya sector in Techaluta de Montenegro (Section 3.3.2). Then, to assess the distribution of economic benefits resulting from bat pollination services, we analysed economic data collected through structured interviews with a sample of representatives from each actor group involved in pitaya production (Sections 3.3.1. and 3.3.3).

3.3.1 Data collection: economic valuation and value chain analysis

We identified actor groups involved in the production of pitayas in Techaluta de Montenegro using semi-structured interviews with key informants, people previously identified to have expert or broad knowledge about the pitaya production sector (Newing, 2010). During the production season in 2017, we collected contact details of potential participants from each actor group by approaching actors at random in both the production area (Techaluta de Montenegro) and subsequent market areas (e.g. Guadalajara). We also used a snowball sampling technique whereby existing participants were asked to recommend other potential participants. Additionally, we randomly approached registered producers from a list of 189 provided by the municipality.

We then conducted structured interviews, using a standard set of pre-prepared interview questions. We asked participants for: characteristics of pitaya plantations and harvest; marketing and fruit prices; a detailed breakdown of financial costs and time spent on pitaya-related activities by both family members and employees; and details of socio-economic background. These topics were selected so we could fully determine aspects of income for each actor group (Kaplinsky and Morris, 2001; M4P, 2008; Sanogo, 2010). To validate responses, we asked each respondent several questions relating to total and monthly income, prices and profits. Interviews allowed accurate data collection while allowing participants privacy to discuss personal issues (Newing, 2010). We carried out pilot interviews in a neighbouring production town (Amacueca) in June 2017 to check and refine interview questions.

We carried out 124 interviews between July and August 2017. Interviews were conducted by trained volunteers and lasted between 40 minutes and 3 hours. Prior to starting the interview, we provided details of the project, data storage, and issues relating to anonymity and confidentiality, and obtained written consent from each participant. We had ethics approval from the University of Southampton ethics committee prior to carrying out data collection.

3.3.2 Economic valuation

To estimate the economic value of bat pollination V_b in pitaya production, we used a production value method (Winfree et al. 2011), which estimates the value of bat pollination assuming that there are no substitutes. This economic value is estimated using the following general model:

$$V_b = D \cdot P \cdot Y \qquad \text{(Eq. 1)}$$

where V_b is the economic value of bat pollination in pitaya fruit production, D is the crop's dependency on bat pollination (i.e. the fractional reduction in crop yield or quality in the absence of bat pollinators), P is crop price (expressed in Mex$ per fruit) and Y is crop yield (in fruits per producer).

Our exclusion experiments showed that bat pollination affects both fruit yield (Y), and fruit quality, in terms of size (Q). Thus, there are two separate elements to the crop's dependency on pollination: D_{yk} and D_{qkw}. We derived D_{yk} from the mixed effects model parameter estimates (see Chapter 2), indicating the difference between pitaya fruit set when bats were excluded (diurnal pollinators only) and fruit set with bats present, which varies across pitaya types k. We derived D_{qkw} from empirical data collected on changes in fruit weights in the absence of bat pollinators in exclusion experiments (Chapter 2 and section 3.3.2) and the subsequent impact on price, which varies across producers w and pitaya type k. Hence, V_b has two additive components:

$$V_b = V_{yb} + V_{qb} \qquad \text{(Eq. 2)}$$

where V_{yb} is the value of the fruit yield attributed to bat pollination (Eq. 3); and V_{qb} is the value of the fruit quality attributed to bat pollination (Eq. 4).

To calculate the value of the fruit yield attributed to bat pollination for each producer, we multiplied the proportion of fruits produced of each pitaya type ($\frac{Y_{kw}}{Y_w}$) by the crop yield dependency specific to each pitaya type (D_{yk}). We then summed the change in fruit yield across pitaya types and multiplied this proportion by the gross revenues from selling pitaya fruits (V_w). To calculate V_{yb}, we then summed the value of the change in yield attributable to bats across all pitaya producers (W) in the study area, i.e.:

$$V_{yb} = \sum^{W} \left(V_w \cdot (\sum^{K} (D_{yk} \cdot \frac{Y_{kw}}{Y_w})) \right) \qquad \text{(Eq. 3)}$$

Y_{kw} was inferred from total fruit production reported by the producer multiplied by the proportion of the cultivar/wild cacti under production.[1] The value of V_{ybw} therefore varies across producers, depending on each producer's total fruit production for each pitaya type (Y_{kw}), as well as their gross revenues from selling the fruits (V_w). We assumed an equal price for all fruits sold by each producer (i.e. the proportion of fruits sold per variety was taken as a proxy for the proportion of revenues per variety), as we did not have data on the number of fruits sold per producer in each price category or per cultivar. In reality, prices received by producers varied according to both fruit size and time of season; however, as producers sold the bulk of their fruits during the peak season for one price, and had fruit production dominated largely by one pitaya type (and therefore of a similar size), we deem this assumption defensible.

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[1] The inference was necessary because producers were unable to provide estimates of the total production or revenue per cultivar or the quantity sold per size (and thus price) category. For each producer, our dataset included: total quantity of fruits sold, gross revenues, number of cacti under production per cultivar, and average prices per fruit size (small, medium, large) and time in season (start, peak, end).

To calculate the value of the fruit quality attributable to bat pollination for each producer, we multiplied proportion of cacti produced of each pitaya type ($\frac{Y_{kw}}{Y_w}$) by the crop quality dependency specific to each pitaya type and producer (D_{qkw}). We then summed the change in fruit quality across pitaya types (K), and multiplied this proportion by the value remaining after subtracting the value of fruit yield attributable to bats from gross revenues from pitaya sales, $V_w - V_{ybw}$. To calculate V_{qb}, we then summed the value of the change in quality attributable to bats across all pitaya producers (W) in the study area, i.e.:

$$V_{qb} = \sum^{W}((V_w - V_{ybw}) \cdot (\sum^{K}(D_{qkw} \cdot \frac{Y_{kw}}{Y_w}))) \qquad \text{(Eq. 4)}$$

We assigned a null value for unstudied cultivars for both increase in fruit yield and size, which accounted for 13% of cacti under production overall.

To calculate D_{qkw}, we first collected data on the size of ten fruits in each of the small, medium and large size bands sold by the roadside in Techaluta de Montenegro in June 2018 to calibrate the weight ranges of fruits in different price categories. We then compared the proportion of fruits in small, medium and large size bands under the nocturnal and diurnal pollination treatments in our exclusion experiments for each pitaya type, and calculated the proportion of fruits that would drop to lower size bands for each pitaya type *k* in the absence of bat pollinators (Table 3.1). We assumed the most conservative size band changes by minimising the number of size bands dropped by fruits i.e. where a large fruit could have become either a medium fruit or a small fruit (as there were more fruits in both smaller bands without bat pollinators), we chose a drop of one band rather than two.

Table 3.1 Percentage of fruits that moved between each size band in the absence of bat pollination for each cultivar and wild cacti, based on weights of fruits collected from exclusion experiments under nocturnal and diurnal pollination treatments.

	Large: no change	Large → medium	Large → small	Medium: no change	Medium → small	Small: no change
Blanco	0	9	24	2	9	56
Mamey	33	0	47	0	0	20
Tenamaxtle	6	25	62	0	7	0
Wild	0	0	16	0	21	63

The drop in size bands implies that the total value of pitaya fruits *V* would be lower in the absence of bat pollination because the fruits would be smaller, and producers would obtain lower prices per fruit. We weighted prices received by each producer at the beginning, middle and end of the season by the approximate volume sold in each time-band. Dependency values were therefore specific to each producer and depended on the weighted prices that each producer could negotiate at each size band: for example, a producer that received the same price for large and medium fruits would have a lower dependency value attributable to the decrease in fruit size in the absence of bat pollination than a producer that sold large fruits for a higher price than medium fruits. We calculated D_{qkw} by multiplying the percentage of fruits that would change size in the absence of bat pollination for each price-size category for each pitaya type S_{qk} by the difference in prices received by each producer. We then summed the differences across the price-size categories (see Appendix B.2 for an example of this calculation):

$$D_{qkw} = \sum^{Q}(S_{qk} \cdot \frac{P_{wqo}}{P_{wqb}}) \qquad \text{(Eq. 5)}$$

where $\frac{P_{wq0}}{P_{wqb}}$ is the fractional change in price received for each pitaya type for each producer, with P_{wq0} indicating the price received per fruit in the absence of bat pollination (for size band q_0), and P_{wqb} indicating the price received per fruit with bat pollination (for size band q_b). S_k is based on the information in Table 3.1, and is the percentage difference in the number of fruits moving between each size band q per variety k in the absence of bat pollination.

To assess the contribution of bat pollination to employment in the pitaya sector, we estimated total extra jobs (J_b) generated by bat pollination by multiplying the total number of employees E of each producer by the proportion of revenue attributable to bats $\frac{V_{bw}}{V_w}$. For example, we assumed that a decreased revenue of 35% would result in a workforce decrease of 35%. Thus:

$$J_b = \sum^{W} \left(\frac{V_{bw}}{V_w} \cdot E_w\right) \qquad \text{(Eq. 6)}$$

where J_b is total extra jobs generated by bat pollination, and E_w is the number of employees of each producer.

To estimate the total gross value of bat pollination services to the pitaya sector in Techaluta de Montenegro, we identified all likely *S. queretaroensis* plantations within the municipal boundaries of Techaluta de Montenegro, using satellite imagery (Google Earth, 2019). We marked the plantations as polygons and exported them to ArcGIS to calculate the area covered in hectares

3.3.3 Value chain analysis

We used the data collected through interviews with different actors to understand the production, processing, marketing, and consumption stages of the pitaya value chain. To better understand the distribution of economic benefit provided by bat pollination services, we assessed the proportion of income attributable to bats, profit, and hourly earnings across actors.

We first estimated the proportion of income attributable to bats for each actor. For all actors that produced fruits themselves we extracted values for the percentage of income attributable to bats from changes in both yield D_{yk} and quality D_{qkw}, from our individual level data collected through interview questions on production and marketing (section 3.3.1). A mixed model from the exclusion experiment detailed in Chapter 2 provided an average estimate of D_y for individuals that did not produce fruits themselves. For actors whose income depended on the quantity but not quality of pitaya fruits, we assumed the proportion of their income attributable to bats was equivalent to D_y. This was assumed for waged workers (work availability depends on fruit volume, but we had no data on the specific volumes of fruits of each cultivar handled by their employers) and plantation owners that rented plantations to others (rent is calculated by number of fruits). For actors whose income depended on both quality and quantity of fruits (e.g. intermediaries and all types of vendors), but that did not produce fruits themselves, we calculated profit margins for small, medium and large fruits during peak production (as the bulk of fruits are sold during this time) by subtracting costs of buying fruits from prices received when selling fruits. We then inferred the overall volume of fruits of each cultivar in the market from the overall proportion of each cultivar under production across our sampled producers; and used data collected in section 3.3.2 on the proportion of fruits of each cultivar in each of the small, medium and large size categories (Table 3.1) to estimate the overall proportions of fruits in the market of each size category with and without bat pollination. We multiplied the proportion of fruits in each size category by the profit margin calculated for each actor, in scenarios of selling 100 fruits in both bat pollinator presence and absence, and took the difference between the two as the per cent increase in profit attributable to increased fruit quality with bat pollination. The proportion of income attributable to bats for product makers was assumed to be equivalent to D_y, as the prices of products did not vary according to the size of fruit used to make them.

We then calculated profit earned by each individual interviewed by subtracting direct costs incurred by pitaya-related activities (costs of renting pitaya plantations, agricultural inputs, salaries and compensations for employees or family members, marketing, transport, tools and equipment, loans, buying pitayas) from gross pitaya income (the sum of any income generated by selling pitaya fruits V, pitaya flowers, and/or pitaya products, as well as income generated by renting out pitaya plantations). Fixed costs e.g. of establishing pitaya plantations were not included in our calculations of costs and profits. For waged workers, costs (e.g. commuting, food, tools and equipment, maintenance) were subtracted from the hours worked in the season multiplied by the hourly wage received.

Finally, we calculated the profit attributable to bats by multiplying profit by the proportion of income estimated to be attributable to bat pollination services. Estimates of profit attributable to bats involved an assumption of constant variable costs per fruit (though we acknowledge that marketing and transport costs will probably not decrease linearly with decreased production).

To incorporate the number of dependents reliant on pitaya-generated income across actor groups, we calculated the per capita monthly income of actors by dividing monthly income by the number of people living in each household. To elucidate the trade-off between profits, working hours and reliance on unpaid labour by family members, we calculated the hourly wages of each actor group by dividing total profit by total hours worked unsalaried on pitaya-related activities by the respondent or family members; except for waged workers where fixed hourly wages received are reported.

To understand the importance of pitaya-generated income, we collected data on whether respondents used it for direct household provisioning or were able to save or invest it for long-term benefit, for example by spending it on school fees. We also asked about other income generating activities throughout the year, and the proportion of yearly income generated by the pitaya. We evaluated constraints to access profitable roles in the pitaya chain by combining qualitative interview data with quantitative costs data.

We tested for differences between groups in profit, hourly wage and per capita monthly income with a Kruskal-Wallis test followed by non-parametric (Dunn) pairwise tests (using R packages ‘FSA’ and ‘rcompanion’; Mangiafico, 2019; Ogle et al. 2019). We also calculated the Gini coefficient between groups in profit and hourly wage (using R package ‘DescTools’; Signorell, 2019). The Gini coefficient is the most commonly used measure of inequality, ranging from 0, indicating complete equality between groups, to 1, indicating ultimate inequality (Stuart and Ord, 1994). Statistical analysis was done in R v. 3.5.3., using R packages ‘dplyr’, ‘tidyr’ and ‘Rmisc’ (Hope, 2019; R Core Team, 2019; Wickham and Henry, 2019; Wickham et al. 2019).

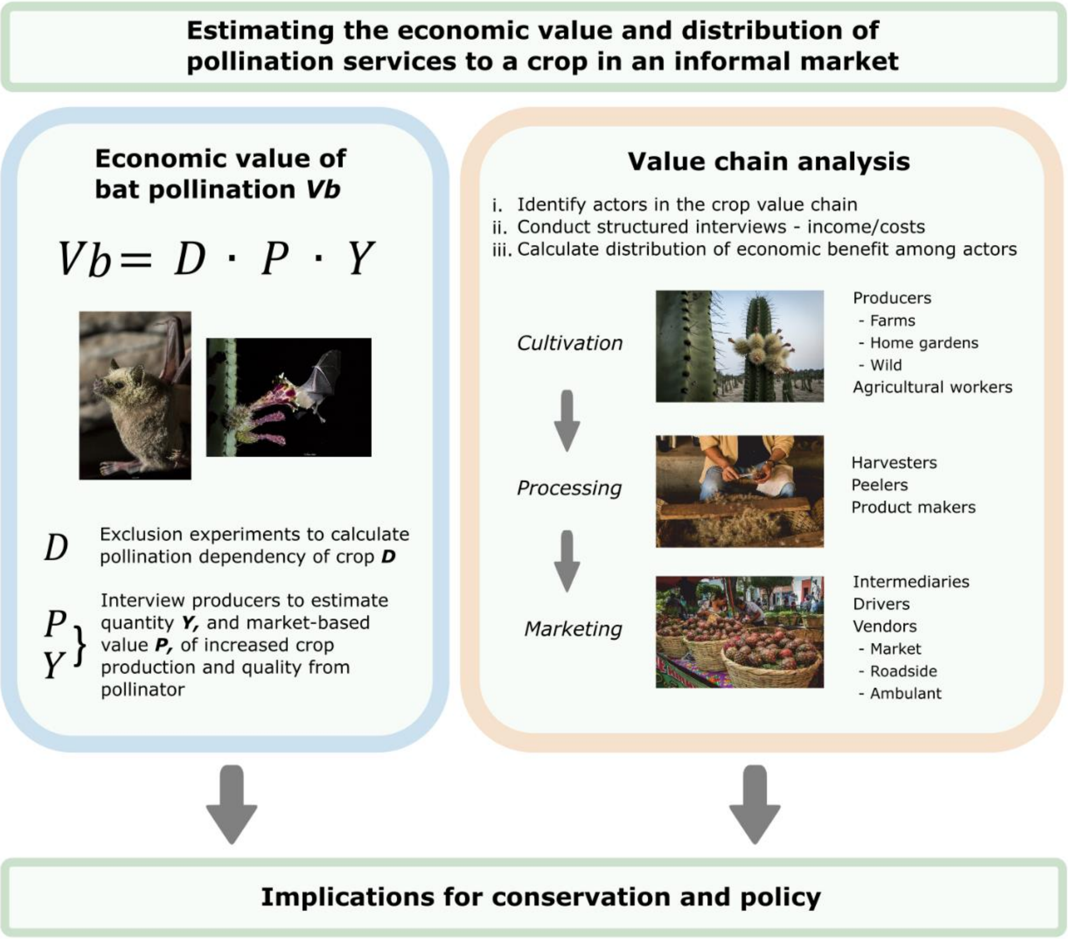


Figure 3.1. Synthesis figure of the valuation approach.

3.4 Results

3.4.1 Economic value of bat pollination service to pitaya production in Techaluta de Montenegro

Pollination by bats resulted in a greater probability of fruit set compared to other taxa in our exclusion experiment, increasing overall probable yield by 35% when averaged across cultivars and wild cacti (GLMM: $\chi^2 = 286.7$, $P < 0.0001$; Tremlett et al. 2020). However, the dependence on bats for fruit set varied between cultivars. Yield increased by 27% for Mamey (GLMM: $p < 0.001$) and 35% for wild individuals (GLMM: $p = 0.002$), but there was no effect of bat pollination on yield for Tenamaxtle (GLMM: $p = 0.65$) and Blanco (GLMM: $p = 0.60$) individuals. Crop dependency on bat pollination D_{yk} was therefore 0.27 for Mamey, 0.35 for wild, and zero for Blanco and Tenamaxtle individuals; and 0.35 when averaged across cultivars D_y. Neither the closed pollination nor pollination by nocturnal insects treatments resulted in fruit set.

Fruit weight decreased by 46% in the absence of bat pollination across all exclusion experiment fruits (excluding the two treatments that did not set fruit and could therefore not be included in analyses of crop quality). The dependence of the pitaya crop on bat pollinators for quality D_{qkw} varied with producer, as it depended on the price charged for fruits of different sizes, but the impact on price was highest for Mamey and Tenamaxtle cultivars, which dropped one or two price bands when bats were excluded (Table 3.1; Fig. 3.2a).

Of the 61 pitaya producers interviewed, 39 owned pitaya plantations, 40 rented pitaya plantations and 20 owned home gardens (some respondents produced fruit under more than one system). The total area under production for each producer ranged in size from 0.03 to 12 ha (mean = 2.58 ha), and fruit production Y_w ha^{-1} ranged from 4,200 fruits ha^{-1} per season to 633,300 (Table 3.2). The most commonly managed cultivars of *S. queretaroensis* were Mamey (63% of total cacti under production across producers interviewed), Tenamaxtle (7%) and Blanco (7%); as well as wild cacti (10%).

Bigger fruits command higher prices than smaller fruits (Fig. 3.2b). Vendors separate fruits into large, medium and small categories, with some adding categories at the extreme (tiny, jumbo). There is no minimum size for a pitaya fruit to enter the market. No other fruit characteristics (e.g. cultivar) affected fruit price at markets we visited. Weights of small fruits measured at markets in 2018 ranged between 21.7 and 42.1g (n = 10), medium fruits between 56.3 and 69.5g (n = 10), and large fruits between 68.1 and 90.6g (n = 10). Fruit prices are highest at the beginning of the season (late May), when there is less fruit available and consumer demand is greatest (Fig. 3.2b). Prices are lowest during peak production (June).

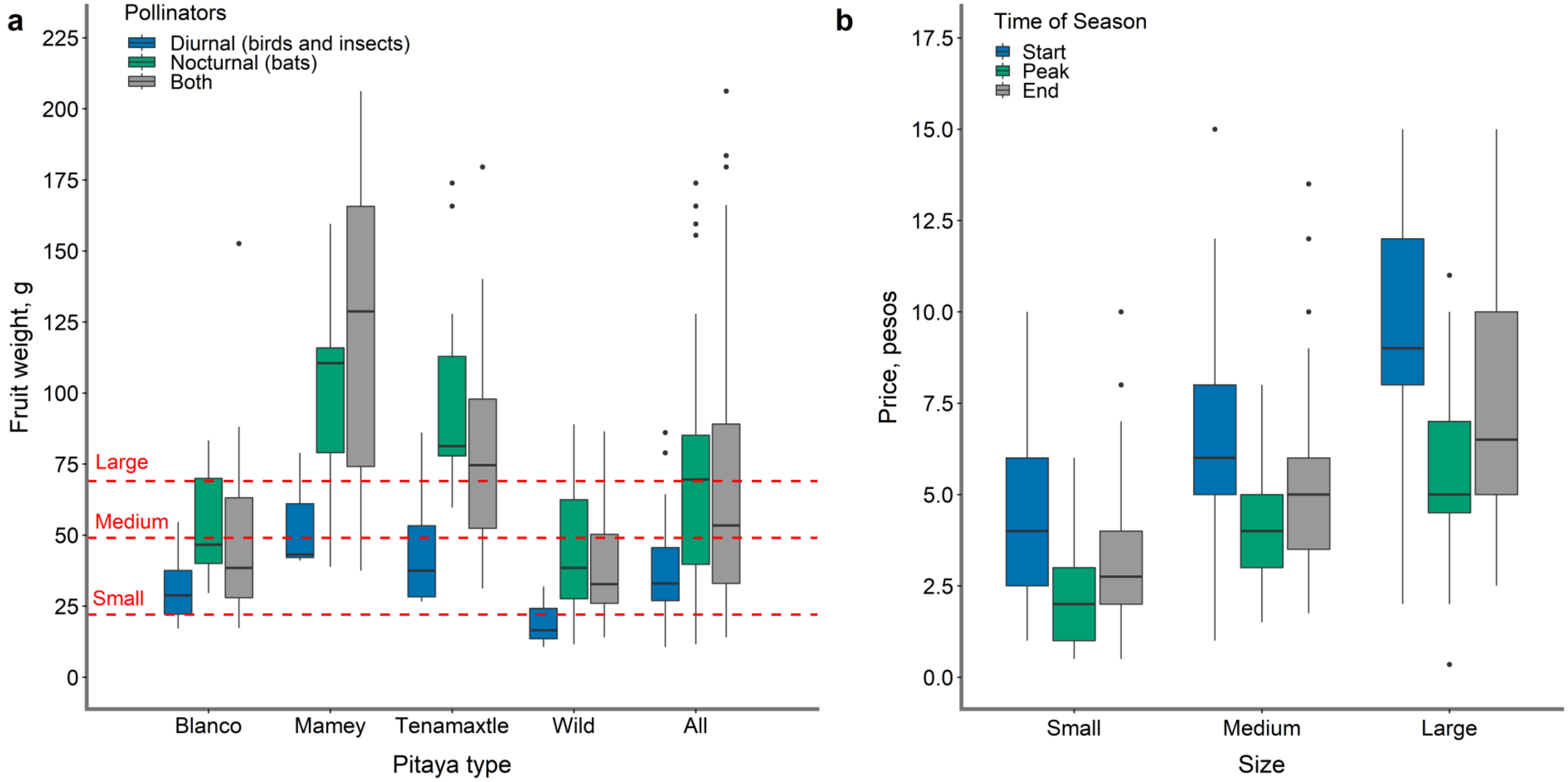


Figure 3.2. a) changes in fruit weight observed in exclusion experiments in 2016 between diurnal and nocturnal pollinators. Red dashed lines indicate lower weight boundaries of different price classes observed in markets in 2018 (small, medium and large); b) final prices (charged to the consumer) of fruits of different price classes (small, medium and large) at different times of the season in 2017: start = late May; peak = June; end = early July

Increased fruit yield resulting from bat pollination across the 61 producers interviewed had a mean total value (before costs) V_{ybw} of Mex$39,900 per producer (range: Mex$600 to 320,300 / US$32 to 16,700; Table 3.2). The mean value of increased fruit size resulting from bat pollination V_{qbw} was Mex$39,500 (range: Mex$0 to 298,400 / US$0 to 12,500; Table 3.2) per producer interviewed. Thus, by increasing fruit yield and size, bat pollination has a mean total market value V_{bw} of Mex$79,300 per producer, or Mex$48,400 (US$2,530) per ha (range: Mex$1700 to 246,400 / US$87 to 12,900; Table 3.2).

The percentage of gross crop value attributable to bat pollination ranged from 5% to 58% across interviewed producers, with bats contributing a mean 39% (± 12 SD) of gross revenues from fruit sales per producer (Table 3.2), or 42% of total gross income summed across producers. Producers with a higher proportion of Mamey and wild cacti were more dependent on bats for total income, because fruit yield increased with bat pollination relative to diurnal pollination for Mamey and wild cacti, but not Tenamaxtle and Blanco. Additionally, producers that received higher prices for large Mamey and Tenamaxtle fruits than medium or small fruits benefited more from bat pollination, as fruits dropped one or two size-price bands in the absence of bat pollination.

Table 3.2. Characteristics of pitaya production and value of bat pollination services across the 61 interviewed producers.

	Size of plantation, ha	Y_w ha^{-1}, # fruits	V_w, Mex$	Price of a small fruit[1], Mex$	Price of a medium fruit[1], Mex$	Price of a large fruit[1], Mex$
Mean ± SD	**2.58** ± 2.83	**51,547** ± 90,914	**187,895** ± 254,146	**2.0** ± 1.0	**3.5** ± 1.2	**5.0** ± 2.0
Range	0.03 – 12.00	4233 – 633,333	4,500 – 1,350,000	0.5 – 5.2	2.0 – 7.3	1.9 – 10.5

	Y_{bw}, # fruits	V_{ybw}, Mex$	V_{qbw}, Mex$	V_{bw}, Mex$	V_{bw} ha^{-1}	% V attributable to bats
Mean ± SD	**12,447** ± 18,743	**39,861** ± 59,915	**39,460** ± 58,356	**79,321** ± 116,023	**48,405** ± 53,112	**39** ± 12
Range	335 – 94,920	610 – 320,355	0 – 298,399	610 – 618,754	1660 – 246,393	5 - 58

[1] Prices weighted by approximate volume sold at different times during the season (different prices are received by farmers at the beginning, middle and end of the season; see Fig. 3.2b).

Y_w ha^{-1}: total number of fruits produced each year (yield) per hectare. **V_w**: gross revenues from fruit sales. **Y_{bw}**: total yield attributable to increase in fruit set with bat pollination relative to other taxa. **V_{ybw}**: total value of yield increase with bat pollination per producer. **V_{qbw}**: total value of size increase with bat pollination per producer. **V_{bw}**: total value of yield and size increase with bat pollination. **V_{bw}** ha^{-1}: value of bat pollination per hectare of pitaya plantation. **%V**: percentage of gross revenues from fruit sales attributable to increases in yield and size of pitayas due to bat pollination.

We estimate that income attributable to bats for the 61 producers interviewed generated approximately 129 extra jobs further down the production chain (e.g. peelers, harvesters), though we acknowledge that job creation is not linearly associated with income. The number of paid workers employed by producers ranged from 0 to 33.

We classified 190 ha of pitaya plantations within the municipal boundaries of Techaluta de Montenegro from satellite images. This is likely to be an underestimation of the likely total area, as we could not distinguish spatially dispersed wild cacti and cacti grown in home gardens. Thus, we conservatively estimate the total gross value of bat pollination services to the pitaya in Techaluta de Montenegro to be approximately Mex$9,200,000, ranging between Mex$315,000 and Mex$46,800,000 (US$480,000: between US$16,500 and US$2,450,000).

3.4.2 Value chain

3.4.2.1 Income and employment

Jobs generated by pitaya production are a chief source of employment in an area lacking many other opportunities and provide an important source of income and a strategy to diversify livelihoods (see Appendix B.3 for a description of all actors and their roles). The pitaya was cited as the principal source of income by 49% of respondents, though only one household was completely reliant on the pitaya; all other households had multiple income streams. Participation in the pitaya chain is therefore a ‘gap-filling activity’ for most people: one that provides a seasonal income during the period of low agricultural activity, thus increasing its relative importance and compatibility with other livelihood activities (Marshall et al. 2006). The actor groups most heavily dependent on pitaya-generated income over the year, and therefore bat pollination services, were intermediaries and market vendors (an estimated 55% and 46% of yearly income respectively), with waged workers reporting between 15% (drivers) and 26% (harvesters) of yearly income coming from work with pitayas (Table 3.3).

However, the pitaya chain is characterised by informal, verbal contracts: just 33% of fruit sellers and 45% of waged workers had a contract arranged prior to the fruiting season, and all were verbal. Participation in the pitaya value chain thus precludes permanent, formal work with benefits such as health insurance and pensions that only accrue to workers in continuous employment, creating a lack of social security for most actors. Despite this, the lack of technical entry requirements, instant generation of cash at low times of the year, and higher wages relative to other low-skilled jobs, makes the pitaya sector an attractive employment option for resource-poor people. Working with pitayas offers a higher daily rate during the pitaya season than many other concurrent available job opportunities, such as agricultural day labouring (Mex$200 per day) or jobs tending plants in large greenhouses that grow berries for the export market (Mex$120 per day).

The discrepancy between the highest and lowest mean hourly wages of actors in the value chain (Gini coefficient = 0.67) indicates inequality in the distribution of both economic benefits and labour costs between actors. The low agricultural requirements of the cacti result in a low labour cost for landowners, particularly those that rent plantations to others for the production season. Actors that had multiple functions in the value chain, such as market vendors that produced and sold fruit themselves, commonly worked very long hours of up to 22 hours a day. The mean hourly wage of plantation owners who rented plantations to others was 22.6 times higher than that of peelers and 5.4 times higher than that of market vendors (Mex$543, Mex$24 and Mex$101 per hour respectively; Table 3.3).

Table 3.3. Income indicators for the different actor groups.

[1] Actor	Peelers*	Agricultural workers*	Ambulant sellers	Drivers*	Product makers	Harvesters*	Home garden owners	Roadside vendors	Plantation owners -do not rent out	Market vendors	Producers – sell fruit with spines	Producers – sell peeled fruit	Intermediaries	Plantation owners – rent out	**Kruskal – Wallis test** [3]
N	12	6	5	4	9	11	20	31	30	19	8	4	4	9	
Income indicators based on calculations in section 3.4:															
Wage/Profit*, Mex$ ± SE	17,201 ^{ab} ± 3,856	7,500 ^{a} ± 2,869	45,156 ^{bcd} ± 6,214	10,369 ^{ab} ± 5,331	27,277 ^{abc} ± 7,048	12,126 ^{a} ± 1,413	49,751 ^{bc} ± 11,717	57,531 ^{c} ± 13,818	78,083 ^{cd} ± 18,504	125,590 ^{d} ± 24,979	17,505 ^{ab} ± 7,350	127,099 ^{bcd} ± 90,386	96,419 ^{cd} ± 47,984	102,409 ^{cd} ± 51,970	$\chi^2 = 64.2$, df = 13, $\boldsymbol{p < 0.0001}$
Hourly wage Mex$ ± SE	24 ^{b} ± 2	25 ^{ab} ± 0	31 ^{ab} ± 8	32 ^{abc} ± 9	35 ^{ab} ± 8	39 ^{abc} ± 7	47 ^{ab} ± 8	47 ^{b} ± 12	90 ^{ab} ± 25	101 ^{abc} ± 24	125 ^{abc} ± 55	165 ^{abc} ± 135	183 ^{ac} ± 61	543 ^{c} ± 234	$\chi^2 = 34.2$, df = 13, $\boldsymbol{p = 0.001}$
Income indicators based on answers to interview questions:															
Per capita monthly income Mex$ ± SE	1003 ± 198	1327 ± 217	1410 ± 370	1234 ± 115	2003 ± 653	1436 ± 228	1664 ± 382	1459 ± 220	2150 ± 409	2921 ± 871	1881 ± 189	1879 ± 221	2917 ± 896	3767 ± 1444	$\chi^2 = 11.0$, df = 13, $p = 0.61$
[2] **Per cent yearly income from pitaya**	23	23	23	15	33	26	35	36	45	46	32	35	55	37	

See following page for footnotes.

[1] The majority of respondents belonged to multiple actor groups, so individual data may be used for several groups (e.g. plantation owners that are also market vendors). Product makers here are those that did not also sell fruits (i.e. were solely product makers). Producers here are those that produce fruit but do not sell it directly to the consumer, but instead to another vendor or intermediary, either peeled or with spines.

*Waged workers.

[2] Per cent of yearly income from the pitaya calculated from the average category rank that actors reported during interviews in answer to the question "What percentage of your average annual income comes from the pitaya?" (1= 0-20%, 2= 20-40%, 3= 40-60%, 4= 60-80% and 5= 80-100%). The mid-point of each category range was used.

[3] Unlike letter superscripts indicate significant differences between mean incomes based on non-parametric (Dunn) pairwise tests at $p < 0.05$, using the Benjamini and Hochberg correction (using R packages "FSA" and "rcompanion" Mangiafico, 2019; Ogle, Wheeler, & Dinno, 2019).

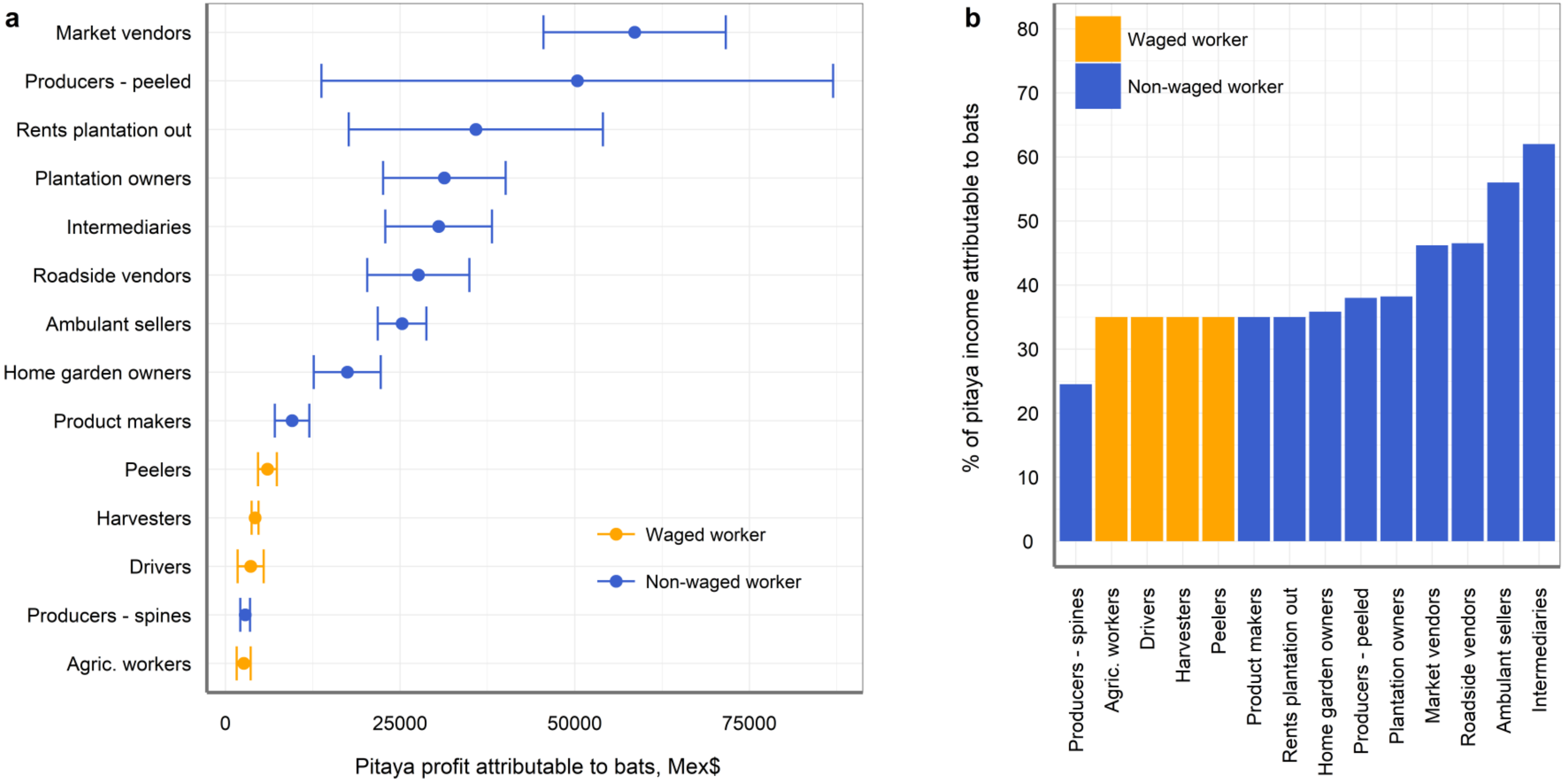


Figure 3.3. a) The profit in Mex$ attributable to bats (±SE) across actor groups, calculated by multiplying profit by the proportion of income attributable to bats for each actor (for waged workers, ‘profit’ is wage received multiplied by hours worked, minus costs), and b) the mean percentage of pitaya-generated income estimated to be attributable to bats for each actor group.

3.4.2.2 Costs

Wages and benefits are a major cost for all the different actors except intermediaries (Appendix B.4). Transport costs (predominantly petrol) and rent are important costs for marketing actors. The costs incurred by intermediaries and market vendors are the highest, while plantation owners have among the lowest costs, thanks to the low agricultural inputs required (Appendix B.4). A mean of Mex$1,260 per ha per year (US$66) was spent on compost, fertilisers, herbicides and pesticides combined. However, there is a high initial fixed cost of establishing pitaya plantations, representing a significant barrier to entry for other actors. Establishment costs are between approximately Mex$9,460 – 72,300 per ha (US$494 – 3,780 per ha), excluding the price of buying land, consisting of the costs of labour and buying cactus branches to plant. Furthermore, there is then a lag time before fruit production of up to 10 years. Access to formal credit is low: six percent of waged workers had access to credit and thirteen percent of non-waged workers. There was no significant difference between actor groups in per capita monthly income (Table 3.3), though those that earned the highest (plantation owners that rent their plantations out to other people, Mex$3,770 ± 1444 SE) had a per capita monthly income of nearly four times those who earned the lowest (peelers, Mex$1,000 ± 198 SE), indicating that access to land may be captured disproportionately by an already economically privileged group.

The majority of the income (84%) associated with pitayas accrues to the local community and is retained as cash income, supporting household activities (Appendix B.5). Cash income generated from the pitaya was allocated to: household food (71% of respondents), rent and bills (54%), investment back into pitaya or other businesses (40%), savings (37%), household goods (36%), childrens' education (30%) and other uses including medical bills and paying debts (19%). Little pitaya-generated income is passed onto the government (7%) as few taxes are paid; most government revenue results from actors buying petrol from the state-owned distributor (Appendix B.5). External agents, for example suppliers of packaging or agricultural inputs, accounted for the remaining 9% of pitaya-generated income (Appendix B.5).

3.4.2.3 Profits

The distribution of profits between actors was unequal (Gini coefficient = 0.60). The highest profits (income minus direct costs) were gained by market vendors who both produced fruits and sold them directly to the consumer, achieving the highest final fruit prices (Table 3.3; Appendix B.6). However, intermediaries, producers and plantation owners all earned a higher hourly wage (Table 3.3) indicating the high labour cost (long working hours) of market vendors. Additionally, many market areas have become saturated, with vendors citing too much competition from other sellers as a primary obstacle to making profit. The barriers to accessing the most profitable marketing situations are access to a vehicle and obtaining selling permits. Plantation owners that rented plantations to others achieved both the highest hourly wage and the second highest profit. As the plantations require little maintenance or input of resources, profit margins are good both for owners renting pitaya plantations out for the season for a fixed sum of money, and for those that harvest and sell the fruit themselves.

Producers that sold peeled fruits to other vendors could earn very high profits but there was substantial variation across respondents (Table 3.3). Profits earned by this group in our study are biased by one producer that had a very high production and took the fruits to Guadalajara to sell direct to market vendors; producers that sold to vendors or intermediaries in Techaluta earned much lower profits. The localised nature of the pitaya market results in a good level of market information throughout the chain and enables direct market access by most actors. This increases the power of producers to earn a fair price and results in intermediaries being uncommon, who frequently earn excessive profits in value chain assessments (Marshall et al. 2006). Nonetheless, the few intermediaries active in the pitaya chain earn a high profit due to the large number of fruits traded, despite earning the lowest profit margin on fruits (Table 3.4) and having the highest costs (Appendix B.4).

A substantial part of pitaya-generated profit for all actor groups could be attributable to the impacts of bat pollination on crop yield and quality (Fig. 3.3a and b). Actors whose profits depended on the quality of fruits as well as quantity were more dependent on bat pollination services than actors who depended on quantity only, as profit margins per fruit decreased with fruit size (Table 3.4), and fruits were smaller in the absence of bat pollination. Intermediaries, and ambulant, roadside and market vendors had the largest mean percentage of profits attributable to bat pollination (62, 56, 47 and 46% of profits respectively; Fig. 3.3b). Actors with the highest value of profit attributable to bat pollination services however, were those that earned the most from working with pitayas: market vendors, producers and plantation owners (Fig. 3.3a; Table 3.3).

Table 3.4. Profit margin (Mex$) per fruit of each size category during peak production (± SD) for actors buying fruit to sell rather than producing their own (cost of buying fruit subtracted from sale price received for fruit).

	Small Mex$	**Medium** Mex$	**Large** Mex$
Intermediaries	0.3 ± 0.3	0.6 ± 0.4	1.1 ± 0.8
Ambulant vendors	1.5 ± 0.4	2.3 ± 1.1	3.6 ± 1.2
Roadside vendors	1.3 ± 2.5	3.3 ± 1.8	4.3 ± 1.1
Market vendors	2.5 ± 0.0	4.1 ± 0.5	6.8 ± 2.5

3.5 Discussion

Our study used an interdisciplinary approach to examine both the value of the direct impacts of bat pollination on crop yield and quality, as well as a disaggregated analysis of the distribution of the economic benefits among actors. We found the value of bat pollination services to be worth approximately US$480,000 in the municipality of Techaluta de Montenegro alone, highlighting the great importance of bat pollinators for the welfare of the rural production region, and the severe economic consequences should bat pollinator populations decline.

L. yerbabuenae populations suffered severe declines in the 1980s, resulting from persecution and disturbance at roosts and loss of foraging habitats (Medellín, 2016). A conservation recovery programme has successfully used environmental education and roost protection schemes to increase population sizes, resulting in delisting of the species by both the Mexican and US governments (Trejo-Salazar et al. 2016; US Fish and Wildlife Service, 2018); though the species remains classified Near Threatened by the IUCN Red List (Medellín, 2016). However, it is vital that public awareness of the ecosystem services provided by bats continues, such as the contribution of bats to food security. This is particularly pertinent in the light of the recent Covid-19 pandemic that has widely negatively associated bats with the virus, driving new threats to bat populations (Fenton et al. 2020; Zhao, 2020; Lu et al. 2021). Our own recent engagement with inhabitants of the pitaya production area indicates growing concern about subsequent negative public perceptions of bat-pollinated fruits.

Economic valuations are one way of raising awareness of the unseen benefits of bats, with local context-specific research providing useful and relevant information to decision makers (Ninan & Inoue, 2013). The value of pitaya-generated income is significant in an area where 49% of people have an income insufficient to provide wellbeing (CONEVAL, 2010). Among individual pitaya farmers within our study region, we found considerable variation in dependence on bat pollination for income, highly impacted by the cultivars grown and the prices charged for fruits of different sizes. However at the community scale, our research showed pitaya production to be heavily dependent on bats, particularly that of the most economically important cultivar; with the spatial and genetic structure of pitaya plantations likely exacerbating the reliance on bat pollinators (Tremlett et al. 2020).

Our multi-faceted approach to estimate the value and distribution of pollination services may be useful for other animal-pollinated crops; particularly those in less formal markets where a lack of registered data on crop production or the value chain necessitates the collection of primary data. We found that pollinator-mediated changes in fruit quality had a high impact on the estimated value of pollination services, demonstrating the importance of conducting detailed field experiments to generate empirical data on the dependency of both crop quality and yield on different pollinators, as well as including multiple cultivars in study designs (Melathopoulos et al. 2015).

Additionally, we have shown that value chain analysis is a useful approach for the evaluation of the social distribution of economic benefits received from ecosystem services, allowing explicit analysis of inequities in income among actor groups and constraints to access roles (Gundimeda et al. 2018; Zhang et al. 2018). To our knowledge there has been no such attempt to disaggregate benefits from pollination services between actors for any crop (Suich et al. 2015). We found that access to the bat pollination service did not have an equalising impact; with some actors receiving a disproportionate share of economic benefit or labour costs, and the chain characterised by a lack of social security throughout. The change of the pitaya from a communally collected resource to an individually owned commodity may disadvantage poorer actors who lack the land or capital to establish plantations themselves or access profitable markets, despite an overall increase in economic wellbeing at the community level (Marshall et al. 2006; Kamanga et al. 2009). Laterra et al (2019) found a lack of financial capital to be the most important source of inequality in access to ecosystem services across Latin America; inequality then increases over time as access to land gradually decreases with resource commercialisation. At the same time, the ease of entry to the pitaya chain (low technical entry requirements, a local market) may lead to excessive competition between small-scale producers and vendors in the production area, limiting profitability.

3.5.1 Conservation and policy implications

Communicating the economic benefits provided by bats helps to raise awareness among the public and policy makers of the importance of bat conservation actions (Cleveland et al. 2006; Boyles et al. 2011; Kunz et al. 2011). Community environmental education programmes can be an important tool to improve understanding of bats by generating more positive attitudes shaped by the benefits bats provide, rather than the damage they may cause (for example by vampire bats, *D. rotundus*, which can transmit bovine paralytic rabies to livestock in Latin America) (López-del-Toro et al. 2009).

Those actors who benefit the most from bat pollination services may be best placed to contribute to bat conservation practically (e.g. land owners) and economically (e.g. consumers). At a local practical level, protection of bat roosts and avoidance of persecution (many bats are killed under the mistaken assumption that they are vampires) will benefit bat populations, maintaining both the provision of pollination services and other bat-mediated ecosystem services such as seed dispersal and pest suppression (Kunz et al. 2011; Williams-Guillén et al. 2016). Additionally, to maintain the provision of bat ecosystem services in pitaya plantations, it is vital that the intensification of the pitaya sector does not result in increased use of pesticides and other agrochemicals. Pitaya production currently is largely small-scale and organic; however, production is expanding yearly, with attempts to export the fruits internationally. Pesticide exposure can have various lethal and sub-lethal effects on bats, including disruption of hormones and the immune system, reproductive failure, and changes to behaviour (Bayat et al. 2014). We found consumers of pitayas to have a higher monthly income and level of education than any of the actors involved in the production chain (Appendix B.3), suggesting that they can afford to contribute to initiatives such as a ‘bat-friendly’ pitaya label (e.g. see Trejo-Salazar et al. 2018: bat-friendly tequila). Such initiatives could add a small surcharge to pitaya prices to feed into conservation efforts such as environmental education programmes or the installation of protection at roost sites.

Until now, there have been no direct economic valuations of bat pollination services provided to crops, though several studies have estimated the value of crop pest suppression by bats. Bat-mediated pest control has been valued between $0 (for coffee and cacao) and $183 (cotton) per ha, representing 0% and 31% of the total crop value respectively (Taylor et al. 2018). The higher value of bat pollination (US$2,500 per ha) revealed by our study suggests that this may be a more effective economic argument for bat conservation in some areas.

This research also has important policy implications for equitable development. In order to ensure that benefits from bat pollination are distributed more fairly across actors, activities could be started at the community, government or NGO level, such as: selling fruits or products collectively; opening up new markets (with assistance to cope with any resulting extra certification or tax requirements) or improving access to existing markets; supporting new actors financially to establish plantations; supporting the introduction of a low-entry health insurance; increased coordination between pitaya farmers; and providing training and equipment to increase product-making capacity. This may become increasingly important as the pitaya sector continues to expand, particularly as it is drought tolerant and therefore likely to be a more sustainable long-term crop than others which require high levels of irrigation, such as avocadoes and berries (Pimienta-Barrios and Nobel, 1994).

3.5.2 Limitations and knowledge gaps

Fruit set and fruit quality between pollination treatments may vary between years, impacted by fluctuations in climate and pollinator availability (Melathopoulos et al. 2015). Economic value will also fluctuate with changes in market prices, and institutional or external environmental factors (López-Hoffman et al. 2014). Nonetheless, our research has clearly demonstrated the economic importance of bats for the pollination of a highly valuable agricultural product.

Additionally, the production value method assumes that crop prices will be unaffected by decreased supply in the case of pollinator loss, and that farmers cannot compensate for reduced pollination supply by reducing input costs or employing substitutive pollination (Winfree et al. 2011). Techaluta de Montenegro contributes 40% of registered pitaya production in Jalisco (SIAP, 2018) and therefore price increases may be seen with decreased fruit supply. However, the pitaya is already a highly priced luxury fruit, and 67% of consumers interviewed in our study said that they would buy fewer pitayas if the price increased. Input costs are already low for pitaya producers and it is unlikely they could be reduced further without loss of employment. Furthermore, bats are wild pollinators that cannot be replaced by a managed service, e.g. from rented bee hives; and the cost of hand-pollination is likely to be prohibitive (Partap and Ya, 2012), though cost estimates are not available for this crop.

It was beyond the scope of this study to consider the distribution of benefits received by actors other than income. Poverty and wellbeing are complex and context dependent, now commonly described with multi-dimensional factors encompassing human and social deprivations as well as economic (Suich et al. 2015). For a better understanding of the impact of bat pollination services on wellbeing, the effect of pitaya-generated income on other objective elements of well-being (such as access to health services), and subjective elements (such as cultural importance or contribution to sense of identity) would need to be quantified.

3.6 Conclusion

The consequences of losing bat pollination services to pitaya production in Techaluta de Montenegro would be severe. By enhancing fruit production and fruit size, bat pollinators contributed around 40% of the total gross income of interviewed pitaya producers in the area, equivalent to US$2,500 per ha annually. This value reflects the high level of dependence of the pitaya crop on bat pollinators for both yield and quality; as well as the high prices achieved for pitayas. The reliance of local employment and income on pitaya production, and thus bat pollination services, is a strong argument for the conservation of bat populations in the production area. However, our value chain analysis showed that barriers to access the most profitable roles should be reduced to enable a fairer distribution of economic benefits among actors, which are currently disproportionately captured by groups already economically or socially advantaged.

3.7 Authors' contributions

MS, VZG and KSHP conceived the research, CJT and MS designed the data collection, CJT collected the data with input from VZG, CJT did the analyses with input from MS, and CJT wrote the paper with inputs from all co-authors, particularly MS. All authors gave final approval for publication.

Chapter 4 Seasonal diet of the lesser long-nosed bat (*Leptonycteris yerbabuenae*) in central Mexico and implications for pollination services

Abstract

The lesser long-nosed bat (*Leptonycteris yerbabuenae*) is the principal pollinator of pitaya fruits (*Stenocereus queretaroensis*: Cactaceae), a major cash crop in central Mexico, enhancing both fruit yield and quality. However, little is known about the year-round diet of *L. yerbabuenae* bats in the pitaya production area and subsequent conservation implications for pollination services provided to the pitaya crop. I collected pollen and faecal samples bimonthly across one entire year from a population of *L. yerbabuenae* and used metabarcoding to identify the plant taxa comprising the diet of *L. yerbabuenae* across seasons.

L. yerbabuenae consumed a range of plant taxa throughout the year, with the highest diversity of plant species consumed during the dry season (November to May). Pitaya flowers were an important resource during the dry season, with a high frequency of *S. queretaroensis* pollen in February (found in 73% and 65% of faecal and pollen samples respectively). However, *L. yerbabuenae* also diversified its diet during the pitaya flowering season, indicating that supplementary food sources were necessary. Plant taxa found in tropical deciduous forest formed an important part of the diet throughout the year, such as canopy trees in the Malvaceae (Bombacoidae), Convolvulaceae, Acanthaceae and Capparaceae plant families.

This research highlights the mutually beneficial relationship between the pitaya crop and its main pollinator; but also emphasises the reliance of *L. yerbabuenae* on plant taxa found in tropical deciduous forest, which is being lost to make way for more pitaya plantations.

To effectively conserve *L. yerbabuenae* and its pollination services in the study area, it is vital to protect patches of different types of vegetation, including tropical deciduous forest, to provide sufficient food resources year-round.

4.1 Introduction

Pollination is a crucial process for maintaining ecosystem function and biodiversity, and is one of the most vulnerable stages to disturbance in the life cycle of plants (Neuschulz et al. 2016). Bats provide an important ecosystem service by pollinating plants, visiting flowers for nectar and pollen rewards and subsequently transferring pollen between individuals, facilitating gene dispersal (Kunz et al. 2011). Nectar-feeding bats are essential to maintain the functioning of many natural and agricultural ecosystems in the tropics (Kunz et al. 2011).

In Mexico, nectar-feeding bats are keystone pollinators of much of the dominant vegetation in arid zones and tropical forests, including columnar cacti (Cactaceae), paniculate agaves (Agavaceae) and canopy trees in the Malvaceae (sub-family Bombacoidae, formerly known as the Bombacaceae) plant families (Soriano and Ruiz, 2002; Fleming et al. 2009). Products from many bat-pollinated plants in Mexico have a high economic and cultural value, such as tequila and mezcal harvested from *Agave* species, and the fleshy fruits collected from columnar cacti (Fleming et al. 2009).

L. yerbabuenae, the lesser long-nosed bat, is a species of nectar-feeding bat distributed from central America to the southern USA (Cole, 1996). The species occurs in thorn scrub and deciduous forest, and roosts in caves and other subterranean habitats in colonies reaching thousands of individuals (Medellín, 2016). *L. yerbabuenae* is listed as near-threatened by the IUCN Red List (Medellín, 2016). The primary recommended conservation action is to avoid further habitat loss: roosting habitat is threatened by mining and recreational activities, while foraging habitat is threatened by land-use change

(Medellín, 2016). Furthermore, the combined effects of land-use and climate change are likely to decrease the environmental suitability of the existing range of *L. yerbabuenae* by around half by the 2050s (Zamora-Gutierrez et al. 2018).

L. yerbabuenae is the principal pollinator of *S. queretaroensis*, a species of columnar cactus endemic to central Mexico that is cultivated commercially for its fleshy fruits, pitayas (Ibarra-Cerdeña et al. 2005). *L. yerbabuenae* enhances both the yield and quality of the pitaya crop (Tremlett et al. 2020; Chapter 2), which is a chief source of employment and income in the main production region in central Mexico, the Sayula Basin (Chapter 3). Declines in bat pollinator populations would have severe socio-economic consequences for the region (Chapter 3). However, to my knowledge there have been no studies examining the year-round diet of *L. yerbabuenae* in the Sayula Basin and its reliance on *S. queretaroensis*, and other plant taxa, for food. Such data would have consequent implications for the conservation of *L. yerbabuenae* populations, its feeding grounds, and the pollination services that they provide to the pitaya crop.

Most studies of the lesser long-nosed bat diet in Mexico have relied on the visual identification of pollen grains in faecal samples (Stoner et al. 2003; Sperr et al. 2011), a time-consuming process requiring a high level of palynological expertise, and typically with a low taxonomic resolution (Bell et al. 2016; Edwards et al. 2019). This has limited the ability to determine to species level certain plant taxa that have morphologically similar pollen within families/genera (e.g. Cactaceae and Bombacoidae); as well as the diversity of plant taxa detected and the proportion of the pollen load that can be analysed (Pornon et al. 2017; Ruppert et al. 2019). Additionally, diet studies have largely occurred only during seasonal occupations of roosts, limiting our knowledge of the food resources used by populations that are resident year-round (Riechers Pérez et al. 2003; Peñalba et al. 2005).

Metabarcoding has been used successfully to investigate the diet of insectivorous and frugivorous bats (e.g. Bohmann et al. 2011; Aziz et al. 2017b; Galan et al. 2017; Aizpurua et al. 2018), but remains a scarcely used technique in the study of the diet of nectarivorous bats (though see Lim et al. 2018 and Edwards et al. 2019). Here, I used metabarcoding to determine the seasonal diet of a population of *L. yerbabuenae* bats in central Mexico. I determined occurrence metrics of plant taxa found in pollen and faecal samples collected across one entire year, to: 1) assess the importance of *S. queretaroensis* in the diet, and 2) investigate the reliance on different vegetation types for foraging resources to inform conservation management to maintain pollination service provision to pitaya production.

4.2 Methods

4.2.1 Study area

Sampling was conducted at a *L. yerbabuenae* cave roost located in the municipality of Atoyac (19.99174, -103.505) in the Sayula Basin (Jalisco, central Mexico). The Sayula Basin consists of a seasonal freshwater lagoon, framed by tropical deciduous forest (25% of total area), semi-arid lowland areas with thorn scrub (2%), human settlements (7%) and agriculture (38%) (Macías-Rodríguez et al. 2018). The sub-basin is surrounded by sierra clad in pine, oak, oak-pine and pine-oak forests (Macías-Rodríguez et al. 2018). The highest floristic diversity is found in the tropical deciduous forest, which mainly occupies the foothills of the lagoon, and is dominated by small trees (e.g. *Bursera fagaroides, B. penicillata*, *Ceiba aesculifolia*, *Cnidoscolus spinosus*, *Ipomoea murucoides* and *Lysiloma divaricatum*) and columnar cacti (*S. queretaroensis* and *S. dumortieri*), with occasional large fig trees (*Ficus sp.*). Thorn scrub occupies the drier, lower areas, and is dominated by spiny species in the Fabaceae (e.g. *Prosopis laevigata*, smooth mesquite) and species of *Opuntia* cacti. The average annual rainfall is 660 mm, which mostly falls between June and

October (around 65% of total annual rainfall occurs between June and August), with the dry season lasting from November to May (Pimienta-Barrios et al. 2004).

The Sayula Basin is one of the most important areas for the commercial production of pitayas, a crop with a high economic and cultural value in the region harvested from *S. queretaroensis* cacti. Pitayas have been cultivated intensively in plantations since the 1970s (Pimienta-Barrios and Nobel, 1994). The relatively high prices received for pitayas and tolerance of *S. queretaroensis* to drought and poor soils, along with the production of fruit at a time when few other crops are available, make it an attractive option for local farmers (Pimienta-Barrios and Nobel, 1994). However, pitaya plantations are typically established on land originally occupied by tropical deciduous forest, which, along with other anthropogenic activities such as deforestation and livestock, is exerting a strong pressure on natural vegetation cover (Macías-Rodríguez et al. 2018).

4.2.2 Sample collection and DNA extraction

I visited the cave every two months from April 2017 to February 2018, making a total of six collecting trips. I placed one 6 m mist-net at the roost entrance to catch bats returning from feeding. I opened the net between 22:00 and 23:00 and closed the net between 02:00 and 07:30, depending on the rate of sample collection. I recorded the species, sex, weight, reproductive status and forearm length for each individual captured. I collected samples of pollen from the fur of captured bats using a cotton swab dabbed in 96% ethanol. The cotton swabs were subsequently placed in tubes with 96% ethanol and stored at -20 ºC. Bats were then placed separately in clean cotton bags for a maximum of one hour to collect faecal samples. Faecal samples were placed in tubes with 96% ethanol, which was poured off after 24 to 36 hours and replaced with fine silica gel following Nsubuga et al. (2004), and then stored at -20 ºC. The exception to this were 29 faecal samples collected from *L.*

yerbabuenae bats in June, which were visually confirmed to contain pitaya fruit remains from *S. queretaroensis* cacti but were not sequenced as part of the metabarcoding study.

I extracted DNA from the faecal samples using a modified CTAB method adapted from Doyle et al. (1991), and from the pollen samples using an ammonium acetate method (Bruford et al. 1998). The Eppendorf tube containing the pollen sample and cotton swab was first vortexed to dislodge the pollen grains from the swab, then the cotton swab removed and the tubes centrifuged to concentrate the pollen at the bottom of the tube. The ethanol was pipetted out and tubes left to air-dry for 15 min. 250 µl of digestion buffer (10 µl 0.5M EDTA, 0.0017g NaCl, 12.5 µl 1M Tris-HCl, 215 µl ddH_20, 12.5 µl 20% SDS) and 10 µl Proteinase K (10mg/ml) was then added to the tubes. Samples were left to digest overnight at 55 ºC in a rotating oven. Once the sample was digested, 300 µl of 4 M ammonium acetate was added and the solution was vortexed and left at room temperature for 15 min. The sample was centrifuged at 13,000 rpm for 10 min and the supernatant aspirated into a clean tube. The DNA was then precipitated by adding 1 ml 100% ethanol and centrifuged for 10 min at 13,000 rpm. The supernatant was removed, and the DNA rinsed in 500 µl of 70% ethanol and air-dried for 30 min. DNA samples were then stored in a low TE buffer.

4.2.3 PCR amplification and sequencing

In silico checks of primer-target homology of the trnL, matK and rbcL regions indicated that the taxonomic resolution was low, particularly for species groups likely to be present in the bats' diet such as agaves (Asparagaceae) and cacti (Cactaceae). Additionally, these regions are impractically long (>500bp) for dietary studies (Moorhouse-Gann et al. 2018). I therefore used primer pair UniPlantF and UniPlantR (Moorhouse-Gann et al. 2018; Table 4.1) to amplify part of the second internal transcribed spacer of nuclear ribosomal DNA

(ITS2), a short region of 160-320 base pairs that provides a high taxonomic resolution (Chen et al. 2010).

PCRs to amplify the target ITS2 region of pollen and faecal samples were carried out in 20 µl reaction volumes, containing: 1 µl (pollen) or 2 µl (faeces) DNA template; 10 µl Qiagen Multiplex PCR Master Mix; 2 µl F primer (at 2 µM); 2 µl R primer (at 2 µM); made up to 20 µl with ddH_2O. Reaction conditions were an initial denaturation step at 95 ºC for 15 min, followed by 34 cycles of 94 ºC for 30 secs, 58 ºC for 30 secs, and 72 ºC for 1 m, and a final extension of 72 ºC for 10 min. The annealing temperature was decided after carrying out a gradient analysis from 50 ºC to 60 ºC on six samples of plant tissue from different families. Successful amplification was determined by visual inspection of a 1% agarose gel stained with ethidium bromide. Each PCR plate contained two PCR blanks, which were subsequently treated in the same way throughout the library preparation as the samples.

I then performed a second PCR step to add uniquely indexed Illumina adaptors to the amplicons from each sample. All samples were processed in duplicate from the first PCR stage (after DNA extraction) resulting in two PCR replicates of each sample, multiplexed into two separate pools. Each pool contained 260 samples including 8 extraction/PCR negatives, and was sequenced on a separate Illumina MiSeq sequencing platform, using 250 bp paired-end reads.

Table 4.1. Sequences of the universal primer pairs used in the study.

Name	**Region**	**Sequence 5’ – 3’**
UniPlantF	ITS2	TGTGAATTGCARRATYCMG
UniplantR	ITS2	CCCGHYTGAYYTGRGGTCDC
S2-F	ITS2	ATGCGATACTTGGTGTGAAT
S3-R	ITS2	GACGCTTCTCCAGACTACAAT

4.2.4 Reference library

The ITS2 region has been criticised for the lower representation of reference sequences available from DNA barcode libraries, and the presence of paralogous ITS copies within an individual genome (Bell et al. 2016; Moorhouse-Gann et al. 2018). Copy numbers are likely to vary among species in pollen DNA, particularly between species that inherit plastid DNA maternally, paternally, or biparentally (Bell et al. 2016), therefore higher sequencing coverage is necessary. To ensure high taxonomic resolution, it is necessary to develop a reference DNA barcoding database of potential dietary species, allowing sequences to be identified with a closest-match approach (Moorhouse-Gann et al. 2018; Edwards et al. 2019).

First, I conducted a literature review of families of plants known to contain bat-pollinated species (Fleming and Valiente-Banuet, 2002; Stoner et al. 2003; Fleming et al. 2009; Lobova et al. 2009; Avila-Cabadilla et al. 2012) and plant species found in the study region (Macías Rodríguez, 2004; Sahagún Godínez et al. 2014; Macías Rodríguez et al. 2018). Where no existing literature on pollination syndrome was found for species found in the study region, I assessed flower suitability by comparing flower and plant form to characteristics associated with chiropterophily. I then collected 75 samples of plant species potentially found in the diet from the Herbario "Luz María Villarreal de Puga" (IBUG) and the botanical collection of Cactaceae and Succulents (CUCBA), both in the Department of Botany and Zoology at the University of Guadalajara (Table C1).

I extracted DNA from the plant reference library specimens using a modified CTAB method adapted from Särkinen et al. (2012). PCR was carried out in volumes of 20 µl with reagents in the same concentrations as detailed above, with 1 µl DNA template, under the same reaction conditions. Sequences were amplified using primer pair UniPlantF and UniPlantR (Table 4.1; Moorhouse-Gann et al. 2018). Where these primers failed to generate clean reference sequences, primer pair S2F and S3R were used to amplify the complete ITS2 region and partial 5.8S and 28S sequences (Table 4.1; Chen et al. 2010). PCR products were sequenced in both directions by an ABI 3730 48-capillary DNA analyzer (Applied Biosystems). Contigs were constructed and consensus sequences generated after editing in BioEdit, and alignment in Mega. In some cases, particularly for members of the Cactaceae, multiple bands of DNA were present on the agarose gel, preventing the generation of clean Sanger sequences. I therefore sequenced 19 plant samples on a 2 x 250bp MiSeq Nano, after following the same library preparation protocol as for the pollen and faecal samples. This has the added benefit of allowing the sequencing of potential multiple ITS polymorphisms within an individual. I successfully generated reference sequences for 61 species, which were then submitted directly to GenBank through the GenBank submission portal https://submit.ncbi.nlm.nih.gov/subs/genbank/ (Table C1). I failed to generate reference sequences for 14 species, which was likely due to poor yield of extracted DNA. A further 69 species that had ITS2 sequences already present on Genbank were also added to the list of reference species (Table C2) as well as 40 that are not currently represented on Genbank (in case of future additions).

4.2.5 Bioinformatics

I followed a pipeline generated by NBAF-S (the NERC Biomolecular Analysis Facility in Sheffield, UK). First, sequencing reads were demultiplexed into sets corresponding to individual faecal or pollen samples using the unique Illumina adaptor indexes added to the amplicons from each sample. I trimmed low-quality base calls and Illumina adaptor sequences using Trimmomatic (Bolger et al. 2014), trimming reads when the average quality dropped below 20 over a 4-base sliding window and discarding reads shorter than 140 bp (with parameters SLIDINGWINDOW:4:20 MINLEN:140). I aligned the trimmed paired reads using FLASH (Magoc and Salzberg, 2011) and converted the output to fasta format using the FASTX-Toolkit. I used mothur (Schloss et al. 2009) to identify and trim off sequences matching the primer sequences used in PCR1, allowing for one mismatch between base pairs. I then used Usearch (Edgar, 2010) to specify a minimum of 10 replicates of each sequence for retention, remove singletons which likely represent sequencing errors, and to cluster sequences with an identity of 98% or more into Molecular Operation Taxonomic Units, or mOTUS (with parameters -minuniquesize 10 -minampsize 2 and -id 0.98). I then BLASTed the clustered sequences against the NCBI nucleotide database containing the reference library sequences generated in section 4.2.4 (with parameters -evalue 0.00001 -perc_identity 80; Altschul et al. 1990). I then filtered these results to exclude those with a low identity, keeping only results with a 95% or higher match. I used BASTA to resolve the taxonomies of plant species identified, returning the taxonomy of the best hit for each sequence (Kahlke and Ralph, 2018).

Reads from the PCR blanks were then checked to provide a baseline for background contamination. The maximum number of reads from each plant species identified in blanks were subtracted from all other samples from the same plate. The maximum number of reads found in pollen blanks was 2520 in the first pool, and 201 in the second, both from *Ipomoea murucoides* (Convulvaceae). The maximum number of reads found in faecal blanks was 5921 in the first pool (*Desmodium sp.*: Fabaceae) and 1291 in the second (*Hibiscus citrinus*: Malvaceae). Blanks were then excluded from further analyses.

Before converting read numbers to percentages for pollen samples, I first excluded plant taxa from families not documented to have bat-pollinated members, to mitigate against a possible disproportionate effect of airborne pollen collected from bats during sample collection (families from Fleming et al. 2009; but updated to reflect current taxonomic classfication). I then converted read numbers to percentages to account for the variation in read depth both between samples and between sequencing runs. I specified a minimum sequence percentage threshold of 1% to determine occurrences. A 1% threshold is suitable for situations where diets are not extremely diverse and where there is a lower read depth (Deagle et al. 2018). I retained for analysis plant taxa found in either replicate at above the 1% threshold. Where species matched with the reference list of plant species already identified as possible dietary components, with ITS2 sequences known to be present on Genbank (section 4.2.5), I proceeded with statistical analyses at the species level, as these plants were likely to be actually found in the diet. Where species were not present in the reference list, I aggregated reads at the genus level to avoid mistakenly assigning reads to plants not found in the study region. This approach may miss some cultivated species that genuinely form part of the diet but are non-natives, but was deemed the most appropriate to best characterise the species assemblages and therefore habitat types most used by the bats.

4.2.6 Statistical analysis

I calculated the presence/absence of plant taxa in samples and used this data to calculate the frequency of occurrence, percent frequency of occurrence and weighted percent frequency of occurrence across samples (Deagle et al. 2018). Frequency of occurrence is the percentage of a sample type (either pollen or faecal) containing a given plant taxon, calculated both across the year and at each sampling month. To calculate percent frequency of occurrence, I rescaled frequency of occurrence so that the sum of all dietary items equalled 100%. To calculate weighted percent frequency of occurrence, I weighted the occurrence of each taxon by the total number of taxa in the sample (e.g. if a sample contained four different taxa, each would have a weight of ¼). Occurrence based diet summaries provide a consistent method of converting sequence reads to dietary data, though they are more conservative than relative read abundance and can over-estimate the importance of food items consumed in small quantities (Deagle et al. 2018). I opted to use occurrence-based metrics owing to possible biases in the amplification of principal dietary items and a lack of mock community data. DNA extraction and amplification from many Cactaceae species is notoriously difficult due to high levels of polysaccharides and secondary metabolites which form insoluble complexes with nucleic acids during extraction and inhibit enzyme action (de la Cruz et al. 1997). Potential differential digestion rates between pollen of different plant taxa in the gut of *L. yerbabuenae* (Herrera and Martinez del Rio, 1998) may also affect read abundance.

To assess sampling completeness for each season, I plotted species accumulation curves using the Vegan package in R (Oksanen et al. 2019), treating data generated from pollen and faecal samples as separate sampling points, and treating each individual bat as a separate "site". The number of plant taxa identified in each sample was used to calculate the curves. Data from each bat was added in random order, with 100 permutations.

4.3 Results

I captured a total of 135 *L. yerbabuenae* bats. The species was present year-round at the roost, though with fluctuating abundance. 95% of bats caught were males, with four females caught in February, two in August and one in April. I collected 74 faecal and 93 pollen samples across the year for sequencing (Table 4.2), with an additional 29 faecal samples collected in June that were visually assessed only. After passing through the bioinformatics steps listed above, the NGS sequencing runs produced a total of 438,163 reads from 80 individual pollen samples and 431,842 reads from 73 individual faecal samples.

Table 4.2. Number of samples from *L. yerbabuenae* bats collected, and number of samples for final analysis after passing through the bioinformatics pipeline.

			Samples collected			**Bioinformatics**		
Season	Sampling month	Bats caught	Faeces	Pollen	Total	Faeces	Pollen	Total
Dry	April (2017)	20	17	20	**37**	17	17	**34**
Wet	June (2017)	35	30*	11	**41**	1	8	**9**
Wet	August (2017)	29	17	20	**37**	17	15	**32**
Wet	October (2017)	13	11	8	**19**	11	7	**18**
Dry	December (2017)	15	12	13	**25**	12	13	**25**
Dry	February (2018)	23	16	21	**37**	15	20	**35**

*29 faecal samples analysed visually, and not sequenced.

The total number of pollen and faecal samples obtained from *L. yerbabuenae* differed throughout the year, with the lowest sample size obtained in October. A higher proportion of captured *L. yerbabuenae* bats carried pollen in the dry season, with pollen samples obtained from 87%, 91% and 100% of bats in December, February and April, respectively (Table 4.2); while we obtained pollen samples from 31%, 69% and 62% of bats in June, August and October.

4.3.1 Seasonal diet of *L. yerbabuenae* in the Sayula Basin

A total of 36 different plant species were identified in diet samples, after retaining only those with a percentage count of reads above the 1% threshold in either sequencing pool, removing reads from pollen samples of plant species from families not documented to have bat-pollinated members, and retaining at species level only those part of the previously identified reference list. Of these plant species, 32 were detected in pollen samples and 23 in faecal samples. In addition, reads were annotated to 199 plants that were not part of the reference list, which were then grouped into 127 genera (within 45 families) for all further analyses (species and genera referred to collectively as plant taxa hereafter). The diversity of plant taxa was higher in the dry season (November to May) than the wet season (June to October), both among and within samples (Table 4.3; Figures 4.1 & 4.2). The highest diversity in the diet was found in February (during the dry season), with 92 plant taxa identified. The lowest diversity of plant species was found in June (during the wet season), with 42 plant taxa identified.

Table 4.3. The total number of plant taxa found in pollen and faecal samples in each sampling month, and the mean number of plant taxa per sample ± SE.

		2017				2018	
		April	**June**	**August**	**October**	**December**	**February**
Total # samples	Pollen	17	8	15	7	13	20
	Faeces	17	1	17	11	12	15
Total # plant taxa	Pollen	78	41	45	27	46	77
	Faeces	21	1	22	24	24	41
Mean # plant taxa	Pollen	20 ± 1	14 ± 2	11 ± 1	8 ± 1	8 ± 1	13 ± 1
	Faeces	3 ± 1	1 ± na	3 ± 1	5 ± 1	5 ± 1	8 ± 1
Season		Dry	Wet	Wet	Wet	Dry	Dry

The most encountered plant taxa in pollen samples across the year were *Tarenaya spinosa*, found in 74% of samples, followed by *Ipomoea murucoides,* found in 64% of samples (Table 4.4; Fig. 4.1). *T. spinosa* is an annual flowering plant native to South America, widely cultivated in gardens, and previously identified as part of the diet of *L. yerbabuenae* bats in coastal tropical deciduous forest in Jalisco (Stoner et al. 2003). *I. murucoides* is a bat-pollinated tree found in tropical deciduous forest, though it can also be planted in hedges or as living fence posts (Macías-Rodríguez et al. 2018). The most encountered plant species in faecal samples across the year were identified as *Cucurbita spp.* (Cucurbitaceae), found in 99% of samples (Table 4.4; Fig. 4.2). It is difficult to distinguish between *Cucurbita spp.* using the primer pair in this study. *Cucurbita* pollen found in samples between October and December may be from *C. argrosperma*, a species of chiropterophilous annual climbing vine that flowers during this time (Stoner et al. 2003). It is also possible that some occurrences of *Cucurbita* pollen represent cultivated species, which are widely grown for squash and pumpkins in Mexico.

The Cactaceae family was an important part of the diet in February (Figs. 4.2 & 4.3). In February, 73% of faecal samples and 65% of pollen samples contained *S. queretaroensis*; 7% of faecal samples and 5% of pollen samples contained *S. dumortieri*; and 13% of faecal samples and 10% of pollen samples contained *Pachycereus pecten-aboriginum* (Table 4.4). This reflects the high abundance in the Sayula Basin of *S. queretaroensis*, which occurs wild in tropical deciduous forest and thorn forest, but is also one of the most important crops in the region (Macías-Rodríguez et al. 2018). Additionally, 97% of faecal samples collected in June contained observed seeds from *S. queretaroensis* cactus fruits, showing the bats consume pitaya fruits during the fruiting season (between May and June) (Pimienta-Barrios and Nobel, 1994).

Pollen from bat-pollinated trees associated with tropical deciduous forest was important both in the dry season (November to May) and the wet season (June to October). There was a high weighted percent frequency of occurrence in pollen samples throughout the year of *I. murucoides* (Convulvaceae), *Crateva palmeri* (Capparaceae), *Ruellia bourgaei* (Acanthaceae) and *Ceiba aesculifolia* (Malvaceae; Table 4.4, Fig. 4.1). Species cultivated either ornamentally or for food were also used in both the dry season (*Pseudobombax ellipticum* and *Ceiba pentandra:* Malvaceae) and wet season (*Mangifera* sp.: Anacardiaceae).

A bipartite interaction matrix showed that plant taxa detected in pollen samples were most distinct in February and August, with many of the plants detected in these months not occurring in any other (Fig. 4.1). This indicates a large change in the composition of the diet of *L. yerbabuenae* between the middle of the wet season (August) and the middle of the dry season (February), as well as an increase in the diversity of plant taxa consumed

(Table 4.3). The diet of *L. yerbabuenae* is varied throughout across the year, with no one species or genera dominating (Figs 4.1, 4.2).

Table 4.4. Frequency of occurrence within each sampling month of plant taxa found in more than one sample, either faecal (F) or pollen (P). Species names are listed only where sequences matched a plant in the reference database; in all other cases, the most closely matched plant genus is listed. Results are listed in descending order of total frequency of occurrence across both sample types. Biological form: H = herb, S = shrub, V = vine, T = tree, Su = succulent. Habitat: TDF = tropical deciduous forest, THF = thorn forest, POF = pine-oak forest, C = cultivated plant. *Taken from the vegetation of the Sayula Basin, Macías-Rodríguez et al. 2018; where not listed, the plant was recorded as cultivated. **Records of seeds found visually in faeces.

Family	**Genus/Species**		**Feb**	**Apr**	**Jun**	**Aug**	**Oct**	**Dec**	**Biological form***	**Habitat***
Cucurbitaceae	*Cucurbita*	F	100	100	100	100	91	100		
		P	80	47		7	14	31		
Convolvulaceae	*Ipomoea murucoides*	F	60	29		6	9	92	T	TDF
		P	55	76	62	60	14	92		
Cleomaceae	*Tarenaya spinosa*	F	33				18			
		P	90	94	100	93	29	8		
Asteraceae	*Stevia*	F		6				8		
		P	20	100	88	73	57	38		
Asteraceae	*Montanoa*	F	7	12		6	9			
		P	45	59	12	47	100	38		
Capparaceae	*Crateva palmeri*	F	33						T	TDF
		P	90	82	50	47	29			
Convolvulaceae	*Tarenaya*	F	13	6						
		P	95	71	75	53				
Acanthaceae	*Ruellia bourgaei*	F	13	18			9	8	T	TDF
		P	5	82	50	33	29	15		
Asteraceae	*Montanoa tomentosa*	F	20	6		6			S	TDF, POF
		P	35	53	88	47		8		

Malvaceae	*Pseudobombax ellipticum*	F	47				18		T	C
		P	95	65	38					
Poaceae	*Molinia*	F	73	18		12	64	92		
Asteraceae	*Ageratina*	F					27			
		P	25	71	38	20	57	15		
Malvaceae	*Ceiba aesculifolia*	F				35			T	TDF
		P	15	6	88	87	14			
Convolvulaceae	*Ipomoea*	F	7	6		6	9	42		
		P	15	18	12	20	43	62		
Malvaceae	*Triumfetta*	F						8		
		P	20	88	75	20	14			
Acanthaceae	*Ruellia*	F	20	12			9			
		P		71	38	27	14	8		
Anacardiaceae	*Mangifera*	F				18				
		P	5	18		100	29			
Fabaceae	*Medicago*	P	10	29	62	33		15		
Malvaceae	*Sida*	F				6				
		P		82	25	27				
Cactaceae	*Stenocereus queretaroensis*	F	73		97**				Su	TDF, THF
		P	65							
Asteraceae	*Parthenium*	P	5	24		33	71			
Asteraceae	*Viguiera*	P		24			86	23		
Fabaceae	*Pithecellobium*	F				12				
		P	10	41	38	13		15		
Asteraceae	*Trigonospermum*	P		76	25	13				
Nyctaginaceae	*Bougainvillea*	P	5	35	25	20	29			
Convolvulaceae	*Distimake*	F	20					8		
		P	30		38					
Asteraceae	*Helianthus*	P	10	41	25			15		

Malvaceae	*Malvastrum*	P	5	35	12	20		15		
Lamiaceae	*Salvia*	P	25	35	12			15		
Cactaceae	*Opuntia*	F		29		12	45			
Convolulaceae	*Ipomoea neii*	F	7						L	TDF
		P	15	12	50					
Asteraceae	*Lagascea*	P	20	18		13		23		
Malvaceae	*Hibiscus citrinus*	F	7					17	H	C
		P	5	24	12			8		
Solanaceae	*Solanum*	P	25	24				23		
Cucurbitaceae	*Cucumis*	F	7	6				8		
		P	10	18	12			8		
Euphorbiaceae	*Croton*	F					9			
		P	5	24		7		23		
Convolvulaceae	*Ipomoea arborescens*	F	7			6		17	T	C
		P				7		31		
Asteraceae	*Roldana*	F						8		
		P	5	18	12			23		
Sapindaceae	*Dodonaea*	P	5	47			14			
Malvaceae	*Robinsonella*	P	10	18	38					
Cucurbitaceae	*Microsechium*	P					43	15		
Oleaceae	*Fraxinus*	F	33			6	9	8		
Moraceae	*Ficus*	P	5	18	25			8		
Moraceae	*Ficus insipida*	P		18	25			8	T	TDF
Euphorbiaceae	*Ricinus*	F	7							
		P	5	6	12	20				
Solanaceae	*Nicotiana glauca*	F	13						S	THF
		P	10	12		7		8		
Malvaceae	*Hibiscus syriacus*	P	20	6			14	8	H	C
Malvaceae	*Pseudabutilon*	P	10				14	23		

Fabaceae	*Phaseolus*	F					9			
		P	15	12				8		
Malvaceae	*Hibiscus phoeniceus*	F						8	H	C
		P	25					8		
Euphorbiaceae	*Euphorbia*	P			25	13				
Cucurbitaceae	*Cucurbita radicans*	F	7	6					V	C
		P	10				14			
Fabaceae	*Prosopis*	F					9			
		P	10	6	12					
Asteraceae	*Bidens*	F	7							
		P	10	6	12					
Musaceae	*Musa*	F				6	18			
		P	10							
Cactaceae	*Hylocereus undatus*	F				6	18		Su	TDF
		P						8		
Solanaceae	*Datura inoxia*	F					18		H	TDF
		P		6		7				
Anacardiaceae	*Schinus*	F	7			6		8		
		P	10							
Asteraceae	*Ambrosia*	P	5	12		13				
Malvaceae	*Hibiscus*	F	7	6						
		P	10					8		
Asteraceae	*Psacalium*	P		18	12					
Fabaceae	*Calliandra*	F						8		
		P				7	14			
Asteraceae	*Lactuca*	P		6		7		15		
Polemoniaceae	*Loeselia*	P			12		14			
Loranthaceae	*Psittacanthus*	F					18			
		P				7				

Malvaceae	*Ceiba pentandra*	P	10	6				8	T	C
Asteraceae	*Chromolaena*	F	7							
		P		18						
Nyctaginaceae	*Mirabilis*	P	10				14			
Cactaceae	*Pachycereus pecten-aboriginum*	F	13						Su	TDF, THF
		P	10							
Passifloraceae	*Passiflora porphyretica*	P	5	6	12				V	TDF
Cordiaceae	*Varronia*	P	5	18						
Poaceae	*Melinis*	F	13				9			
Solanaceae	*Nicotiana*	F		6						
		P	10	6						
Cactaceae	*Stenocereus dumortieri*	F	7				9		Su	TDF, THF
		P	5							
Solanaceae	*Datura stramonium*	F	7					8	H	THF
		P	5							
Fabaceae	*Desmodium*	P			12			8		
Cibotiaceae	*Cibotium*	F					18			
Combretaceae	*Combretum igneiflorum*	P		18					V	TDF
Moraceae	*Morus*	P		6	12					
Malvaceae	*Pseudobombax palmeri*	P		18					T	TDF
Cucurbitaceae	*Sechium*	F	7							
		P	5	6						
Asparagaceae	*Agave attenuata*	F						17	Su	TDF, THF, POF
Fabaceae	*Vigna*	P	5	12						
Brassicaceae	*Brassica*	F	7					8		
Passifloraceae	*Passiflora*	F						8		
		P				7				
Solanaceae	*Capsicum*	F					14			

Malvaceae	*Ceiba acuminata*	F		6				8	T	TDF
Nyctaginaceae	*Commicarpus*	P				6		8		
Malvaceae	*Hibiscus rosa-sinensis*	P		6				8	H	C
Fabaceae	*Pachyrhizus*	P		6				8		
Cucurbitaceae	*Sechiopsis*	P		6				8		
Asteraceae	*Taraxacum*	P					14			
Opiliaceae	*Agonandra*	F	13							
Ericaceae	*Arbutus*	F	7							
		P				7				
Malvaceae	*Gaya*	P	5					8		
Rubiaceae	*Hintonia*	P				13				
Convolvulaceae	*Merremia*	F						8		
		P	5							
Piperaceae	*Piper*	F	13							
Asparagaceae	*Agave*	P	5			7				
Fabaceae	*Coursetia*	P	5			7				
Asteraceae	*Dendroviguiera*	P			12					
Amaranthaceae	*Iresine interrupta*	F		6		6				
Polygalaceae	*Monnina*	F		6		6				
Rhamnaceae	*Sageretia*	P			12					
Cucurbitaceae	*Wilbrandia*	F	7							
		P	5							
Myrtaceae	*Eucalyptus*	P	5	6						
Passifloraceae	*Passiflora pavonis*	P	5	6					V	C
Rosaceae	*Prunus*	P	5	6						
Convolvulaceae	*Ipomoea purpurea*	P	10						V	TDF, THF
Salicaceae	*Salix*	P	10							

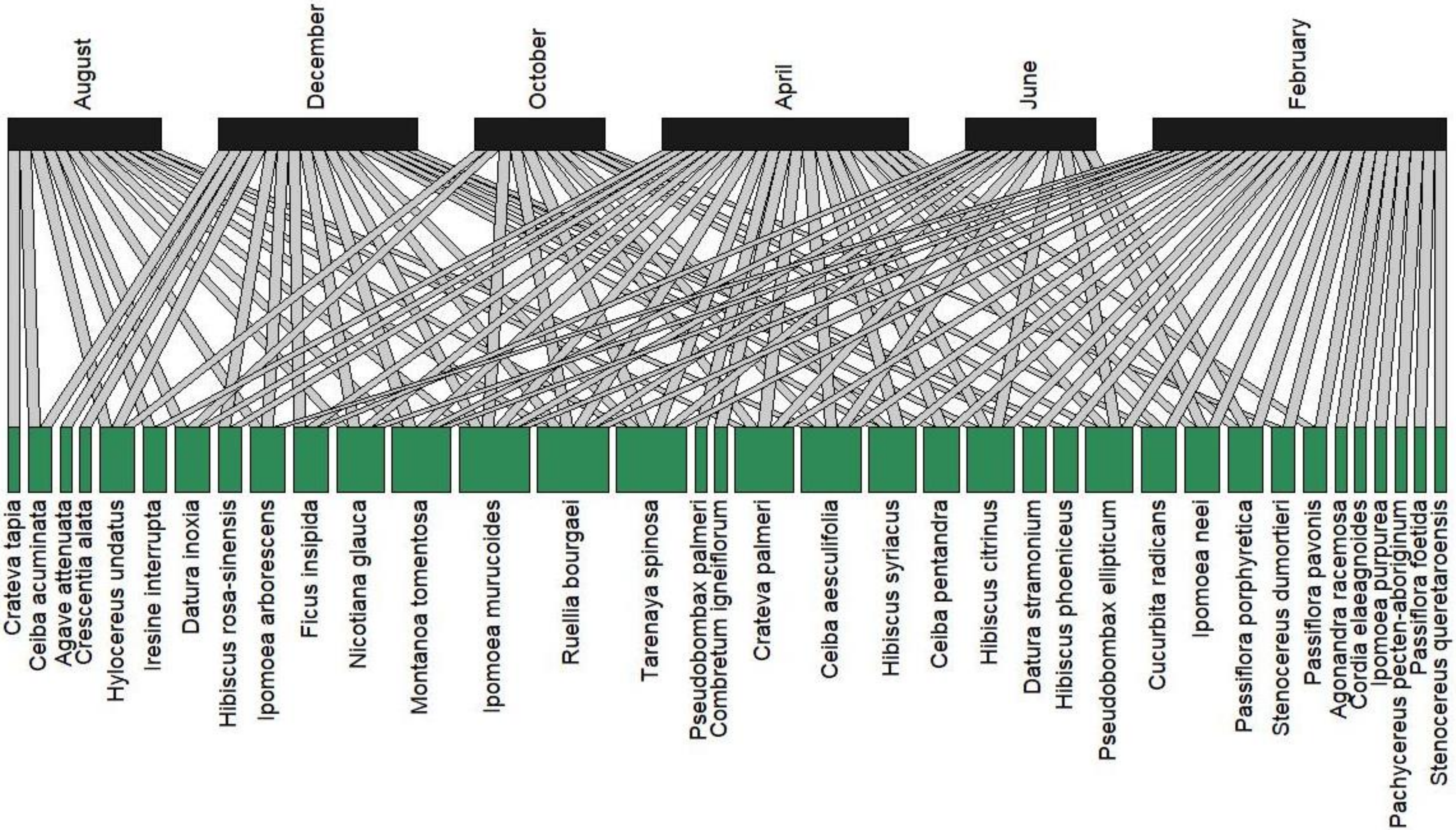

August
December
October
April
June
February
Crateva tapia
Ceiba acuminata
Agave attenuata
Crescentia alata
Hylocereus undatus
Iresine interrupta
Datura inoxia
Hibiscus rosa-sinensis
Ipomoea arborescens
Ficus insipida
Nicotiana glauca
Montanoa tomentosa
Ipomoea murucoides
Ruellia bourgaei
Tarenaya spinosa
Pseudobombax palmeri
Combretum igneiflorum
Crateva palmeri
Ceiba aesculifolia
Hibiscus syriacus
Ceiba pentandra
Hibiscus citrinus
Datura stramonium
Hibiscus phoeniceus
Pseudobombax ellipticum
Cucurbita radicans
Ipomoea neei
Passiflora porphyretica
Stenocereus dumortieri
Passiflora pavonis
Agonandra racemosa
Cordia elaeagnoides
Ipomoea purpurea
Pachycereus pecten-aboriginum
Passiflora foetida
Stenocereus queretaroensis

Figure 4.1. A bipartite interaction matrix visualising plant species visited by *L. yerbabuenae* bats throughout the year, using weighted per cent of occurrence data of plant species in both pollen and faeces samples combined. Only plant species matching those in the reference database are presented here. The green rectangles represent plant species, with the width proportional to the sum of the occurrences in different sampling months. Black rectangles represent sampling months. Lines connect plant species with the sampling months they occur in, with the width proportional to the sum of occurrences in that month. As few crossings of interactions are plotted as possible, meaning that months closer together have a more similar species assemblage than those further apart (Dormann, Gruber, & Fründ, 2008).

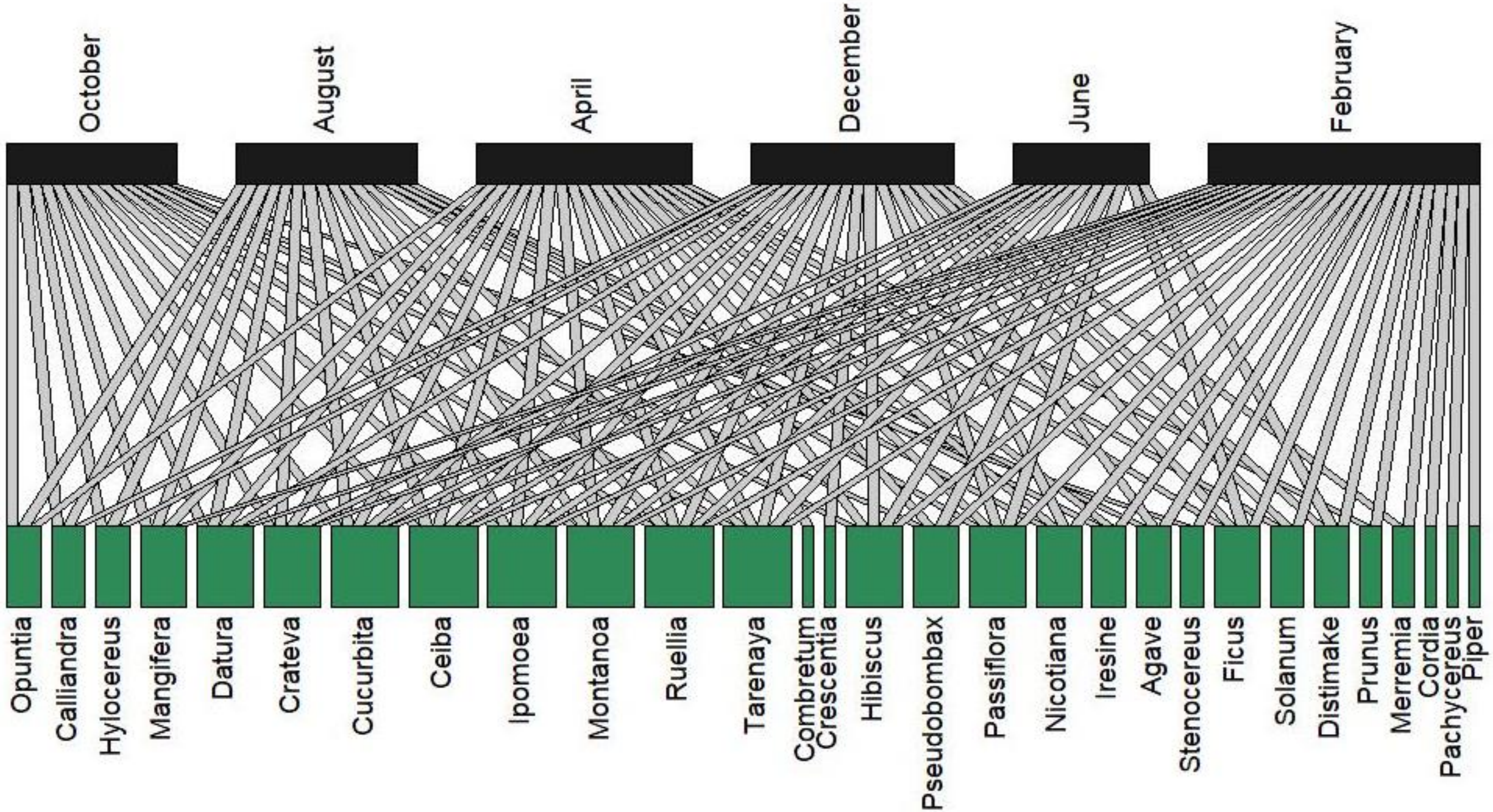

October
August
April
December
June
February
Opuntia
Calliandra
Hylocereus
Mangifera
Datura
Crateva
Cucurbita
Ceiba
Ipomoea
Montanoa
Ruellia
Tarenaya
Combretum
Crescentia
Hibiscus
Pseudobombax
Passiflora
Nicotiana
Iresine
Agave
Stenocereus
Ficus
Solanum
Distimake
Prunus
Merremia
Cordia
Pachycereus
Piper

Figure 4.2. A bipartite interaction matrix visualising plant genera visited by *L. yerbabuenae* bats throughout the year, using weighted per cent of occurrence data of plant genera in both pollen and faeces samples combined. Only plant genera matching those in the reference database are presented here.The green rectangles represent mOTUs, with the width proportional to the sum of the occurrences in different sampling months. Black rectangles represent sampling months. Lines connect genera with the sampling months they occur in, with the width proportional to the sum of occurrences in that month. As few crossings of interactions are plotted as possible, meaning that months closer together have a more similar species assemblage than those further apart (Dormann, Gruber, & Fründ, 2008).

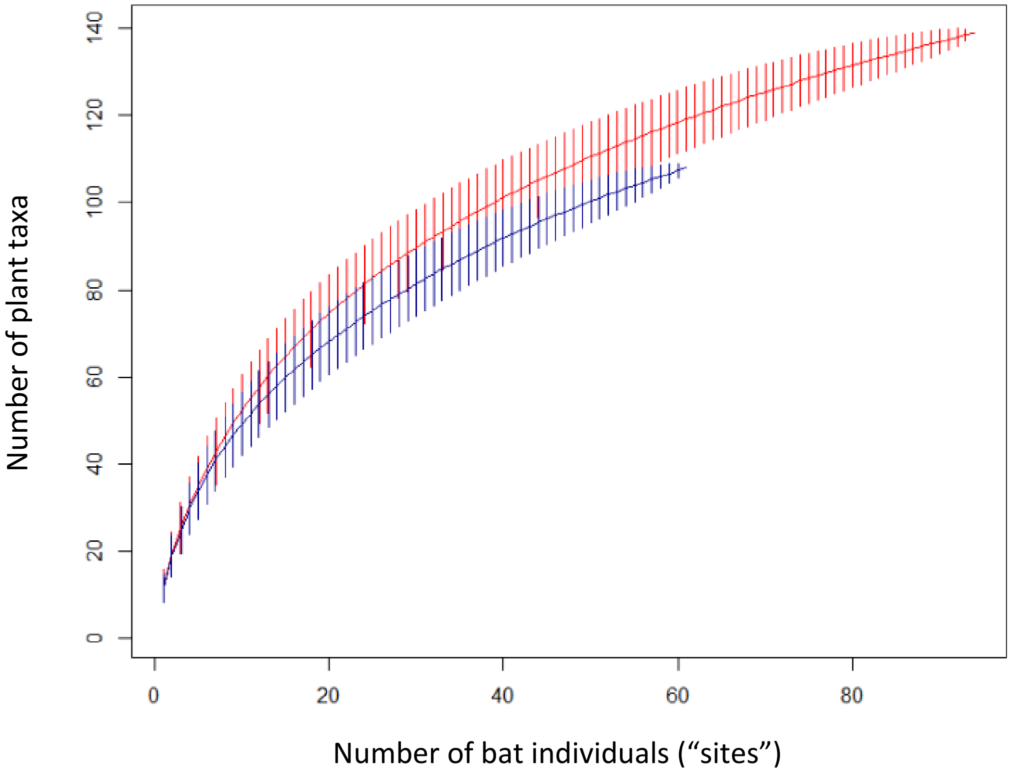


Figure 4.3. Species accumulation curves showing the increase in detection of plant taxa with bat individual sampled (pollen and faecal samples considered collectively) during the wet season (blue) and the dry season (red).

Species accumulation curves indicated that sampling was less complete in the dry than the wet season (Figure 4.3), despite a higher number of detected plant taxa in samples in the dry season when the sampling effort (number of bat individuals) was accounted for (Table 4.3). As plant taxa were aggregated at the genus level if they did not match with species listed in the reference database, this is likely to be a conservative estimate of sampling completeness. This suggests that further sampling is necessary for a full understanding of the plant taxa in the diet of *L. yerbabuenae.*

4.4 Discussion

The lesser long-nosed bat *L. yerbabuenae* has a diverse diet, feeding from a variety of plant taxa throughout the year. The use of metabarcoding in this study allowed the identification of a higher diversity of plant taxa in the diet of lesser long-nosed bats than plant species found in previous studies that used visual identification of pollen grains alone. I identified a total of at least 36 different plants to species, compared with 19 to 28 plant species visually identified from pollen remains in faeces and on the fur of *L. yerbabuenae* bats in tropical dry forest on the central Pacific coast of Mexico (Stoner et al. 2003; Sperr et al. 2011). The high number of plant taxa found in pollen and faeces samples that were not previously identified as potential parts of the diet, as well as the species accumulation curves, indicates that the true diversity of plant taxa consumed by lesser long-nosed bats is likely to be even higher.

The high frequency of *S. queretaroensis* pollen in samples collected in February suggests that pitaya plantations in the Sayula Basin represent valuable foraging grounds for *Leptonycteris* bats, providing pollen and nectar rewards during the flowering season (February to April). *S. dumortieri* and *Pachycereus pecten-aborigininum* are also found in tropical deciduous forest and thorn forest in the Sayula Basin, flowering and fruiting between February and June, but occur at much lower densities than *S. queretaroensis* (Valiente-banuet et al. 2004; Cruz and Pavón, 2013; Macías-Rodríguez et al. 2018). *Leptonycteris* bats are specialised to digest a high proportion of pollen grains of columnar cacti, obtaining important dietary components such as nitrogen and protein (Herrera and Martinez del Rio, 1998; Roulston and Cane, 2000; Munoz-Romo et al. 2005). In return, *Leptonycteris* bats act as the principal pollinator of the pitaya crop, enhancing both fruit yield and quality (Tremlett et al. 2019; Chapter 2). The high presence of *S. queretaroensis* fruit seeds in June indicates that pitaya fruits are also an important food resource. Cactus fruits are documented to form part of the diet of *L. yerbabuenae* bats when seasonally abundant during the summer months in central Mexico, at a time of lower chiropterophilous flower availability (Stoner et al. 2003; Rojas-Martínez et al. 2012). Pitaya fruit production peaks in May in the study region, at the end of the dry season, and the high water content of the pulp may be a valuable resource for bats at this time, as well as a potential source of energy (Fleming and Nassar, 2002). *L. yerbabuenae* bats may also be important seed dispersers of wild *S. queretaroensis* cacti (García-Ruiz et al. 2018).

However, despite the high availability of *S. queretaroensis* pollen and nectar resources in the Sayula Basin in February, lesser long-nosed bats diversify their diet with plant species associated with tropical deciduous forest and thorn forest during the pitaya flowering season. This suggests that access to resources found in the pitaya plantations are not sufficient and bats must supplement their diets with other plant taxa. I found a high occurrence of pollen from bat-pollinated tree species associated with tropical deciduous forest in both the dry and wet seasons, though plant diversity was higher in the dry season. Despite the high costs of producing the large volumes of nectar associated with bat-pollinated flowers (Fleming et al. 2009), chiropterophily is a prominent pollination system in tropical deciduous forest (Frankie et al. 2004). Flowering phenology is highly variable between different areas of tropical deciduous forest; but bat-pollinated plants tend to flower sequentially across the year, often with a longer flowering duration than seen in plants pollinated by other taxa, producing only a few flowers each day (Heithaus et al. 1975; Stoner et al. 2003; Frankie et al. 2004; Cortés-Flores et al. 2017). I found pollen from bat-pollinated flowers in the diet of *L. yerbabuenae* throughout the year. Water availability is one of the most important abiotic factors influencing the flowering phenology of plants in tropical deciduous forest; however, various bat-pollinated plants incur high costs by producing flowers during the dry season, prompted by increased daylength (Cortés-Flores et al. 2017). This provides a continuous supply of floral resources for bat pollinators, and encourages the availability of bats as pollinating agents that are able to reside year-round.

I found the lowest diversity of plant taxa, and the lowest percentage of bat individuals captured with pollen, in the wet season. This is comparable to studies of *L. yerbabuenae* diet in tropical deciduous forest on the central Pacific coast of Mexico, which found the highest diversity of plant species consumed in the dry season and increased consumption of fruits and insects in the wet season, during the months of lowest chiropterophilic flower abundance (Stoner et al. 2003; Sperr et al. 2011). No insects were detected visually in faecal samples in this study, but *L. yerbabuenae* bats showed a high consumption of cactus fruits in the wet season. Additionally, the high occurrence of *Cucurbita spp.* in the faecal samples and the low occurrence of Cucurbitaceae detected in pollen samples collected from the fur during the wet season, suggests that some of the occurrences of this family in faecal samples may be the result of bats feeding from cucurbit fruits rather than visiting flowers (for example, hanging fruits from cucurbit vines are eaten by Phyllostomidae bats in South America (Kalko and Condon, 1998)).

Several plant families not documented to have bat-pollinated species were identified in the diet. The presence of anemophilous (wind-pollinated) plant taxa, such as the Poaceae and Pinaceae, probably represents pollen accidentally inhaled or ingested by bats while grooming or drinking from nectar sources. There was also a high occurrence of species matched to the Asteraceae family, though Fleming et al. (2009) identified just three species of Asteraceae documented to be pollinated by bats in the New World. The Asteraceae family is one of the largest and widespread plant families, and so background airborne pollen levels are likely to be high. The Asteraceae has been found in studies of the diet of lesser long-nosed bats in both southern and northern parts of their range in Mexico, but the occasional nature of the presence of the Asteraceae pollen grains in samples was deemed an indicator of aerial origin (Riechers Pérez et al. 2003; Peñalba et al. 2005); though bats can be opportunistic feeders and visit flowers with a morphology associated with pollination by insects or birds (Fleming et al. 2009).

4.4.1 Implications for pollination services and bat conservation

The diverse diet of the lesser long-nosed bat in the Sayula Basin emphasises the need to ensure that food resources are maintained at a landscape scale to support bat populations year-round (Fleming and Nassar, 2002), particularly by conserving habitat within the foraging range of *L. yerbabuenae* roosts to maintain a high species richness of food plants (Burke et al. 2019). Tropical deciduous forests in Mexico harbour an extremely rich floristic diversity, with high numbers of endemic species (Banda et al. 2016). However, they are severely impacted by climate change and human activities such as logging, agriculture and cattle-ranching; with scant formal protection (Maas, 1995; Trejo and Dirzo, 2000; Miles et al. 2006; Mendoza-Ponce et al. 2019). In the Sayula Basin, tropical deciduous forest are under strong pressure from anthropogenic activities (Macías-Rodríguez et al. 2018).

Loss of foraging habitat from conversion of land for intensive agriculture and livestock production, and the degradation and fragmentation of remaining habitat, is one of the major threats to bats worldwide (Frick et al. 2019). Over-harvesting of wild cacti and agaves also reduces the food resources available to *Leptonycteris* bats (Cole and Wilson, 1996). The apparent importance of both *S. queretaroensis* flowers and fruit in the diet of the resident population of *L. yerbabuenae* suggests that pitaya plantations are a valuable supplementary resource for nectar-feeding bats; however *S. queretaroensis* plantations are largely established in areas originally occupied by flower-rich tropical deciduous forest and area under production is increasing yearly (Macías-Rodríguez et al. 2018; SIAP, 2018). The maintenance of the *L. yerbabuenae* population and the pollination services provided to the pitaya crop is reliant on the protection of remaining tropical deciduous forest to provide foraging resources, or the restoration of agricultural land to tropical deciduous forest, as the abundance of *L yerbabuenae* has been found to be similar between early and late successional stages of tropical deciduous forest (Avila-Cabadilla et al. 2012; Avila-Cabadilla et al. 2014). This is important both in the flowering and fruiting season of *S. queretaroensis* (July to January), to supplement resources provided by pitaya plantations, as well as throughout the rest of the year. The high diversity of plant taxa consumed by *L. yerbabuenae* indicates that they are important pollinators in tropical deciduous forest, and declines in bat pollinator populations may have severe ecological effects.

Disturbance and fragmentation of tropical deciduous forest can also negatively impact the reproductive success of bat-pollinated trees. For example, changes to bat foraging behaviour can result in decreased visitation rates to flowers (Quesada et al. 2004), a reduced number of pollen donors contributing to conspecific pollen loads carried by bats (Fuchs et al. 2003), and decreased flower specialisation resulting in the delivery of large amounts of incompatible pollen in mixed pollen loads (Sritongchuay et al. 2019). Bat-pollinated plants often occur naturally at low densities and are self-incompatible (e.g. canopy trees in the Bombacoidae and arid-zone cacti and agaves), and are therefore reliant on bats as highly mobile pollen dispersal agents (Herrerías-Diego et al. 2006; Fleming et al. 2009; Quesada et al. 2013).

Additionally, there is a continued need for sustainable pitaya production practices, avoiding the use of pesticides and other agrochemicals which are dangerous to bats (Bayat et al. 2014; Williams-Guillén et al. 2016). Enrichment planting of bat-pollinated trees could also increase resources available to nectar-feeding bats, as well as retaining diverse agroforestry systems and live fences (Chazdon et al. 2011; Berthinussenet al. 2019).

4.4.2 Limitations and uncertainties

There was a marked difference in the plant taxa detected in faecal samples and pollen samples, with faecal samples dominated by members of the Cucurbitaceae plant family, and pollen samples by species from tropical deciduous forest such as members of the Bombacoideae (Malvaceae) and Cactaceae. This could be partly due to the efficiency of *L. yerbabuenae* at extracting pollen contents, emptying over three-quarters of columnar cactus pollen grains within an hour of ingestion (Herrera and Martinez del Rio, 1998). It may also be due to the detection of fruit in faecal samples, as it is not possible to differentiate between parts of the plant that are consumed with metabarcoding: the presence of plant taxa could represent pollen or fruits. This reduces the ability to make inferences about pollination implications from the faecal samples if no phenological information is available for some plant species. Additionally, sampling and DNA extraction protocols, and differences in primer fit between taxa, can influence DNA amplification and the success in detecting different taxa (Deiner et al. 2015).

I did not use any insect primers in our study, though arthropods have been found in the diet of lesser long-nosed bats throughout their range. Insect consumption by lesser long-nosed bats in central Mexico has been found to be low (Stoner et al. 2003; Sánchez and Medellín, 2007), and we did not visually detect insects in faeces. I therefore felt it better to concentrate our limited resources on sequencing a replicate of the pollen and faecal samples with plant primers to maximise read depth and detection of plant taxa.

4.5 Conclusion

While our study highlights the mutually beneficial relationship between bat pollinator and pitaya crop; it also emphasises the need for landscape-scale conservation management to ensure a high diversity of floristic resources to maintain *L. yerbabuenae* bats throughout the year, particularly those associated with tropical deciduous forest.

Chapter 5 General discussion

5.1 Ecological and economic importance of bat pollination services

Bats play key roles in both natural and agricultural systems and provide a range of ecosystem services that benefit humans, but are disregarded as pests throughout large parts of the world. Bats are commonly viewed with suspicion and dislike for many cultural, symbolic and religious reasons; and the value of bats to the maintenance of ecosystems and human wellbeing is largely underestimated (Mickleburgh et al. 2002; Kingston, 2016). Pest suppression provided by bats to crops is the best studied of the potential ecosystem services provided by bats in agricultural systems, with insectivorous bats documented to predate on pests of a range of crops including global staple foods such as corn and rice (e.g. Maine and Boyles, 2015; Puig-Montserrat et al. 2015), and there have been several attempts to estimate the economic value of this service (e.g. Cleveland et al. 2006, Boyles et al. 2011, Maas et al. 2013, Taylor et al. 2018). However, the effect of bat predation on arthropod pest abundance and/or crop damage, and the resulting economic value of bat-mediated pest suppression, is still not well understood (Williams- Guillén et al. 2016). Pollination services provided by bats to crops are even more poorly documented, despite bats playing a key role as pollinators in the tropics (Kunz et al. 2011). Increased awareness of the benefits provided by bats is a key first step to improve perceptions of bats and conserve bat populations.

The primary aim of this thesis was to conduct research to highlight the importance of ecosystem service provision by bats and encourage their conservation, with three main objectives: assess the 1) ecological and 2) economic importance of bat pollination services to a highly valuable regional fruit crop (pitayas, *Stenocereus queretaroensis*) grown in central Mexico; and 3) investigate resource use and subsequent conservation implications for the principal species of pitaya-pollinating bat.

There is scant literature available examining the contribution of bat pollinators to food security and crop production compared to the wealth of research on the importance of insect pollinators (Potts et al. 2016b). Bats have been demonstrated to pollinate crops such as durian *Durio zibethinus* and bitter beans *Parkia spp.* in south-east Asia (Bumrungsri et al. 2008, 2009), though the impact of bat pollination on fruit quality and market value remains largely unknown. In the New World, bats are key pollinators of wild Cactaceae, *Agave* and *Musa* spp., playing a vital role in maintaining genetic diversity in wild relatives of domestic species even where the crop species themselves are propagated vegetatively under cultivation (Hopkins and Maxted, 2011; Williams- Guillén et al. 2016). As bat-pollinated crops are often regional and found in lower income countries, the poor documentation of bat pollination services to crops may be in part due to the neglect of small-holder agriculture in pollination research, despite smallholdings contributing 16% of global farmland area and 83% of the world's agricultural population (Steward et al. 2014). This thesis helps to fill this knowledge gap by assessing the contribution of bat pollination to pitaya production: pitayas are a regionally valuable crop grown largely as smallholder agriculture, but provide a chief source of employment in the production area.

Exclusion experiments showed that bat pollination enhanced both the yield and quality of the pitaya crop relative to other pollinating taxa (Chapter 2). Importantly, I found that consideration of both is essential to fully understanding the benefits of bat pollination – crop quality has not been considered in previous efforts to quantify the contribution of bats to crop production, which has likely resulted in underestimating of the benefits provided. Pollination by bats resulted in enhanced pitaya fruit set and quality: pitaya yield decreased by 35%, and fruit weight by 46%, when bats were excluded from pitaya flowers and pollination was carried out by birds and diurnal insects. The impact of bat pollination on fruit quality was high, comparable to existing research assessing the impact of insect pollination e.g. on the quality of apples (Garrett et al. 2014), strawberries (Klatt et al. 2014), and multiple cash crops in Burkina Faso (Stein et al. 2017). The decrease in both yield and quality of the pitaya crop suggested likely socio-economic repercussions in the case of bat pollinator population decline.

The estimated market value of the increased fruit yield and quality associated with bat pollination confirmed the high economic value of bat pollination services to pitaya producers (Chapter 3). The total worth of bat pollination services to pitaya producers was $2,500 per hectare, or around 40% of the gross income of pitaya producers sampled. This economic value was a result as much of the enhanced fruit quality resulting from bat pollination as the higher yield; the difference in market value of bigger bat-pollinated fruits was worth approximately the same as the value of the increased crop yield to pitaya farmers. I found bat pollination services to have a considerably higher value per hectare than studies valuing crop pest suppression (Taylor et al. 2018; Williams-Guillén et al. 2016). Therefore, quantifying pollination services could provide a stronger economic argument for bat conservation than other ecosystem services in areas with bat-pollinated crops. For example, the value of bat pollination to durian production in West Sulawesi was roughly estimated at over $117/ha/fruiting season, through increases in fruit set relative to pollination by other taxa (Sheherazade et al. 2019); however, this did not account for changes in fruit quality and so the true value of bat pollination services to the durian sector is likely to be higher.

However, the dollar value worth of a service is not the whole story, and local context is important. For example, Wanger et al. 2014 found that the value of bat-mediated pest suppression by one species of insectivorous bat to rice production in Thailand had an estimated value of just $0.13 per hectare per year. This is much lower than value I found of pollination services to the pitaya, but represents a substantial contribution to food security, with just one species of bat preventing the loss of enough rice to feed the entire population of Thailand for a week (Williams-Guillén et al. 2016). Though historically important for the subsistence of Mexican peoples, today pitayas themselves do not form a significant part of peoples' diets, though they provide a good source of vitamins and minerals at a certain time of the year. They do however still have a very high cultural importance, which was beyond the scope of this research to attempt to value. Furthermore, the pitaya is now a high-value fruit, commanding similar prices to other luxury fruits such as apples and pears, and forms a crucial part of the local economy, providing many jobs in an area with few other opportunities. On the other hand, extrapolating the value of all bat-mediated crop pest suppression services nation-wide (such as the estimated value of nearly US$23 billion annually in the USA; Boyles et al. 2011) will likely produce higher estimates than for aggregated pollination services, as a greater range of crops will benefit from bat-mediated pest limitation services than from bat-mediated pollination services. But again, local context is crucial – crops dependent on bats for pollination may not often be globally, or even nationally important, but still be highly significant at a regional level, as in the case of the pitaya. The relative purchasing power of the same amount of currency (valuations of bat ecosystem services are often reported in US$) will also differ markedly between countries, which should be considered for meaningful comparisons. It is also important to re-emphasise that economic values of bat-mediated ecosystem services will also fluctuate with changes in market prices, and institutional or external environmental factors (López-Hoffman et al. 2014).

The value chain analysis showed that the economic benefit from bat pollination services was not distributed equitably between actors in the pitaya commodity chain (Chapter 3). Privileged actors with land and capital can establish pitaya plantations and access profitable marketing situations, with pitayas moving from a communally collected wild resource to a privately owned commodity. The pitaya chain is also characterised by a lack of social security, which is one of the primary deprivations in the production area (CONEVAL, 2010). Initiatives at the community, NGO and government level are necessary for improved access to the pitaya chain and a fairer distribution of the economic benefits of bat pollination services.

Chapter 4 highlighted the mutualistic relationship between the pitaya crop and *L. yerbabuenae*: in return for the pollination services provided to the pitaya crop, pitaya plantations provided important flower and fruit resources for several months of the year. However, *L. yerbabuenae* diversified its diet throughout the year, visiting a range of plants from tropical deciduous forest as well as ornamental and cultivated species. This demonstrates the need for landscape-scale conservation to provide sufficient year-round foraging resources, and maintain the provision of pollination services by *L. yerbabuenae* bats to pitaya producers.

5.2 Implications for conservation

Mexico is one of the most biodiverse countries in the world but also has one of the highest rates of habitat loss, which is a leading driver of biodiversity declines (Fuller et al. 2007; Visconti et al. 2011). In addition, large changes in environmental conditions are projected with consequent severe ecological disturbances and species turnover (Peterson et al. 2002; Zamora-Gutierrez et al. 2018). Pollination systems are vulnerable to disturbance (Neuschulz et al. 2016) and there is increasing pressure on bat pollinator populations in the context of changing land use and climatic conditions. Bats inhabiting arid and semi-arid regions such as *L. yerbabuenae* are particularly vulnerable to predicted climate change, with decreased humidity and increased temperature impacting ecosystems such as deciduous forests and shrublands (Zamora-Gutierrez et al. 2018). It is vital to protect such ecosystems to maintain foraging habitat for nectarivorous bats, as indicated in Chapter 4.

Nectarivorous bats in Mexico are also threatened by active persecution. Bats are commonly killed under the mistaken belief that all bats are 'vampiros', which can transmit bovine paralytic rabies to cattle (Williams-Guillén et al. 2016). Local farmers and agricultural technicians are often unable to distinguish between bat species or functional groups that provide beneficial ecosystem services such as pollination and pest control (nectarivores, insectivores) and disservices such as crop raiding and disease transmission (frugivores, sanguivores), and therefore cull indiscriminately (Williams-Guillén et al. 2016). Knowledge of farmers in Mexico of the pollination services provided by bats is poor and bats are badly perceived (López-del-Toro et al. 2009), and land is often valued for potential agricultural production over less tangible benefits such as the provision of ecosystem services (Castillo et al. 2005).

Reducing negative perceptions of bats is an ever more pertinent issue, with widespread alarmist and negative coverage of bats in the media, often inaccurate or misleading; particularly around zoonotic disease transmission. Bats are often said to harbour a disproportionate amount of zoonotic viruses, though this has recently been shown to be a simple function of the diversity of bats - the number of viruses across different reservoir taxonomic orders was explained by the number of animal host species within the order (Mollentze and Streicker, 2020). The current Covid-19 pandemic provides a good demonstration of the vilification of bats. The virus causing Covid-19 is most closely related to bat coronaviruses, but it is likely that intermediate hosts facilitated transmission to humans (such as civets and camels in the case of previous zoonotic viruses SARS and MERS respectively; Fenton et al. 2020). However, the widespread reporting of bats as the originator of the virus has driven new threats to bat populations. Where bats were already poorly perceived, such as Latin America, persecution is likely to increase further, such as recent arson attacks on bat roosts in Peru due to fears of Covid-19 transmission (Fenton et al. 2020; Lu et al. 2021).

The majority of the inhabitants of the pitaya production area interviewed as part of the value chain analysis did not know that bats are the principal pollinators of the pitaya (Appendix B.7 and B.8). Community environmental education programmes, therefore, could be a powerful tool to induce positive perceptions of bats in the area by informing people of ecosystem services provided, that are directly relevant to the local context; an approach that could also be applicable in other areas. For example, a study in Fiji found a high similarity between plant species valued by humans, and those plant species pollinated or dispersed by bats, suggesting that conservation approaches tailored to local communities and resource use may be an effective way to combat the negative perception of bats (Scanlon et al. 2014).

5.3 Future of the pitaya sector

Pitaya production is increasing yearly (SIAP, 2018). To maintain the value of the pitaya crop and bat pollinator populations, several management recommendations emerged from this thesis:

1) The spatial and genetic composition of the pitaya plantations likely impacted crop yield. Pitaya farmers may be able to improve yields by including a mix of cultivars distributed throughout each plantation, and/or by growing cacti from seed instead of from cuttings. This would increase the genetic variability present within the plantation and thus increase the probability of pollinators delivering pollen from a different individual, necessary for successful outcrossing. Increased genetic variability also increases the resilience of plant populations to environmental pressures such as climate change and disease (Zhu et al. 2000).

2) Landowners such as pitaya farmers may have cave roosts on their land. The loss or disturbance of subterranean habitats such as caves, which are used for mining and recreation, is a key threat to *L. yerbabuenae* bats (Frick et al. 2019; Medellín, 2016). Protection of cave roosts would benefit nectarivorous cave-dwelling bat species such as *L. yerbabuenae, L. nivalis* and *Choeronycteris mexicana* and encourage the continued provision of pollination services. It would also benefit other guilds of cave-dwelling bats that likely provide additional ecosystem services to pitaya farmers, such as pest control (Medellín et al. 2017).
3) In the Sayula Basin, pitaya plantations are largely established on land originally covered by tropical deciduous forest (Macías-Rodríguez et al. 2018), and disturbance and fragmentation of forest areas could decrease foraging resources available for nectar-feeding bat populations. Actions to ensure a high floristic diversity of foraging resources, such as the protection of remaining forest fragments and prevention of over-harvesting of wild cacti and agaves, are important at a landscape scale.
4) Finally, to improve access to the pitaya chain and encourage a fairer distribution of economic benefit between actors, activities could be started at the community, government or NGO level, such as: selling fruits or products collectively; opening up new markets (with assistance to cope with any resulting extra certification or tax requirements) or improving access to existing markets; supporting new actors financially to establish plantations; supporting the introduction of a low-entry health insurance; and providing training and equipment to increase product-making capacity.

5.4 Future research direction

This thesis represents one of the first efforts to quantify the ecological and economic importance of bats to crop production. There are many important bat-pollinated plants that are used commercially by humans around the world. Detailed assessments of the contribution of bats to both yield and quality, as well as the subsequent economic benefit, would be a valuable addition to research demonstrating ecosystem service provision by bats and the need to conserve bat populations. For example, such assessments could be carried out for further Mexican crops such as other fleshy cactus fruits and mezcals (alcoholic beverages produced from agave plants); as well as crops from other countries such as durian and bitter beans (commercially important crops in South-east Asia; Bumrungsri et al. 2008; Sheherazade et al. 2019), and balsa wood (the lightest known commercial timber wood, native to South and Central America; Kunz et al. 2011). Bats may also provide further ecosystem services to pitaya agriculture in the form of crop pest suppression. The decreased pitaya yield I observed after excluding bat pollinators is similar to the decline of 31% found by Maas et al. (2013) after excluding all flying vertebrates which feed on arthropod crop pests from Indonesian shade cacao (though these effects are not universal – another study found no effect of excluding bats on cacao yield in Indonesian smallholder agroforestry; Gras et al. 2016). It would be interesting to assess whether insectivorous bats make a further contribution to pitaya crop yield to further strengthen the narrative of bat-mediated ecosystem services in the production region.

Additionally, experiments to identify the provenance of pollen resulting in cases of fruit set (indicating successful outcrossing) or aborted fruit (indicating unsuitable pollen deposition), would provide useful information to inform management to increase and/or maintain the genetic diversity present in the pitaya plantations. During the fieldwork, pitaya farmers commonly complained that incidence of both pests and disease had increased in recent years, and it is possible that the system of clonal propagation might be increasing the susceptibility of cacti (Zhu et al. 2000). Research to determine the genetic composition of plants within plantations and the incidence of pests and disease, would be highly valuable to pitaya farmers. Furthermore, this would facilitate the above-mentioned investigation of other potential ecosystem services provided by bats to pitaya farmers, such as suppression of crop pests.

It was beyond the scope of this research to consider the distribution of benefits received by actors other than income. For a better understanding of the impact of bat pollination services on wellbeing, the effect of pitaya-generated income on other objective elements of well-being (such as access to health services), and subjective elements (such as cultural importance or contribution to sense of identity) would also be analysed. Additionally, broader macro-environmental factors that impact the system should be considered (Zhang et al. 2018). This would include factors that limit access by actors to resources, opportunities and decision-making, such as a discussion of gender, and dimensions of justice, governance and organisation in the pitaya value chain (McDermott et al. 2013; Tirado von der Pahlen et al. 2018).

I also collected samples of pollen and faeces from two other nectar-feeding bat roosts in the Sayula Basin area, which have been processed in the same way as the samples in Chapter 4. This data will allow me to investigate seasonal changes in diet and resource partitioning throughout the year between *L. yerbabuenae,* two other species of nectivorous bats (*Choeronycteris mexicana and L. nivalis*) and one species of nectar-feeding bat that can switch to fruit and insects when resources are scarce (*Anoura geoffroyi*). Nectar-feeding bats typically share more resources during periods of high flower availability and increase specialisation during times of low flower availability (Sperr et al., 2011; Sritongchuay et al. 2019). This data will also allow me to build a pollination network of the mutualistic interactions between nectarivorous bats in the Sayula Basin and the plants they pollinate.

5.5 Conclusion

It is vitally important to educate the public and decision-makers as to the benefits provided by bats, such as the suppression of crop pests and other arthropods, seed dispersal, the provision of guano, and pollination. The subject of this thesis provides an excellent example of the high importance of an ecosystem service provided by bats and the direct economic benefit generated for the local community. Bat pollinators enhanced the yield and quality of a valuable local crop, providing an important cash income to inhabitants of the pitaya production area. Our novel, multi-faceted approach may provide a useful framework for other animal-pollinated crops; particularly those in less formal agrosystems and markets where a lack of registered data necessitates the collection of primary data. Such research then provides an alternative narrative around bats to that of ecosystem disservices which may currently form the dominant association. Management actions to protect bat pollinator populations and the habitats they rely on for roosting and foraging are essential to maintain the ecosystem services provided.

Appendix A Supplementary material Chapter 2

A.1 Further description of the ecology of *Stenocereus queretaroensis*

Stenocereus queretaroensis is a species of arborescent columnar cactus, reaching heights of ten metres (Pimienta-Barrios and Nobel, 1994). Wild populations grow on shallow, rocky soils at elevations of 1300 – 1600 m, while cultivated populations are found at slightly lower altitudes (Pimienta-Barrios and Nobel, 1994). *S. queretaroensis* flowers between mid-March and the end of May, with peak flowering in mid-April. Flowers display chiropterophilous characteristics: they are large, white and robust. Flowers have a total length of 10 to 14cm and grow in accessible positions from areolas on the upper side of branches (Pimienta-Barrios and Nobel, 1994). *S. queretaroensis* flowers asynchronously, with several opening each night on each plant throughout the blooming period. Flowers begin to open in the evening, at around 19.40, with anthesis and stigma receptivity occurring soon after (Ibarra-Cerdeña et al. 2005; Tremlett et al. unpublished data). The flowers last for one night only before desiccation, closing at mid-afternoon the next day. Flowers produce large volumes of nectar, with peak nectar production and peak nectar sucrose levels corresponding with the hours of peak bat activity (Ibarra-Cerdeña et al. 2005; Tremlett et al. unpublished data).

A.2 **Table A1.** Characteristics of study cultivars (‘Blanco’, ‘Mamey’, ‘Tenamaxtle’) and wild plants of *Stenocereus queretaroensis.*

*Calculated from open control fruits collected from exclusion experiments. **Data from interviews conducted in 2017 focussing on the economic value of the pitaya sector. Price is averaged across the production season and is based on size. Other information sourced from Pimienta-Barrios, 1999.

	Blanco	Mamey	Tenamaxtle	Wild
Characteristics	Longer shelf life Sweetness	Large fruits Sweetness	Large fruits Sweetness	High yield from well-established plants
Colour of pulp	White	Red	Red	Variable
Fruit weight, g*	51.0 ± 36.1	121.0 ± 56.1	79.7 ± 38.0	38.4 ± 19.0
Price, pesos**	5.1 ± 2.5	7.5 ± 3.3	7.5 ± 3.3	3.1 ± 2
% production**	8	56	7	8

A.3 Table A2. Correlation statistics showing relationship between pitaya fruit weight and size parameters.

Using Kendall rank correlation coefficient, calculated using R (2019).

	Fruit length, mm	Fruit width, mm	Fruit weight, g	Pulp weight, g
Fruit length, mm	1.00	0.64	0.72	0.64
Fruit width, mm	0.64	1.00	0.87	0.82
Fruit weight, g	**0.72**	**0.87**	1.00	**0.85**

A.4 **Table A3.** Mean number of ovules from 15 flowers ± standard deviation.

	Mean number of ovules ± SD (n = 15)	
Blanco	1130.1	± 454.0
Mamey	1614.5	± 452.3
Tenamaxtle	1430.3	± 258.4
Wild	1476.8	± 351.9

A.5 **Table A4.** Seed set under different pollination treatments ± standard error.

	Blanco		**Mamey**		**Tenamaxtle**		**Wild**		**All**	
	N fruits	Seed set ± SE	N fruits	Seed set ± SE	N fruits	Seed set ± SE	N fruits	Seed set ± SE	N fruits	Seed set ± SE
Nocturnal pollination (NP)	9	0.94 ± 0.13	10	0.64 ± 0.09	14	0.99 ± 0.05	19	0.60 ± 0.06	52	0.77 ± 0.04
Diurnal pollination (DP)	11	0.21 ± 0.04	3	0.19 ± 0.07	16	0.37 ± 0.07	3	0.10 ± 0.01	33	0.28 ± 0.04
Diurnal insects (DI)	5	0.21 ± 0.05	1	0.11 ± na	8	0.43 ± 0.13	1	0.13 ± na	15	0.32 ± 0.08
Open control (OC)	14	0.80 ± 0.08	12	0.70 ± 0.06	19	0.77 ± 0.08	19	0.56 ± 0.06	64	0.70 ± 0.04

A.6 **Table A5.** Estimates from Generalized Linear Mixed Model investigating effect of pollination treatment on fruit set, calibrated to each cultivar.

See Table 1, main text, for model outputs.

	Blanco		**Mamey**		**Tenamaxtle**	
Predictors	Estimates	95% CI	Estimates	95% CI	Estimates	95% CI
NP	1.28	0.60 – 1.96	-0.35	-0.94 – 0.25	1.32	0.67 – 1.96
OC	1.62	0.91 – 2.32	-0.01	-0.61 – 0.58	1.65	0.98 – 2.32
DI	-1.27	-1.99 – -0.54	-2.90	-3.69 – -2.11	-1.24	-1.92 – -0.55
DP	-0.17	-0.82 – 0.47	-1.80	-2.47 – -1.14	-0.14	-0.74 – -0.46

Appendix B Supplementary material Chapter 3

B.1 Overview of the stages in the pitaya value chain

Cultivation

The main actors at the cultivation stage are plantation owners and renters, and home garden owners (Table B3 describes all actors and their roles). Additionally, day labourers are employed in the management of the plantations. Agricultural inputs are low: only 24% of plantation owners interviewed used pesticides (mostly a powder applied to deter ants) or fertilisers, and 16% irrigated the cacti. Over half (54%) of plantation owners also grew crops other than pitayas. The timing of pitaya harvest in the dry season (April - June) allows producers to grow other crops during the rainy season (July - September), such as maize (22% of plantation owners) and beans (14%). 35% of plantation owners also grew fruit trees such as guamuchil, mango, avocado, orange and guava.

Processing

The main actors at the processing stage are waged workers that harvest and peel the fruits, and product makers. Pitayas are harvested manually within a day of ripening, usually in the early hours of the morning to sell the fruits the same day. Pitayas are packed with foliage (e.g. alfalfa) to keep them fresh for transportation to markets. The spines are removed from fruits by peelers before they are sold to the consumer. Most fruits are sold to the consumer fresh, but a small proportion are increasingly used to make products such as cakes, jams and punch.

Marketing

The main marketing actors are roadside and market vendors, ambulant sellers that sell on foot or from a vehicle, drivers that transport fruits, and sales assistants. The market area for pitayas is very localised, with 98% of fruits sold by interviewed vendors within the state of

Jalisco, both in the production area and nearby towns and cities. 69% of all fruits sold by interviewed vendors were sold at markets in Guadalajara, the largest city in Jalisco (80km north-east of Techaluta de Montenegro). The most important of these is a traditional seasonal market dedicated to pitayas, ‘Las 9 esquinas’, which accounted for 23% of total fruits sold. 10% of total fruits are sold to consumers in Techaluta de Montenegro, from stalls by the side of the main road.

Most fruits are sold direct to the consumer for immediate payment. Markets are informal, and contractual agreements with commercial enterprises are rare, due to difficulties with transport and packaging, and lag-times in payment for goods by large companies to vendors with high overheads to pay. Some fruits are exchanged for goods in shops or with travelling salesmen.

B.2 Example calculation of value of dependency of fruit quality on bat pollination

Below is an example of how we calculated the dependency of fruit quality on bat pollination D_{qkw} for one producer, ‘Producer A’:

Producer A receives 0.5 pesos for small fruits, 3 pesos for medium fruits and 3 pesos for large fruits. He produces 10,000 fruits each year. He has 600 Blanco cacti (6%), 8400 Mamey cacti (84%), 1000 Tenamaxtle cacti (10%) and 0 wild cacti (0%). We use these percentages of cacti as a proxy for the percentage of fruits of each pitaya type. His total income from selling fruits was Mx$20,000, of which Mx$2,268 can be attributed to increased fruit yield resulting from bat pollination.

To calculate D_q for this producer for Blanco fruits, we first add up the difference in fruit price resulting from changes in price/size categories in the absence of bat pollination. So, for example, if large fruits remained in the large category, the producer would receive 100% of the original price ((large price / large price) * 100 , i.e. here (3 pesos / 3 pesos) * 100, = 100%).

Table B1. Percentage of fruits that move between each size-price category in the absence of bat pollination for each cultivar and wild cacti.

	Large: no change	Large → medium	Large → small	Medium: no change	Medium → small	Small: no change
Blanco	0	9	24	2	9	56
Mamey	33	0	47	0	0	20
Tenamaxtle	6	25	62	0	7	0
Wild	0	0	16	0	21	63

We can see from Table B1 however, that no fruits remained in the large category for Blanco fruits in the absence of bat pollination, so we would multiply this price difference by zero (Table B2). 9% of the fruits moved from the large category to the medium category, but there is no difference in price received by the producer between large and medium fruits. We therefore multiply the proportion of price change (0.00) by the proportion of fruits to make this category change (0.09; Table B2). There is a decrease of 83% in price between large and small fruits, so there is a 0.83 drop in potential earnings for fruits that would be large under natural pollination conditions but in the absence of bats are small. The proportion of fruits to move from large to small is 0.24, so we get a total drop in value of 0.1992 for these fruits (0.83 * 0.24). We do this for all the category changes and add up the totals (Table B2).

Table B2. Example of the calculation used to calculate $\boldsymbol{D_q}$ for producer A for Blanco fruits.

	Large: no change	Large → medium	Large → small	Medium: no change	Medium → small	Small: no change
Prop. Blanco fruits that change size categories in the absence of bat pollination (Table 1)	0	0.09	0.24	0.02	0.09	0.56
Difference in fruit price between size categories (producer specific)	100 – (3/3 * 100) = 0	100 – (3/3 * 100) = 0	100 – (0.5/3 * 100) = 0.83	100 – (3/3 * 100) = 0	100 – (0.5/3 * 100) = 0.83	100 – (0.5/0.5 * 100) = 0
Change in potential earnings due to size category change	0 * 0 = 0	0 * 0.09 = 0	0.24 * 0.83 = 0.199	0 * 0.02 = 0	0.83 * 0.09 = 0.075	0 * 0.56 = 0

Thus, the total D_q for producer A for Blanco fruits is 0.27 – i.e. the value of his Blanco fruits drops by 27% in the absence of bats. We get this total by adding up the changes in potential earnings due to size category change (last row of Table B1).

To calculate the value of bat pollination resulting from increased fruit quality V_{qb}, we multiply the income remaining after we have subtracted the value of bat pollination resulting from increased fruit yield from total income ($V_b - V_{yb}$), by the coefficient for the change in fruit quality D_q for Blanco fruits multiplied by the proportion of Blanco cacti under production by Producer A. So, in this example: ((Mx$20,000 – Mx$2,268) * (0.06*0.27)) = Mx$297. To estimate the proportion of the income of Producer A that will be lost from decreases in size of Blanco fruits in the absence of bat pollination, we divide this by total remaining income: Mx$298 / Mx$17,732 = 0.0162 * 100 = 1.62%. So for

Producer A, Mx$297, or 1.62% of the income remaining after we have subtracted V_{yb}, is attributable to increases in quality (size) of Blanco fruits as a result of bat pollination.

We repeat this process for each of the pitaya types, and sum them to get the total value associated with increases in fruit size V_{qb} for each producer. You can see that if the producer charged more for the large fruits than for the medium fruits, then there would be a higher overall difference in the amount of money that he/she would lose from having smaller fruits in the absence of bat pollination. Likewise, for producers that have higher proportions of cacti with fruits that show a greater number of size category changes in the absence of bat pollination (i.e. Mamey and Tenamaxtle).

B.3 Table B3. Summary of stakeholders, their functions and characteristics

Stakeholders	N	Functions/Activities	Characteristics
Production:			
Plantation owners	39	▪ Own plantations of *S. queretaroensis*: bought or inherited ▪ Some harvest fruits to sell on to consumers or other vendors ▪ Some rent out plantations for the season, mostly for a fixed sum agreed in advance	▪ Majority male (61%) and middle-aged (mean age = 53). ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 55% ▪ 71 % have access to a vehicle
Plantation renters	40	▪ Rent plantations for the season ▪ Harvest fruits to sell to consumers or to other vendors	▪ Majority male (68%). ▪ Mean age = 46. Median age = 46 ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 63% ▪ 90% have access to a vehicle
Home garden owners	20	▪ Own small numbers of cacti in backyards ▪ Harvest fruit for home consumption, to make into products, and to sell to vendors or to consumers	▪ Majority female (57%). ▪ Mean age = 57. Median age = 56 ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 62% ▪ 71% have access to a vehicle
Wild fruit collectors	1	▪ Harvest fruit from cacti on ejido (common) land ▪ Rare commercial activity in study area: other paid work associated with pitayas preferentially chosen ▪ Some families harvest fruits for own consumption	

Agricultural workers	6	▪ Paid a fixed daily rate for labour on pitaya plantations such as weeding, planting cacti, building fences. Day labourer	▪ Mostly male (83% - check interview 112 – should be included as female TA?) ▪ Mean age = 50. Median age = 43 ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 67% ▪ 50% have access to a vehicle
Consultant	1	▪ Offers consultancy service, primarily overseeing the establishment of new pitaya plantations for absent landowners who have saved money working abroad ▪ Respected pitaya producer, well-known for excellent technical knowledge, high quality fruits, and healthy cacti	
Processing:			
Harvesters	11	▪ Paid either a fixed hourly or daily rate. Temporary labourer ▪ Harvest fruits during the night or early morning ▪ Transport to peelers or vendors	▪ Male (100%), of all ages. ▪ Commonly either work as a harvester in addition to normal day job (e.g. in construction during the day, harvesting at night). Highest proportion compared to other actor groups manage to save at least some of their earnings (90%). ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 45% ▪ 36% have access to a vehicle
Peelers	12	▪ Paid either a fixed hourly or daily rate (or rarely, by fruits peeled). Temporary labourer ▪ Peel fruits (remove the spines) and pack for transport	▪ Mostly female (92%), of all ages ▪ Paid between 20 and 30 pesos per hour: working conditions vary more than pay ▪ Do not usually work during the rest of the year, are occupied in the household ▪ Work long hours during pitaya production, both peeling fruits and continuing to be responsible for domestic duties such as childcare, cleaning and cooking. ▪ Percentage of yearly income from pitayas = 20%

			▪ Pitaya principal income = 33% ▪ 33% have access to a vehicle
Managers	1	▪ Manage teams of peelers and harvesters ▪ Only employed by larger scale producers	
Product makers	25	▪ Use pitayas to make products such as cakes, jams, punch and salsas ▪ Pitayas can be sourced from own production, bought, or gifted	▪ Mostly female (76%), mean age = 46 ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 40%# ▪ 76% have access to a vehicle
Marketing/retailing:			
Intermediaries	4	▪ Buy fruit directly from producers and sell to other vendors ▪ Some have fixed situations (e.g. stall at wholesale market) and vendors come to them to buy fruit; others have pre-agreed arrangements and deliver the fruit to vendors ▪ Buy and sell by boxes; pay immediately and receive payment immediately	▪ Mostly male (75%) and middle-aged (mean age = 46). ▪ Percentage of yearly income from pitayas = 40-60% ▪ Pitaya principal income = 50% ▪ 100% have access to a vehicle
Roadside vendors	31	▪ Sell peeled fruits, and often other products such as flowers and cakes, direct to consumers from roadside stalls ▪ Some rely on drivers to stop cars to buy fruits; some rely on pedestrian passers-by ▪ Take immediate payment for products	▪ Majority female (61%). ▪ Mean age = 49. Median age = 50 ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 55% ▪ 81% have access to a vehicle
Market vendors	19	▪ Sell peeled fruits, and often other products such as flowers and cakes, direct to consumers from stalls at a market ▪ Take immediate payment for products	▪ Pretty evenly split between men and women (53% female). ▪ Mean age = 47. Median age = 50 ▪ Percentage of yearly income from pitayas = 40-60% ▪ Pitaya principal income = 68% ▪ 89% have access to a vehicle

Ambulant vendors	5	▪ Sell peeled fruits moving from place to place either on foot or with a vehicle ▪ Take immediate payment	▪ Majority female (60%). ▪ Mean age = 40. Median age = 45 ▪ Percentage of yearly income from pitayas = 20-40% ▪ Pitaya principal income = 80% ▪ 100% have access to a vehicle
Drivers	4	▪ Transport fruits from production area to market area. Temporary labourer ▪ Often make multiple trips per day ▪ Some own their own vehicle, some drive their employer's vehicle	▪ All male ▪ Mean age 41. Median age = 39 ▪ Percentage of yearly income from pitayas = 0-20% ▪ Pitaya principal income = 25% ▪ 75% have access to a vehicle
Sales assistants	3	▪ Paid either a fixed hourly or daily rate. Temporary labourer ▪ Sell fruits from roadside or market stalls	▪ All female ▪ Mean age = 24. Median age =25 ▪ Percentage of yearly income from pitayas = 0-20% ▪ Pitaya principal income = 67% ▪ 33% have access to a vehicle
Consumption:			
Consumers	20	▪ Consume fruits; mostly passers-by for home consumption ▪ Some restaurants buy fruits to make into e.g. drinks or desserts	▪ Mean monthly income around $12,500; a higher income than any of the actors involved in the rest of the value chain ▪ 78% had reached a level of schooling of undergraduate degree or above ▪ Indicates that pitayas are a luxury fruit ▪ Travelled a mean time of 38 minutes to buy pitayas, almost always by car

B.4 Table B4. Mean percentage of total costs for actors in expense categories, and total costs (Mx$ ± SD)

Actor	N	Agricultural inputs	Rent	Financial	Marketing	Tools and equipment	Buying fruits	Transport	Employee wages and benefits	Total costs Mx$
Home garden owner	7	20.9	0	0	0	16.3	0	31.0	**31.7**	11,242 ± 166,73
Plantation owner	20	31.9	0	0	1.1	12.2	0	24.3	**32.2**	19,319 ± 36624
Intermediaries	2	0	0	0	2.9	0.7	**86.5**	4.2	5.9	317,775 ± 319
Ambulant sellers	5	0.4	25.9	0	0.4	4.8	18.3	23.7	**26.5**	84,006 ± 27,766
Roadside vendor	27	0.8	17.4	0.7	1.5	22.1	11.9	16.6	**28.9**	77,320 ± 95,914
Market vendor	19	0.8	19.3	0	2.0	4.8	19.5	17.8	**35.9**	253,529 ± 262,765

n.b. Home garden owners did not sell fruits at market or roadside, nor rent plantations. Market vendors did not sell fruits by the roadside and vice versa. Plantation owners did not also sell fruits at market or by the road, or rent plantations. Intermediaries did not also rent or own plantations. Main cost categories for each actor type are emphasised in bold.

B.5 **Table B5. Distribution of economic activities between government, external and local actors.** Value = the total value cited by interviewees, as either profits or costs. % = the proportion of the expense of each actor group represented by item within the actor category.

Government	**Value, pesos**	**%**	**External agents**	**Value, pesos**	**%**	**Local community**	**Value, pesos**	**%**
Export tax	2,650	0	Profits from outside vendors	357,430	27	Wages and benefits	3,411,655	27
Production tax	0	0	Selling permits	47,010	4	Plantation rents	1,922,500	15
Income tax	0	0	Agricultural inputs	48,855	4	Buying fruits	1,677,140	13
VAT	870	0	Tools and equipment	426,027	32	Profits	5,433,067	44
Property tax	30,217	3	Car insurance	128,400	10			
Certification	11,740	1	Packaging	328,249	25			
Road tolls	17,687	2						
Petrol	931,685	89						
Stall rents	47,015	5						
Total	**1,041,864**	**7**	**Total**	**1,335,971**	**9**	**Total**	**12,444,362**	**84**

B.6 Figure B1. Mean final prices for fruits received by different actors

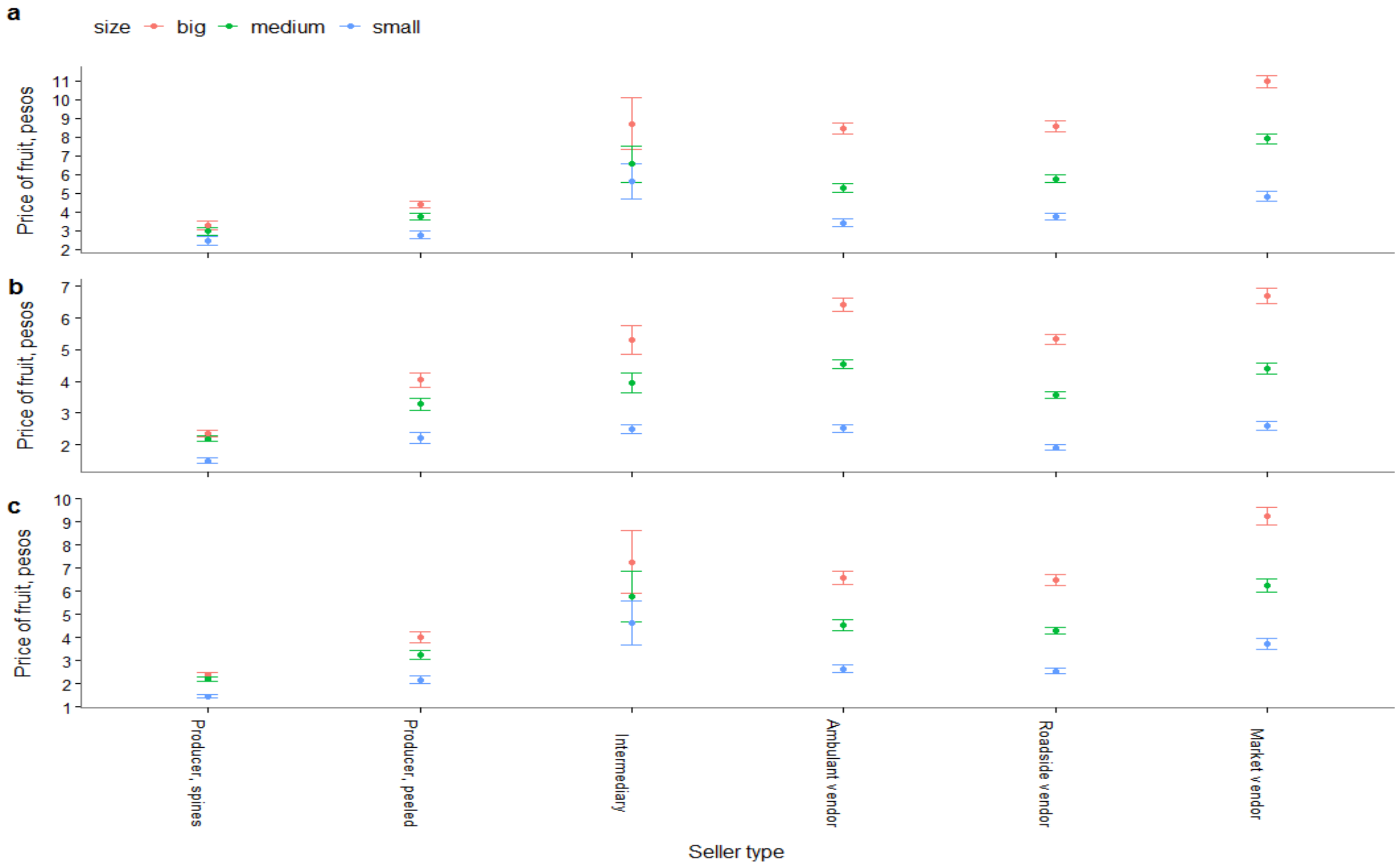
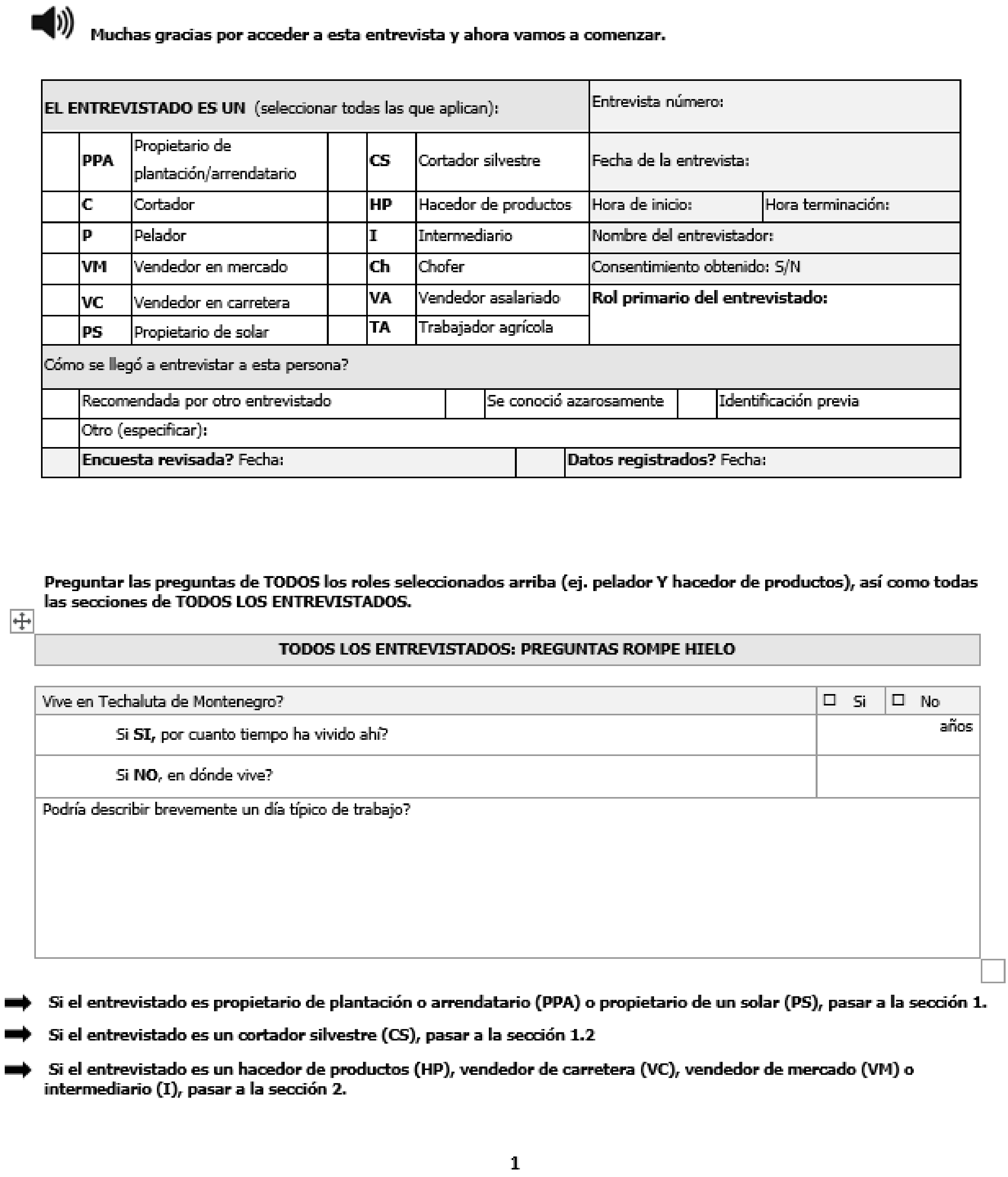


Figure B1. The mean final price of fruits (±SE) received by different stakeholders at the **a**) start of season, **b**) peak season, **c**) end of season.

B.7 Interview questions



Muchas gracias por acceder a esta entrevista y ahora vamos a comenzar.

EL ENTREVISTADO ES UN (seleccionar todas las que aplican):						Entrevista número:	
	PPA	Propietario de plantación/arrendatario		**CS**	Cortador silvestre	Fecha de la entrevista:	
	C	Cortador		**HP**	Hacedor de productos	Hora de inicio:	Hora terminación:
	P	Pelador		**I**	Intermediario	Nombre del entrevistador:	
	VM	Vendedor en mercado		**Ch**	Chofer	Consentimiento obtenido: S/N	
	VC	Vendedor en carretera		**VA**	Vendedor asalariado	**Rol primario del entrevistado:**	
	PS	Propietario de solar		**TA**	Trabajador agrícola		

Cómo se llegó a entrevistar a esta persona?					
	Recomendada por otro entrevistado		Se conoció azarosamente		Identificación previa
	Otro (especificar):				
	Encuesta revisada? Fecha:		**Datos registrados?** Fecha:		

Preguntar las preguntas de TODOS los roles seleccionados arriba (ej. pelador Y hacedor de productos), así como todas las secciones de TODOS LOS ENTREVISTADOS.

TODOS LOS ENTREVISTADOS: PREGUNTAS ROMPE HIELO

Vive en Techaluta de Montenegro?	☐ Si	☐ No
Si **SI**, por cuanto tiempo ha vivido ahí?	años	
Si **NO**, en dónde vive?		
Podría describir brevemente un día típico de trabajo?		

➡ **Si el entrevistado es propietario de plantación o arrendatario (PPA) o propietario de un solar (PS), pasar a la sección 1.**

➡ **Si el entrevistado es un cortador silvestre (CS), pasar a la sección 1.2**

➡ **Si el entrevistado es un hacedor de productos (HP), vendedor de carretera (VC), vendedor de mercado (VM) o intermediario (I), pasar a la sección 2.**

1

Ahora le hare unas preguntas sobre su plantación o solar y sus pitayos.

PPA, PS	**PROPIETARIOS DE PLANTACIONES Y ARRENDATARIOS (PPA), PROPIETARIOS DE SOLARES (PS):** **1. CARACTERÍSTICAS DE LAS PLANTACIONES DE PITAYA**

PPA	1.1.1	Posee o renta una pitayera?	1. Posee (ir a 1.1.2)	2. Renta (ir a 1.2)
	1.1.2	Si **POSEE en 1.1.1**, compró o heredo la tierra?	1. Compró	2. Heredó
	1.1.3	Si **POSEE en 1.1.1**, se la renta a alguien más?	1. Si	2. No
PPA	1.2	Cuántas plantaciones posee o renta?	1.2.1 _____ posee	1.2.2 _______ renta
PPA	1.3	Cuál es el tamaño total de la(s) plantación(es) que posee/renta?	1.3.1. _____ posee	1.3.2. _______ renta ☐ m^2 ☐ hectáreas
PS	1.4	Cuál es el tamaño total de su solar?	_______	☐ m^2 ☐ hectáreas
PPA **PS**	1.5	Cuántos pitayos hay en total?	1.5.1 _______ posee	1.5.2 _______ renta 1.5.3 _______ solar
PPA **PS**	1.6	Cuál es el edad de los pitayos? Llenar el cuadro de abajo:		

	1.6.1	0-10 años	Cantidad:	1.6.4	30-40 años	Cantidad:
	1.6.2	10-20 años	Cantidad:	1.6.5	40-50 años	Cantidad:
	1.6.3	20-30 años	Cantidad:	1.6.6	50+ años	Cantidad:

PPA **PS**	1.7	Qué porcentaje de las diferentes variedades de pitaya tiene en total?

1. Mamey _______%	2. Blanco ________ %	3. Tenamaxtle _____%	4. Silvestre__________%
5. Morado_______%	6. Amarillo________%	7. Otro (especificar:) __________%	

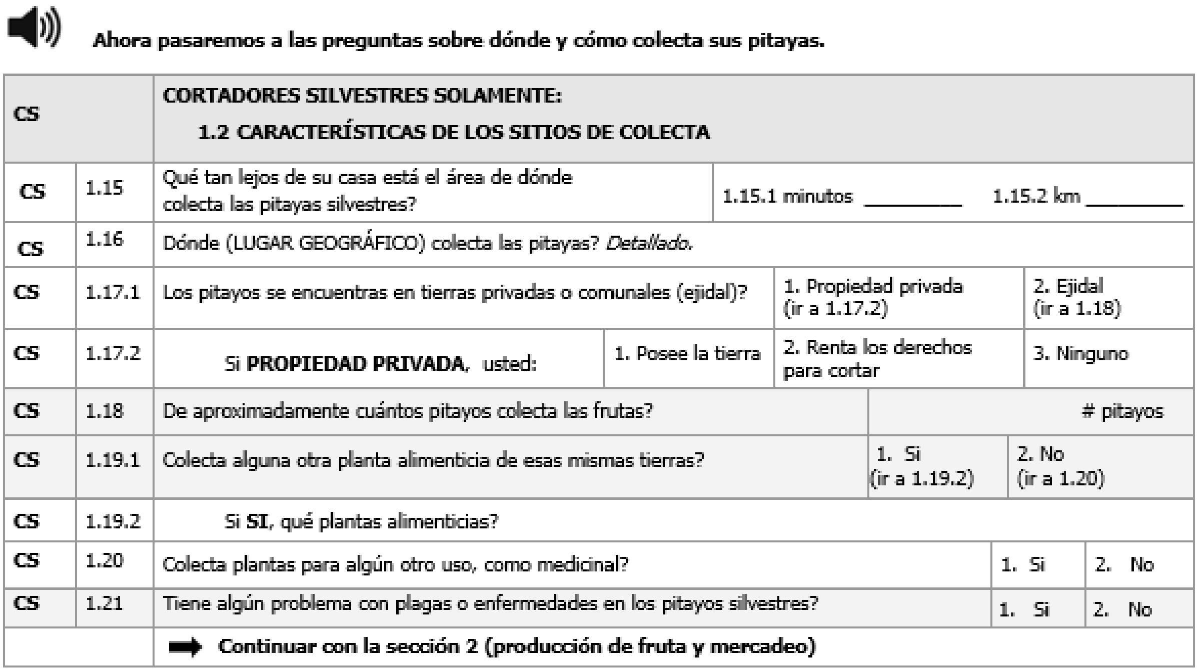
PO **PS**	1.8.1	Planta otros cultivos en la misma tierra?	1. Si (ir a 1.8.2)	2. No (ir a 1.9)
	1.8.2	Si **SI**, cuáles?		
PO **PS**	1.9	Por qué prefiere cultivar pitayas en lugar de algún otro cultivo?		
PPA **PS**	1.10	Ofrece algún cuidado a los pitayos de sus plantaciones/solar?	1. Si (ir a 1.11)	2. No (ir a 1.12.1)
PPA **PS**	1.11	Si **SI**, qué actividades realiza? Seleccionar todas las actividades de manejo		

1.	Rotación de suelo	4.	Aplicación de composta
2.	Irrigación	5.	Aplicación de pesticidas/insecticidas
3.	Deshierbar	6.	Adición de fertilizantes
7.	Otro (especificar):		

PO **PS**	1.12.1	Cómo propaga los pitayas?	1. No lo hago (ir a 1.13)	2. Semilla (ir a 1.12.2)	3. Brazos (ir a 1.12.2)
PO **PS**	1.12.2	**Si propaga los pitayas,** provienen las semillas/brazos de sus propias plantaciones?	1. Si	2. No	
PPA **PS**	1.13	Tiene algún problema con plagas o enfermedades en sus pitayos?	1. Si	2. No	
PPA **PS**	1.14	De dónde obtiene consejos o asistencia técnica para el cuidado de sus pitayos o para combatir las plagas y enfermedades?			

1. Vendedor de productos agrícolas	2. Otros agricultores	3. Conocimiento empírico
4. Gobierno/servicios de apoyo gubernamentales		

➡ **Continuar con la sección 2 (producción de fruta y mercadeo)**



Ahora pasaremos a las preguntas sobre dónde y cómo colecta sus pitayas.

CS		**CORTADORES SILVESTRES SOLAMENTE:** **1.2 CARACTERÍSTICAS DE LOS SITIOS DE COLECTA**			
CS	1.15	Qué tan lejos de su casa está el área de dónde colecta las pitayas silvestres?	1.15.1 minutos _________	1.15.2 km _________	
CS	1.16	Dónde (LUGAR GEOGRÁFICO) colecta las pitayas? *Detallado.*			
CS	1.17.1	Los pitayos se encuentras en tierras privadas o comunales (ejidal)?	1. Propiedad privada (ir a 1.17.2)	2. Ejidal (ir a 1.18)	
CS	1.17.2	Si **PROPIEDAD PRIVADA**, usted:	1. Posee la tierra	2. Renta los derechos para cortar	3. Ninguno
CS	1.18	De aproximadamente cuántos pitayos colecta las frutas?	# pitayos		
CS	1.19.1	Colecta alguna otra planta alimenticia de esas mismas tierras?	1. Si (ir a 1.19.2)	2. No (ir a 1.20)	
CS	1.19.2	Si **SI**, qué plantas alimenticias?			
CS	1.20	Colecta plantas para algún otro uso, como medicinal?	1. Si	2. No	
CS	1.21	Tiene algún problema con plagas o enfermedades en los pitayos silvestres?	1. Si	2. No	
		➡ **Continuar con la sección 2 (producción de fruta y mercadeo)**			

PPA, PS, CS, VC, VM, I, HP	**PROPIETARIOS DE PLANTACIONES O ARRENDATARIOS (PPA), PROPIETARIOS DE SOLARES (PS), CORTADORES SILVESTRES (CS), VENDEDORES DE MERCADO (VM), VENDEDORES DE CARRETERA (VC), INTERMEDIARIOS (I), HACEDORES DE PRODUCTOS (HP)** **2. PRODUCCIÓN DE FRUTA Y MERCADEO**

TODOS	2.1	Usted corta, vende y/o compra pitayas? Seleccionar todas las opciones aplicables.			
		☐ Corta (hacer preguntas 2.2 – 2.4)	☐ Vende (hacer preguntas 2.5 - 2.31)	☐ Compra (hacer preguntas 2.32 – 2.37)	☐ No (ir a 2.39.1)
		Preguntas de CORTA			
TODOS	2.2	Cuántos días aproximadamente duró la producción de fruta este año?	días		
PPA, PS, CS	2.3.1	Cuántas frutas cortó este año aproximadamente?	frutas		
	2.3.2	Esto fue más, menos o similar al año pasado?	1. Más (ir a 2.3.3)	2. Menos (ir a 2.3.3)	3. Similar (ir a 2.4)
	2.3.3	Si **MÁS** o **MENOS**, a qué cree que se deba esta diferencia?	1. Clima	2. Plagas/enfermedades	3. Otro
	2.3.4	Favor de dar más detalles: (*ej. más o menos lluvia? Temperaturas más cálidas o frías?*)			
PPA, PS, CS	2.4	Qué porcentaje (%) de la fruta es:			
		1. vendida:	2. regalada:	3. perdida: *(No cosechada)*	4. Para consumo de vivienda:

		Preguntas de VENTA				
TODOS	2.5	Qué tan seguido vende pitayas/productos durante la temporada de producción de frutas?				
		1. Todos los días	2. Algunas veces a la semana	3. Una vez por semana	4. Menos de una vez a la semana	
		5. Cuando necesito dinero	6. Cuando tengo demasiadas pitayas	7. Otro (especificar):		
PPA, PS, CS, VC, VM, I	2.6.1	Vende los pitones de la pitaya (flores secas)?	1. Si (ir a 2.6.2)	2. No (ir a 2.7)		
	2.6.2	Si **SI a 2.6.1**, cuántas flores vende por día en promedio?				
	2.6.3	Si **SI a 2.6.1**, en cuánto vende las flores?	pesos			
HP	2.7	Qué productos hace?				
		1. Ponche	2. Mermelada	3. Nieve	4. Gelatina	5. Rompope
		6. Pan de pitaya	7. Yogurt	8. Otro (especificar):		
HP, VC, VM, I,	2.8	Cuántos productos vende diariamente en promedio?				
		1. Ponche:	2. Mermelada:	3. Nieve:	4. Gelatina:	5. Rompope:
		6. Pan de pitaya:	7. Yogurt:	8. Otro (especificar):		
HP, VC, VM, I	2.9	Cuál es el precio de los productos que vende?				
		1. Ponche:	2. Mermelada:	3. Nieve:	4. Gelatina:	5. Rompope:
		6. Pan de pitaya:	7. Yogurt:	8. Otro (especificar):		
VC, VM	2.10	Cuánto del precio de venta queda con usted? *A veces, los puestos venden los productos de otros por una comisión.*				
		1. Ponche:	2. Mermelada:	3. Nieve:	4. Gelatina:	5. Rompope:
		6. Pan de pitaya:	7. Yogurt:	8. Otro (especificar):		

HP	2.11	**Dónde** (lugar geográfico) se venden los productos a los consumidores? (especificar lo más posible): Si los productos van a más de un lugar, estimar la proporción de los productos vendidos en cada lugar.					
PPA, PS, CS, VC, VM, I	2.12	**Dónde** (lugar geográfico) se venden las frutas a los consumidores? (especificar lo más posible): Si las frutas van a más de un lugar, estimar la proporción de la fruta vendida en cada lugar.					
VC, VM, I,	2.13	**Cómo** vende la fruta?					
		1. Puesto en carretera	2. Puesto en mercado	3. En un carro	4. Otro (especificar):		
HP	2.14	Cómo vende los productos?					
		1. Puesto en carretera	2. Puesto en mercado	3. Puesto de alguien más	4. Otro (especificar):		
PPA, PS, CS, VC, VM, I	2.15	**Por qué** eligió vender la fruta/productos en la manera que lo hace?					
VC, VM, I, HP	2.16.1	Necesita un permiso en donde vende pitayas/productos actualmente?	1. Si (ir a 2.16.2)	2. No (ir a 2.17.1)			
	2.16.2	Si **SI**, por cuánto tiempo es el permiso?					
PPA, PS, CS, VC, VM, I	2.17.1	Cuántas frutas vendió esta temporada aproximadamente?	Frutas				
	2.17.2	Fué más, menos o similar al año pasado?	1. Más	2. Menos	3. Similar		
	2.18.1	Cuantas frutas vendió en pro medio por día esta temporada? Anotar abajo.					
		Inicio de temporada:	Durante la privanza:	Fin de temporada:			
	2.18.2	Cómo varían los precios entre tamaños, hora del día o momento de la temporada? Anotar los precios abajo					
		Inicio de temporada:		**Durante la privanza:**		**Fin de temporada:**	
		1. día (am)	**2. tarde (pm)**	**3. día (am)**	**4. tarde (pm)**	**5. día (am)**	**6. tarde (pm)**
		Chico: Mediano: Grande:	Chico: Mediano: Grande:	Chico: Mediano: Grande:	Chico: Mediano: Grande:	Chico: Mediano: Grande:	Chico: Mediano: Grande:
TODOS	2.19.1	El precio que dá cambia dependiendo de quién compra la fruta/producto?	1. Si (ir a 2.19.2)	2. No (ir a 2.20.1)			
	2.19.2	Si **SI a 2.19.1**, cómo? Dar detalles.					
	2.20	Quién determina el precio de las pitayas/productos que vende?	1. Comprador	2. Vendedor	3. Depende		
	2.21.1	Cómo se establece el precio de la pitaya/productos?	1. Regateo (ir a 2.25.2)	2. Predeterminado (ir a 2.26)			
	2.21.2	Si **REGATEO**, qué afecta el precio final acordado? Seleccionar todas las que aplican.					
		1. Demanda de comprador	2. Disponibilidad de la fruta	3. Otros productores	4. Hora del día	5. Otro (especificar):	
TODOS	2.22	Cuánto vendió en pesos en total esta temporada?	pesos				
TODOS	2.23.1	Cree que obtiene un precio justo por la venta de pitayas/productos?	1. Si (ir a 2.24.1)	2. No (ir a 2.23.2)			
TODOS	2.23.2	Si **NO a 2.20.1**, por qué no?					
TODOS	2.24.1	Quién cree que obtiene las mejores ganancias de la venta de pitayas?					
TODOS	2.24.2	Por qué?					
TODOS	2.25.1	El precio de la pitaya fluctúa entre años?	1. Si (ir a 2.25.2)	2. No (ir a 2.26)			
TODOS	2.25.2	Cómo?					

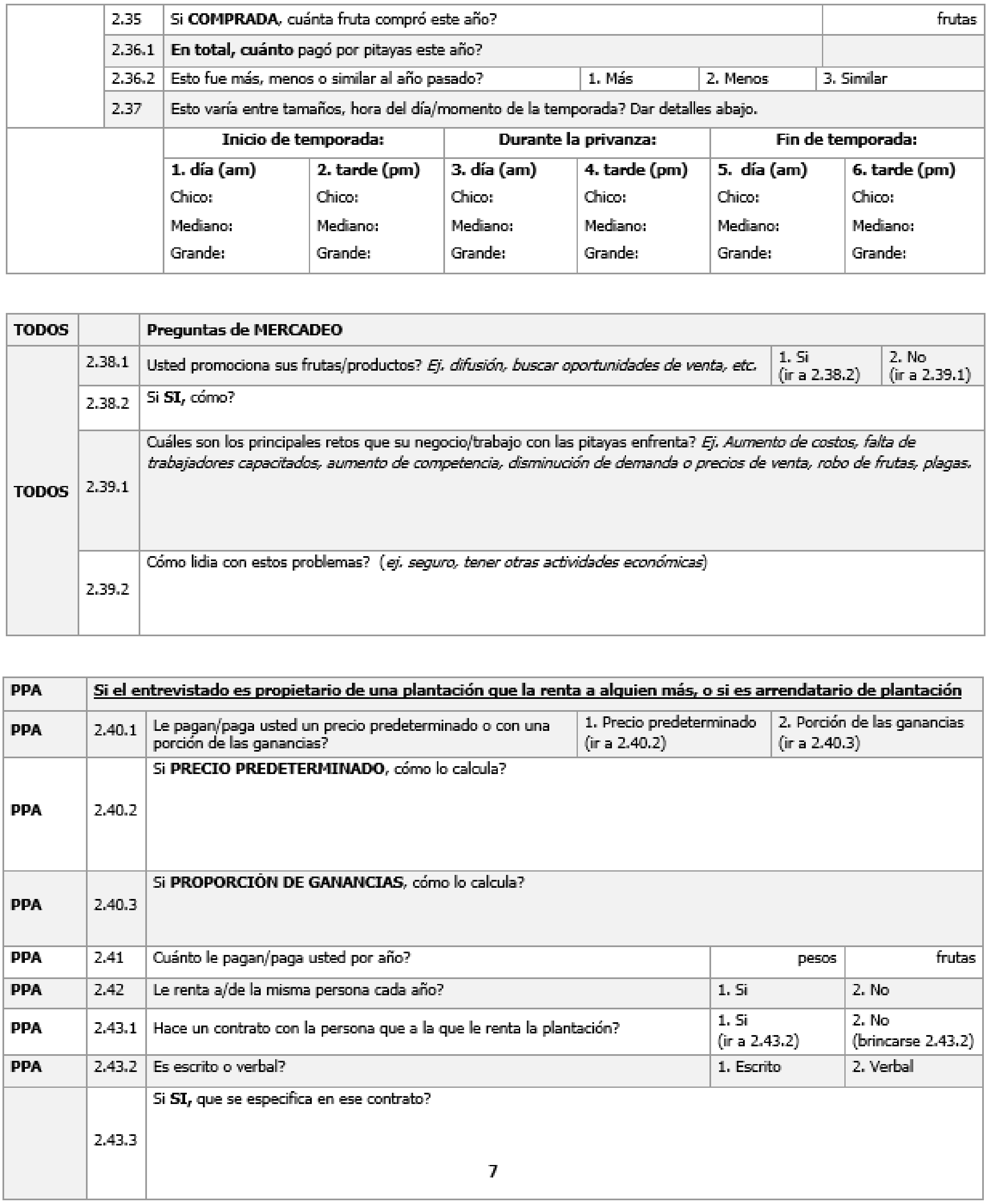
TODOS	2.26	Quién compra las frutas/productos? Anotar **un porcentaje del total de frutas vendidas** en cada respuesta apropiada:			
		1. Intermediarios:	2.Vendedores en mercado:	3.Hacedores de productos:	4.Personas de paso:
		5.Amigos/familia/conocidos:	6.Vendedores ambulantes:	7.Otro (especificar):	

ALL	2.27.1	Tiene contratos **de venta** pre-establecidos o tratos antes de la temporada de cosecha?	1. Si (ir a 2.27.2)	2. No (ir a 2.28)
	2.27.2	Si **SI**, son verbales o escritos?	1. Verbales	2. Escritos
	2.27.3	Si **SI**, que se especifica en estos acuerdos/contratos?		

Seleccionar:		Detalles:	
1.	Precio		pesos
2.	Cantidad de fruta/productos	productos	frutas
3.	Regularidad de la entrega	Cada: ☐ Día ☐ Semana ☐ Mes	
4.	Hora de entrega		
5.	Fecha de entrega		
6.	Tamaño de la fruta		
7.	Variedad de la fruta		
8.	Duración del contrato		
9.	Formas de pago		
10.	Penalizaciones	Si se selecciona, ir a 2.27.4	

ALL	2.27.4	Si **SI** a penalizaciones, que pasa si no puede hacer alguna entrega según el contrato? *Ej. Si la calidad de fruta es inadecuada.*			
PPA	2.28	Qué pasa si las ganancias de la venta de frutas no son suficientes para pagar la renta de la plantación?			
		1. El arrendatario asume las perdidas	2. Reembolso parcial del dueño al arrendatario	3. El dueño reembolsa todo el dinero al arrendatario	
ALL	2.29.1	Le pagan de manera inmediata las frutas/productos que vende?	1. Si (ir a 2.30)	2. No (ir a 2.29.2)	
	2.29.2	Si **NO a 2.29.1**, cuánto tardan en pagarle en promedio?	días		
ALL	2.30.1	Qué proporción de la fruta o productos no se vende? (*solo las frutas que fueron traídas para vender)*			
	2.30.2	Qué hace con la fruta/productos que no se venden?			
		1. Se regalan	2. Se tiran	3. Se utilizan para hacer productos	4. Se venden para hacer productos (ir a 2.13.3)
ALL	2.30.3	Si se venden para hacer productos, cuánto le pagan por la fruta?	por fruta		

		Preguntas de COMPRA			
VC, VM, I, HP	2.32	Cómo consigue la fruta que vende/con la que hace productos?			
		1. Comprada (ir a 2.33)	2. Cosecha propia o de un familiar (ir a **mercadeo**)	3. Otro (especificar):	
VC, VM, I, HP	2.33	Si **COMPRADA**, a quién le compra la fruta? Seleccionar todas las opciones que aplican.			
		1. Miembro de la familia/amigo	2. Intermediario	3. Vendedor de carretera/mercado	4. Propietario de solar
		5. Directo de la plantación	6. Desconocido	7. Otro (especificar):	
VC, VM, I, HP	2.34	Si **COMPRADA**, de dónde (**lugar geográfico**) proviene la fruta y en **qué proporción**?			



	2.35	Si **COMPRADA**, cuánta fruta compró este año?			frutas
	2.36.1	**En total, cuánto** pagó por pitayas este año?			
	2.36.2	Esto fue más, menos o similar al año pasado?	1. Más	2. Menos	3. Similar
	2.37	Esto varía entre tamaños, hora del día/momento de la temporada? Dar detalles abajo.			

	Inicio de temporada:		**Durante la privanza:**		**Fin de temporada:**	
	1. día (am) Chico: Mediano: Grande:	**2. tarde (pm)** Chico: Mediano: Grande:	**3. día (am)** Chico: Mediano: Grande:	**4. tarde (pm)** Chico: Mediano: Grande:	**5. día (am)** Chico: Mediano: Grande:	**6. tarde (pm)** Chico: Mediano: Grande:

TODOS		**Preguntas de MERCADEO**		
TODOS	2.38.1	Usted promociona sus frutas/productos? *Ej. difusión, buscar oportunidades de venta, etc.*	1. Si (ir a 2.38.2)	2. No (ir a 2.39.1)
	2.38.2	Si **SI,** cómo?		
	2.39.1	Cuáles son los principales retos que su negocio/trabajo con las pitayas enfrenta? *Ej. Aumento de costos, falta de trabajadores capacitados, aumento de competencia, disminución de demanda o precios de venta, robo de frutas, plagas.*		
	2.39.2	Cómo lidia con estos problemas? (*ej. seguro, tener otras actividades económicas*)		

PPA	**Si el entrevistado es propietario de una plantación que la renta a alquien más, o si es arrendatario de plantación**			
PPA	2.40.1	Le pagan/paga usted un precio predeterminado o con una porción de las ganancias?	1. Precio predeterminado (ir a 2.40.2)	2. Porción de las ganancias (ir a 2.40.3)
PPA	2.40.2	Si **PRECIO PREDETERMINADO**, cómo lo calcula?		
PPA	2.40.3	Si **PROPORCIÓN DE GANANCIAS**, cómo lo calcula?		
PPA	2.41	Cuánto le pagan/paga usted por año?	pesos	frutas
PPA	2.42	Le renta a/de la misma persona cada año?	1. Si	2. No
PPA	2.43.1	Hace un contrato con la persona que a la que le renta la plantación?	1. Si (ir a 2.43.2)	2. No (brincarse 2.43.2)
PPA	2.43.2	Es escrito o verbal?	1. Escrito	2. Verbal
	2.43.3	Si **SI,** que se especifica en ese contrato?		

Ahora haremos unas preguntas sobre quién hace cada trabajo con las pitayas. Es importante saber quién ayuda aunque no reciban salario.

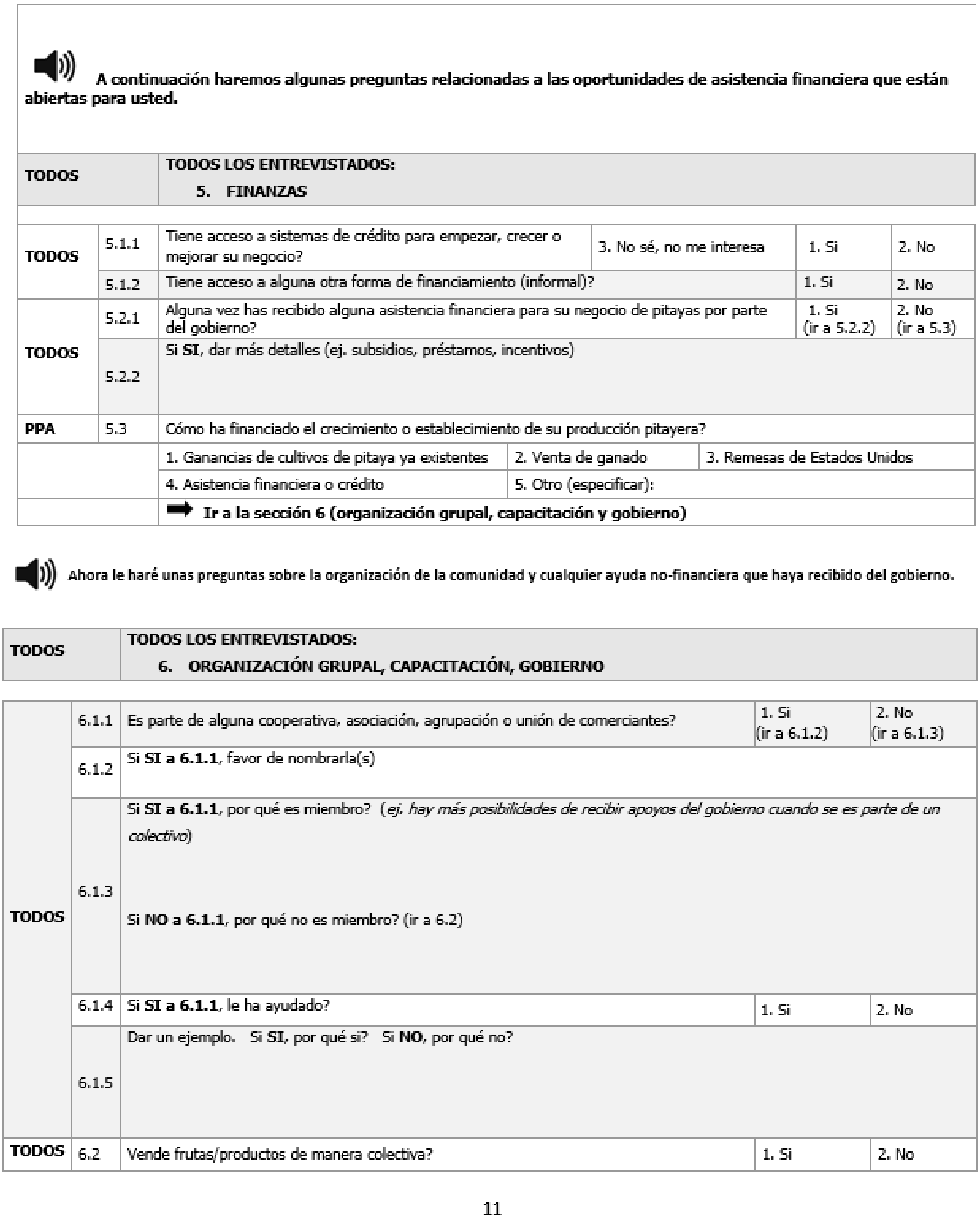
TODOS	**3. TIEMPO Y USO DE MANO DE OBRA**

		Tarea	Persona	Número de personas	Horas/día/ persona trabajadas	Días/año trabajados
PPA, PS, TA	3.1	Actividades de manejo ej. Aplicación de pesticidas, rotación de suelo	Dueño de plantación			
			Arrendatario de plantación			
			Miembros de la familia			
			Empleado pagado (todo el año)			
			Empleado pagado (temporal)			
			Otro (especificar):			
TODOS	3.2	Corta de frutas	Dueño de plantación			
			Arrendatario de plantación			
			Miembros de la familia			
			Empleado pagado (todo el año)			
			Empleado pagado (temporal)			
			Otro (especificar):			
TODOS	3.3	Pela de frutas	Dueño de plantación			
			Arrendatario de plantación			
			Miembros de la familia			
			Empleado pagado (todo el año)			
			Empleado pagado (temporal)			
			Otro (especificar):			
TODOS	3.4	Ventas	Dueño de plantación			
			Arrendatario de plantación			
			Miembros de la familia			
			Empleado pagado (todo el año)			
			Empleado pagado (temporal)			
			Otro (especificar):			

Ahora haremos preguntas para describir de manera detallada los costos asociados con el trabajo de la pitaya. Esta información es muy importante ya que nos permitirá entender los ingresos promedios atribuibles a las pitayas en Techaluta.

TODOS	**TODOS:** **4. COSTOS**			
Tipo de costo:				Costo en **pesos**/temporada
TODOS	4.1	Tierra/plantación(es)	Renta	
PPA, PS	4.2.1	Manejo/aporte	Pesticidas	
	4.2.2		Fertilizantes	
	4.2.3		Herbicidas	
	4.2.4		Composta	
	4.2.5		Brazos de pitaya/semillas	
	4.2.6		Otro (especificar)	
TODOS	4.3.1	Mano de obra	Compensación a miembros de la familia	
	4.3.2	*(Corrobore las respuestas con la sección 3)*	Trabajadores permanentes-salarios	
	4.3.3		Trabajadores temporales-salarios	
	4.3.4		Pagos de seguro social, medicinas, doctor	
	4.3.5		Otros beneficios, ej. comidas	
TODOS	4.4	Certificación	Certificación	
TODOS	4.5.1	Impuestos	Exportación	
	4.5.2		Producción	
	4.5.3		Ingresos	
	4.5.4		IVA	
	4.5.5		Tierra/propiedad (predial)	
VC, VM	4.6.1	Mercadeo	Renta de puesto	
	4.6.2		Almacenamiento	
	4.6.3		Cuota del Mercado, permiso de venta	
TODOS	4.7.1	Transporte	Seguro de vehículo	
	4.7.2		Gasolina	
	4.7.3		Mantenimiento de vehículo	
	4.7.4		Transporte público (ej. al y del trabajo)	
	4.7.5		Casetas	
TODOS	4.8.1	Equipo y herramientas	Compra de equipos y herramientas	
	4.8.2		Mantenimiento de equipos y herramientas	
	4.8.3		Empaque (ej. alfalfa, bolsas o envoltura plástica)	
TODOS	4.9.1	Finanzas	Pagos de créditos (formal o informal) e intereses para su trabajo con las pitayas	
	4.9.2		Pagos de seguro agrario para su(s) pitayera(s)	
		TOTAL		

HP		**HACEDORES DE PRODUCTOS SOLAMENTE:** **COSTOS:** **FAVOR DE LISTAR TODOS LOS PRODUCTOS QUE SE HACEN, EL TIEMPO Y COSTOS DE MANUFACTURA DE CADA UNO**			
		Nombre del producto *(Corroborar respuestas con 2.7)*	Tiempo para hacer el producto *Unidad?*	Cuántos se pueden hacer a la vez? *Unidad?*	Costo en pesos de los ingredientes del producto (incluyendo las pitayas)
HP	4.10				
HP	4.11				
HP	4.12				
HP	4.13				
HP	4.14				
HP	4.15				
HP	4.16				



🔊 **A continuación haremos algunas preguntas relacionadas a las oportunidades de asistencia financiera que están abiertas para usted.**

TODOS	**TODOS LOS ENTREVISTADOS:** **5. FINANZAS**				

TODOS	5.1.1	Tiene acceso a sistemas de crédito para empezar, crecer o mejorar su negocio?	3. No sé, no me interesa	1. Si	2. No
	5.1.2	Tiene acceso a alguna otra forma de financiamiento (informal)?		1. Si	2. No
TODOS	5.2.1	Alguna vez has recibido alguna asistencia financiera para su negocio de pitayas por parte del gobierno?		1. Si (ir a 5.2.2)	2. No (ir a 5.3)
	5.2.2	Si **SI**, dar más detalles (ej. subsidios, préstamos, incentivos)			
PPA	5.3	Cómo ha financiado el crecimiento o establecimiento de su producción pitayera?			
		1. Ganancias de cultivos de pitaya ya existentes	2. Venta de ganado	3. Remesas de Estados Unidos	
		4. Asistencia financiera o crédito	5. Otro (especificar):		
		➡ **Ir a la sección 6 (organización grupal, capacitación y gobierno)**			

🔊 **Ahora le haré unas preguntas sobre la organización de la comunidad y cualquier ayuda no-financiera que haya recibido del gobierno.**

TODOS	**TODOS LOS ENTREVISTADOS:** **6. ORGANIZACIÓN GRUPAL, CAPACITACIÓN, GOBIERNO**

TODOS	6.1.1	Es parte de alguna cooperativa, asociación, agrupación o unión de comerciantes?	1. Si (ir a 6.1.2)	2. No (ir a 6.1.3)
	6.1.2	Si **SI a 6.1.1**, favor de nombrarla(s)		
	6.1.3	Si **SI a 6.1.1**, por qué es miembro? (*ej. hay más posibilidades de recibir apoyos del gobierno cuando se es parte de un colectivo*) Si **NO a 6.1.1**, por qué no es miembro? (ir a 6.2)		
	6.1.4	Si **SI a 6.1.1**, le ha ayudado?	1. Si	2. No
	6.1.5	Dar un ejemplo. Si **SI**, por qué si? Si **NO**, por qué no?		
TODOS	6.2	Vende frutas/productos de manera colectiva?	1. Si	2. No

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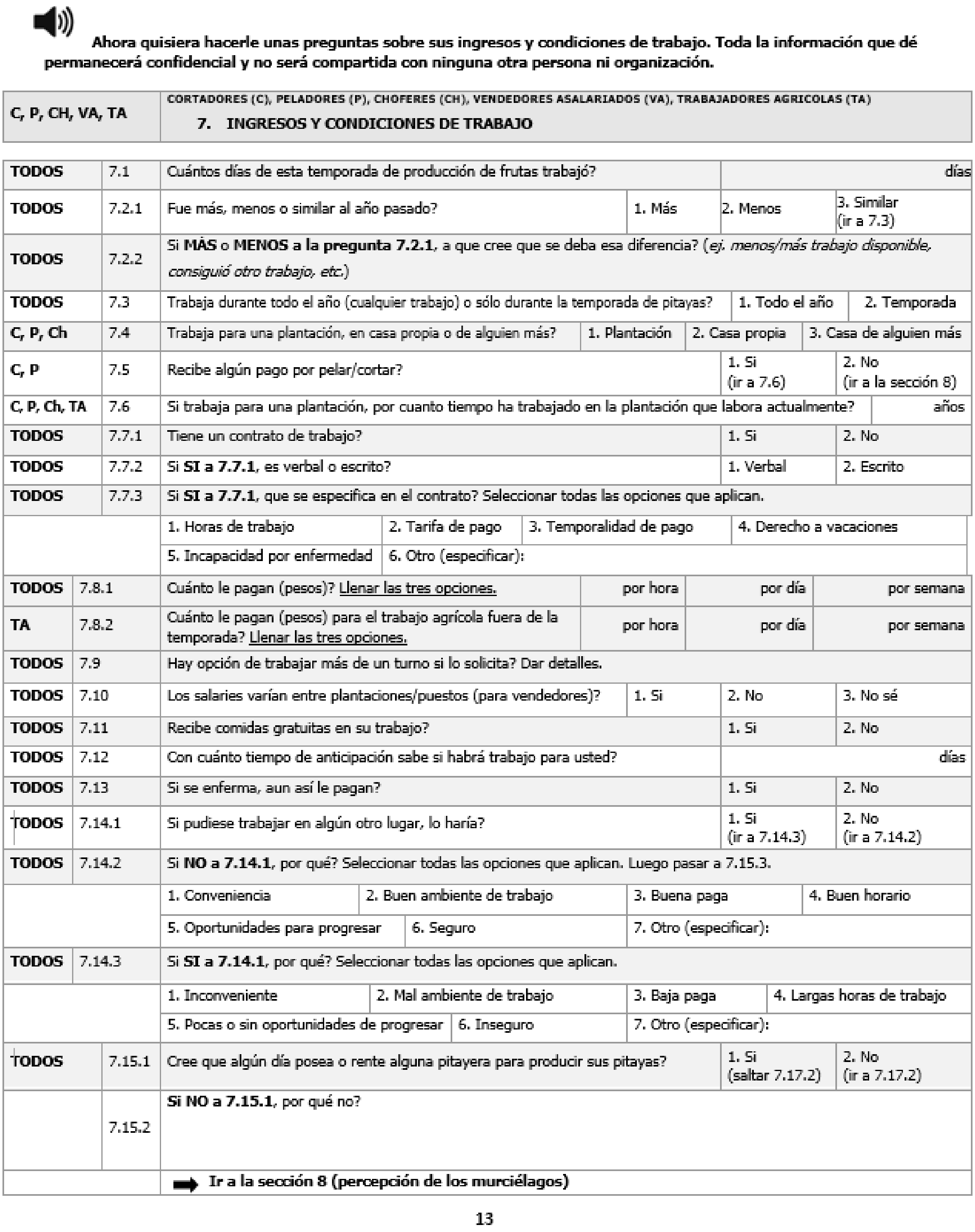
TODOS	6.3.1	Qué opina del nivel de organización en Techaluta de Montenegro para mejorar las condiciones o proveer más oportunidades para las personas que trabajan con pitayas?	1. Bueno	2. Regular	3. Malo
TODOS	6.3.2	A qué cree que se deba?			
TODOS	6.4.1	Alguna vez ha recibido capacitación en temas relacionados a negocios?	1. Si	2. No (ir a 6.5.1)	
TODOS	6.4.2	Si **SI**, dar detalles (**qué tipo de capacitación***? Ej. para hacer productos, composta*)			
	6.4.3	**Quién promovió u organizó esa capacitación?**			
TODOS	6.5.1	Qué tecnología o equipo necesita para llevar a cabo su trabajo?			
TODOS	6.5.2	Tiene acceso a estas tecnologías o equipo?	1. Si (ir a 6.6)	2. No (ir a 6.5.3)	
TODOS	6.5.3	Si **NO** a 6.5.2, por qué no?			
TODOS	6.6	El gobierno local ha tomado acciones que le han ayudado **O** le han obstaculizado el trabajo con las pitayas? Dar detalles			
TODOS	6.7.1	Cómo cree que el sector pitayero podría mejorar para mejorar las condiciones de trabajo y expandir los mercados?			
TODOS	6.7.2	Quién cree que debería estar a cargo de eso?			
		➡ **C, P, L: Ir a la sección 7 (ingresos y condiciones de trabajo)** ➡ **PPA, VM, VC, PS, CS, HP, I: Ir a la sección 8 (percepción de los murciélagos)**			



🔊 **Ahora quisiera hacerle unas preguntas sobre sus ingresos y condiciones de trabajo. Toda la información que dé permanecerá confidencial y no será compartida con ninguna otra persona ni organización.**

C, P, CH, VA, TA	**CORTADORES (C), PELADORES (P), CHOFERES (CH), VENDEDORES ASALARIADOS (VA), TRABAJADORES AGRICOLAS (TA)** **7. INGRESOS Y CONDICIONES DE TRABAJO**

TODOS	7.1	Cuántos días de esta temporada de producción de frutas trabajó?	días		
TODOS	7.2.1	Fue más, menos o similar al año pasado?	1. Más	2. Menos	3. Similar (ir a 7.3)
TODOS	7.2.2	Si **MÁS** o **MENOS a la pregunta 7.2.1**, a que cree que se deba esa diferencia? (*ej. menos/más trabajo disponible, consiguió otro trabajo, etc.*)			
TODOS	7.3	Trabaja durante todo el año (cualquier trabajo) o sólo durante la temporada de pitayas?	1. Todo el año	2. Temporada	
C, P, Ch	7.4	Trabaja para una plantación, en casa propia o de alguien más?	1. Plantación	2. Casa propia	3. Casa de alguien más
C, P	7.5	Recibe algún pago por pelar/cortar?	1. Si (ir a 7.6)	2. No (ir a la sección 8)	
C, P, Ch, TA	7.6	Si trabaja para una plantación, por cuanto tiempo ha trabajado en la plantación que labora actualmente?	años		
TODOS	7.7.1	Tiene un contrato de trabajo?	1. Si	2. No	
TODOS	7.7.2	Si **SI a 7.7.1**, es verbal o escrito?	1. Verbal	2. Escrito	
TODOS	7.7.3	Si **SI a 7.7.1**, que se especifica en el contrato? Seleccionar todas las opciones que aplican.			
		1. Horas de trabajo	2. Tarifa de pago	3. Temporalidad de pago	4. Derecho a vacaciones
		5. Incapacidad por enfermedad	6. Otro (especificar):		
TODOS	7.8.1	Cuánto le pagan (pesos)? <u>Llenar las tres opciones.</u>	por hora	por día	por semana
TA	7.8.2	Cuánto le pagan (pesos) para el trabajo agrícola fuera de la temporada? <u>Llenar las tres opciones.</u>	por hora	por día	por semana
TODOS	7.9	Hay opción de trabajar más de un turno si lo solicita? Dar detalles.			
TODOS	7.10	Los salaries varían entre plantaciones/puestos (para vendedores)?	1. Si	2. No	3. No sé
TODOS	7.11	Recibe comidas gratuitas en su trabajo?	1. Si	2. No	
TODOS	7.12	Con cuánto tiempo de anticipación sabe si habrá trabajo para usted?	días		
TODOS	7.13	Si se enferma, aun así le pagan?	1. Si	2. No	
TODOS	7.14.1	Si pudiese trabajar en algún otro lugar, lo haría?	1. Si (ir a 7.14.3)	2. No (ir a 7.14.2)	
TODOS	7.14.2	Si **NO a 7.14.1**, por qué? Seleccionar todas las opciones que aplican. Luego pasar a 7.15.3.			
		1. Conveniencia	2. Buen ambiente de trabajo	3. Buena paga	4. Buen horario
		5. Oportunidades para progresar	6. Seguro	7. Otro (especificar):	
TODOS	7.14.3	Si **SI a 7.14.1**, por qué? Seleccionar todas las opciones que aplican.			
		1. Inconveniente	2. Mal ambiente de trabajo	3. Baja paga	4. Largas horas de trabajo
		5. Pocas o sin oportunidades de progresar	6. Inseguro	7. Otro (especificar):	
TODOS	7.15.1	Cree que algún día posea o rente alguna pitayera para producir sus pitayas?	1. Si (saltar 7.17.2)	2. No (ir a 7.17.2)	
	7.15.2	**Si NO a 7.15.1**, por qué no?			
		➡ **Ir a la sección 8 (percepción de los murciélagos)**			

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Ahora me gustaría preguntarle algunas cosas para saber su opinión sobre los murciélagos.

TODOS	**TODOS LOS ENTREVISTADOS:** **8. PERCEPCIÓN DE LOS MURCIÉLAGOS**

TODOS	8.1	¿Cuantas especies de murciélagos piensa que hay en México?	especies	
TODOS	8.2.1	¿De estas especies, cuantas piensa que son beneficiosos a las personas, y cuantos perjudicial?		
		beneficiosos	perjudicial	
TODOS	8.2.2	¿Porque piensa que son buenos o malos?		
TODOS	8.3	Nombre de todas las cosas de que piensa se alimentan los murciélagos.		
TODOS	8.4.1	¿Piensa que los murciélagos comen las pitayas?	1. Si	2. No
TODOS	8.4.2	Si **SI**, que proporción de la cosecha cree que se comen?		%
TODOS	8.5.1	Toma alguna medida para proteger a las pitayas de los murciélagos?	1. Si (ir a 8.5.2)	2. No (ir a 8.6)
TODOS	8.5.2	Si **SI**, cuáles? (ej. *Cortar las frutas en un momento específico, matar a los murciélagos*)		
TODOS	8.6	¿Sabía que los murciélagos polinizan las pitayas antes de saber de nuestro trabajo en Techaluta?	1. Si	2. No
TODOS	8.7	¿Piensa que los murciélagos deben ser protegidos?	1. Si	2. No
		➡ **Continuar con la sección 8.2 (pitayas)**		

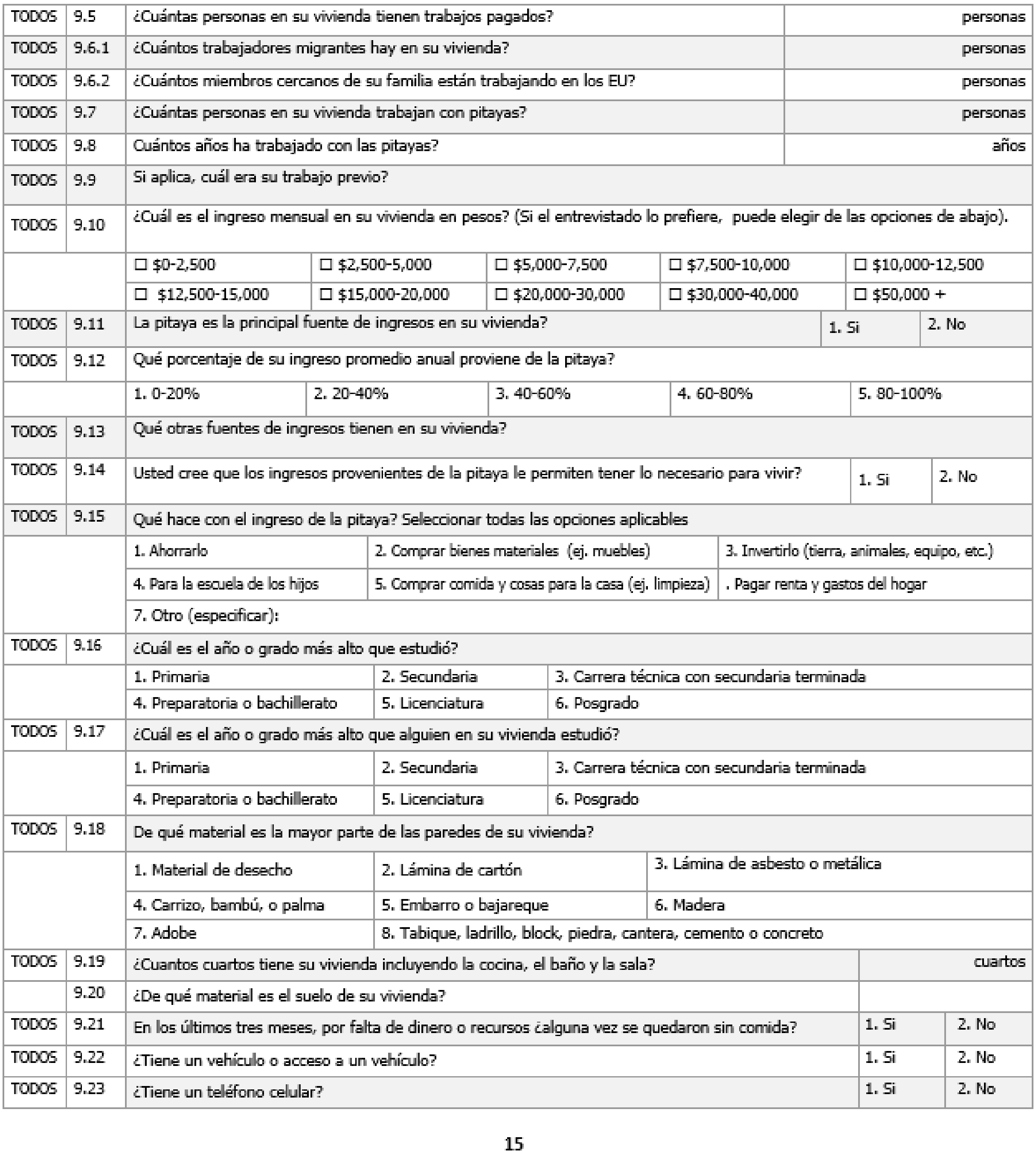
TODOS	**TODOS LOS ENTREVISTADOS:** **8.2 PITAYAS**

TODOS	8.8.1	Come pitayas?		1. Si (ir a 8.8.2)	2. No (ir a 8.9)
TODOS	8.8.2	Si **Si**, por qué?	1. Calorías	2. Nutrición	3. Otro (especificar):
TODOS	8.9	Cree que las pitayas son parte importante de su dieta? Asigne un número en una escala del 1 al 5, dónde 1 es muy importante y 5 nada importante)			
		➡ **Ir a la sección 9 (características socioeconómicas del hogar)**			

Para terminar, me gustaría hacerle algunas preguntas sobre algunos detalles personales, algunas características de su hogar y sus ingresos.

TODOS	**TODOS LOS ENTREVISTADOS:** **9. CARACTERÍSTICAS SOCIOECONÓMICAS DEL HOGAR**

TODOS	9.1	Sexo:		1. Hombre 2. Mujer
TODOS	9.2	Edad:		años
TODOS	9.3	Número de personas (incluyendo todos los niños y adultos) viviendo en la casa:		
		Menos de 15 años	Entre 15-64 años	65 o más años



TODOS	9.5	¿Cuántas personas en su vivienda tienen trabajos pagados?				personas
TODOS	9.6.1	¿Cuántos trabajadores migrantes hay en su vivienda?				personas
TODOS	9.6.2	¿Cuántos miembros cercanos de su familia están trabajando en los EU?				personas
TODOS	9.7	¿Cuántas personas en su vivienda trabajan con pitayas?				personas
TODOS	9.8	Cuántos años ha trabajado con las pitayas?				años
TODOS	9.9	Si aplica, cuál era su trabajo previo?				
TODOS	9.10	¿Cuál es el ingreso mensual en su vivienda en pesos? (Si el entrevistado lo prefiere, puede elegir de las opciones de abajo).				
		☐ $0-2,500	☐ $2,500-5,000	☐ $5,000-7,500	☐ $7,500-10,000	☐ $10,000-12,500
		☐ $12,500-15,000	☐ $15,000-20,000	☐ $20,000-30,000	☐ $30,000-40,000	☐ $50,000 +
TODOS	9.11	La pitaya es la principal fuente de ingresos en su vivienda?			1. Si	2. No
TODOS	9.12	Qué porcentaje de su ingreso promedio anual proviene de la pitaya?				
		1. 0-20%	2. 20-40%	3. 40-60%	4. 60-80%	5. 80-100%
TODOS	9.13	Qué otras fuentes de ingresos tienen en su vivienda?				
TODOS	9.14	Usted cree que los ingresos provenientes de la pitaya le permiten tener lo necesario para vivir?			1. Si	2. No
TODOS	9.15	Qué hace con el ingreso de la pitaya? Seleccionar todas las opciones aplicables				
		1. Ahorrarlo	2. Comprar bienes materiales (ej. muebles)	3. Invertirlo (tierra, animales, equipo, etc.)		
		4. Para la escuela de los hijos	5. Comprar comida y cosas para la casa (ej. limpieza)	. Pagar renta y gastos del hogar		
		7. Otro (especificar):				
TODOS	9.16	¿Cuál es el año o grado más alto que estudió?				
		1. Primaria	2. Secundaria	3. Carrera técnica con secundaria terminada		
		4. Preparatoria o bachillerato	5. Licenciatura	6. Posgrado		
TODOS	9.17	¿Cuál es el año o grado más alto que alguien en su vivienda estudió?				
		1. Primaria	2. Secundaria	3. Carrera técnica con secundaria terminada		
		4. Preparatoria o bachillerato	5. Licenciatura	6. Posgrado		
TODOS	9.18	De qué material es la mayor parte de las paredes de su vivienda?				
		1. Material de desecho	2. Lámina de cartón	3. Lámina de asbesto o metálica		
		4. Carrizo, bambú, o palma	5. Embarro o bajareque	6. Madera		
		7. Adobe	8. Tabique, ladrillo, block, piedra, cantera, cemento o concreto			
TODOS	9.19	¿Cuantos cuartos tiene su vivienda incluyendo la cocina, el baño y la sala?				cuartos
	9.20	¿De qué material es el suelo de su vivienda?				
TODOS	9.21	En los últimos tres meses, por falta de dinero o recursos ¿alguna vez se quedaron sin comida?			1. Si	2. No
TODOS	9.22	¿Tiene un vehículo o acceso a un vehículo?			1. Si	2. No
TODOS	9.23	¿Tiene un teléfono celular?			1. Si	2. No

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B.8 Table B8. The number of people in each actor group interviewed for the value chain analysis that knew that bats pollinate the pitaya.

Role in value chain	Yes	No	% knew bats pollinate pitayas
Plantation owner	16	25	39
Plantation renter	11	28	28
Home garden owner	4	17	19
Market vendor	8	11	42
Roadside vendor	6	25	19
Intermediary	2	2	50
Ambulant vendor	0	5	0
Product maker	6	19	25
Harvester	5	6	45
Driver	0	4	0
Peeler	3	9	25
Sales assistant	1	2	33
Agricultural worker	2	4	33
TOTAL MEAN	**64**	**157**	**41**

B.9 Indicators of poverty Techaluta de Montenegro (CONEVAL, 2010)

Indicators	**Percentage**	**Number of people**	**Average number of poverty indicators**
Poverty			
Population living in poverty	47.7	1,784	2.0
Population in moderate poverty	42.5	1,589	1.7
Population in extreme poverty	5.2	194	3.9
Population vulnerable due to social poverty indicators	45.4	1,699	1.7
Population vulnerable due to income	1.4	52	0.0
Population not poor or vulnerable	5.5	205	0.0
Social deprivation			
Population with at least one social poverty indicator	93.1	3,482	1.8
Population with at least three social poverty indicators	17.9	668	3.6
Social poverty indicators			
Lack of education	25.9	969	2.5
Access to health services	20.0	749	2.6
Access to social security	80.7	3,019	1.9
Quality and space of household	8.0	299	3.9
Access to basic household services	16.7	623	3.2
Access to food	18.0	675	3.2
Economic wellbeing			

Population with income insufficient to provide minimum level of wellbeing	17.8	666	2.1
Population with income insufficient to provide wellbeing	49.1	1,836	1.9

Poverty indicators are:

1. Low monthly income per capita.
    - Considered low if it does not cover the economic cost of basic wellbeing (provision of food, transport, education, health, leisure and everyday goods and products). Considered very low if it does not cover the cost of food alone.
2. Average education gap in the household
    - Children between 3 and 15 years should be attending school; adults born before 1982 should have completed primary school; adults born after 1982 should have completed secondary school.
3. Access to health services
    - No health insurance or provision by work or state.
4. Social security
    - People in work should have access to medical cover and pension schemes.
    - People over 65 should receive some sort of pension.
5. Quality and space of household
    - House should be built of solid and stable materials.
    - No more than 2.5 people per room.
6. Access to basic household needs
    - Drainage, clean water, electricity and fuel for cooking.
7. Access to food
    - Lack of food considered if during the last three months the diet eaten was not varied; if meals were not eaten when people were hungry; or if not enough food was eaten to assuage hunger
8. Social cohesion
    - Made up of: quality of social networks, discrimination, social participation, dependence, and economic inequality.

Appendix C Supplementary material Chapter 4

C.1 Table C1. Samples of plant tissue collected for reference database and Genbank accession numbers for plants successfully sequenced.

CUCBA = botanical collection of Cactaceae and Succulents, IBUG = Herbario "Luz María Villarreal de Puga", both in the Department of Botany and Zoology at the University of Guadalajara, Mexico.

Family	**Species**	**Source**	**GenBank accession number**
Agavaceae	*Agave angustifolia*	CUCBA	MW374669
	Agave attenuata	CUCBA	MW374670
	Agave guadalajarana	CUCBA	MW374671
	Agave inaequidens	CUCBA	MW374672
	Agave schidigera	CUCBA	MW374673
	Agave vilmoriniana	CUCBA	MW374674
	Yucca jaliscensis	CUCBA	MW374726
Cactaceae	*Acanthocereus occidentalis*	CUCBA	MW374668
	Hylocereus purpusi	CUCBA	MW374729
	Opuntia joconostle	CUCBA	MW374713
	Opuntia atropes	CUCBA	MW374712
	Opuntia robusta	CUCBA	MW374714
	Pachycereus pecten-aboriginum	CUCBA	MW374728
	Pilocereus allensis	CUCBA	MW374734
	Stenocereus dumortieri	CUCBA	MW374733
	Stenocereus standleyi	CUCBA	MW374732
	Stenocereus queretaroensis	IBUG	MW374727
Acanthaceae	*Ruellia bourgaei*	IBUG	MW374724
	Annona longiflora	IBUG	MW374676
	Annona reticulata	IBUG	MW374731
Apocynaceae	*Thevetia ovata*	IBUG	MW374678
Bignoniaceae	*Crescentia alata*	IBUG	MW374730
Capparaceae	*Crateva palmeri*	IBUG	MW374687
	Crateva tapia	IBUG	MW374688
Combretaceae	*Combretum farinosum*	IBUG	MW374682

	Combretum fruticosum	IBUG	MW374683
	Combretum igneiflorum	IBUG	MW374684
	Combretum laxum	IBUG	MW374685 MW374686
Convolvulaceae	*Ipomoea ampullacea*	IBUG	MW374703
	Ipomea arborescens	IBUG	MW374704
	Ipomoea corymbosa	IBUG	MW374705
	Ipomoea intrapilosa	IBUG	MW374706
	Ipomoea murucoides	IBUG	MW374707
	Ipomoea stans	IBUG	MW374708
	Ipomoea tricolor	IBUG	MW374709
	Ipomoea tyrianthina	IBUG	MW374710
	Merremia aegyptia	IBUG	MW374711
Cucurbitaceae	*Cucurbita argyrosperma*	IBUG	MW374689
	Cucurbita foetidissima	IBUG	MW374690
	Cucurbita radicans	IBUG	MW374691
Malvaceae	*Ceiba acuminata*	IBUG	MW374679
	Ceiba aesculifolia	IBUG	MW374680
	Ceiba pentandra	IBUG	MW374681
	Hibiscus biseptus	IBUG	MW374696
	Hibiscus citrinus	IBUG	MW374697
	Hibiscus phoeniceus	IBUG	MW374698
	Hibiscus rosa-sinensis	IBUG	MW374699
	Hibiscus syriacus	IBUG	MW374700
	Hibiscus tiliaceus	IBUG	MW374701
	Pseudobombax ellipticum	IBUG	MW374722
	Pseudobombax palmeri	IBUG	MW374723
Passifloraceae	*Passiflora foetida*	IBUG	MW374716
	Passiflora exsudans	IBUG	MW374715
	Passiflora mexicana	IBUG	MW374717
	Passiflora pavonis	IBUG	MW374718
	Passiflora podadenia	IBUG	MW374719
	Passiflora porphyretica	IBUG	MW374720
	Passiflora subpeltata	IBUG	MW374721
	Datura inoxia	IBUG	MW374693
	Datura stramonium	IBUG	MW374695
	Datura quercifolia	IBUG	MW374694

C.2 **Table C2. Additional diet reference plant species that may present in the Sayula Basin.** Sequences at the ITS2 region already present in Genbank indicated with a Y.

Family	**Species**	**GenBank**
Acanthaceae	*Ruellia lactea*	Y
	Ruellia pilosa	Y
Amaranthaceae	*Iresine calea*	N
	Iresine gossypina	N
	Iresine interrupta	N
Anacardiaceae	*Mangifera spp. (fruit)*	Y
	Rhus allophyloides	Y
	Rhus radicans	N
	Spondias purpurea (fruit)	Y
Apocynaceae	*Asclepias angustifolia*	N
	Asclepias auriculata	N
	Asclepias curassavica	Y
	Asclepias fournieri	N
	Asclepias glaucescens	N
	Asclepias linaria	N
	Plumeria rubra	Y
	Stemmadenia donnell-smithi (fruit)	N
	Tabernaemontana divaricata	Y
Arialaceae	*Oreopanax xalapensis*	Y
Asteraceae	*Montanoa bipinnatifida*	Y
	Montanoa tomentosa	Y
Bignoniaceae	*Tecoma stans*	Y
Boraginaceae	*Cordia alba*	N
	Cordia cana	N
	Cordia elaeagnoides	Y
	Cordia gerascanthus	Y
	Cordia seleriana	N
Bromeliaceae	*Tillandsia achyrostachys*	Y
	Tillandsia dasyliriifolia	N
	Tillandsia macdougallii	N
	Tillandsia makoyana	Y
	Tillandsia pamelae	N
	Tillandsia plumosa	N

	Tillandsia recurvata	N
	Tillandsia schiedeana	Y
Cactaceae	*Hylocereus undatus*	Y
	Nopalea cochenillifera	Y
	Opuntia ficus-indica	Y
	Opuntia fuliginosa	Y
	Opuntia jaliscana	N
	Opuntia pubescens	Y
	Opuntia pumila	Y
	Opuntia undulata	N
Campanulaceae	*Lobelia fenestralis*	Y
	Lobelia hartwegii	N
	Lobelia laxiflora	Y
	Lobelia tenera	Y
Cleomaceae	*Tarenaya spinosa / Cleome spinosa*	Y
	Cleomella jaliscensis	N
Convulvulaceae	*Ipomoea alba*	Y
	Ipomoea bracteata	Y
	Ipomoea capillacea	Y
	Ipomoea coccinea	Y
	Ipomoea congesta	N
	Ipomoea costellata	Y
	Ipomoea mairetii	Y
	Ipomoea muricatisepala	N
	Ipomoea neei	Y
	Ipomoea nil	Y
	Ipomoea painteri	N
	Ipomoea parasitica	Y
	Ipomoea pauciflora	Y
	Ipomoea purpurea	Y
	Ipomoea trichocarpa	N
	Merremia quinquefolia	Y
Fabaceae	*Acacia cochliacantha*	N
	Acacia pennatula	N
	Albizzia occidentalis	N
	Bauhinia pauletia	N
	Bauhinia ungulata	N
	Calliandra formosa / Zapoteca formosa	Y
	Inga vera	Y

Malvaceae	*Ceiba aesculifolia*	Y
	Guazuma ulmifolia	Y
	Helicteres baruensis	N
Moraceae	*Chlorophora tinctoria / Maclura tinctoria*	Y
	Ficus cotonifolia (fruit)	N
	Ficus crocata (fruit)	Y
	Ficus goldmanii (fruit)	Y
	Ficus insipida (fruit)	Y
	Ficus pertusa (fruit)	Y
	Ficus petiolaris (fruit)	Y
Onagraceae	*Ludwigia peploides*	Y
Opiliaceae	*Agonandra racemosa*	Y
Orchidaceae	*Laelia autumnalis*	Y
	Laelia catarinensis	N
	Laelia speciosa	Y
Piperaceae	*Piper arboretum (fruit)*	Y
Rosaceae	*Prunus serotina (fruit)*	Y
Rubiaceae	*Chiococca alba*	Y
Rutiaceae	*Ptelea trifoliata*	Y
Sapindaceae	*Cardiospermum halicacabum*	Y
Solanaceae	*Cestrum anagyris*	N
	Cestrum aurantiacum	N
	Cestrum confertiflorum	N
	Cestrum lanatum	N
	Cestrum nitidium	N
	Cestrum terminale	N
	Cestrum thyrsoideum	Y
	Cestrum tomentosum	Y
	Datura ceratocaula	Y
	Datura discolor	Y
	Lycium carolinianum	Y
	Nicotiana glauca	Y
	Solanum aphyodendron	Y
	Solanum erianthum	Y
	Solanum lanceolatum	Y
	Solanum rudepannum	Y
Verbenaceae	*Lantana camara*	Y

C.3 Pilot sequencing

In 2018 we piloted the use of trnL and ITS markers on ten of the pollen samples collected as part of this study, from April (n = 3), June (n = 3) and July (n= 4). DNA was extracted from the pollen samples using a modified CTAB method adapted from Doyle et al. (1991). We used primers trnLc and trnLh to amplify part of the single-locus region of the chloroplast trnL (UAA) intron (Table S3; Taberlet et al. 2007). This is a robust marker for plant dietary analysis and can be amplified from highly degraded DNA such as from animal faeces (Taberlet et al. 2007; Kraaijeveld et al. 2015; Mallott et al. 2018). We used primers ITS-p4 and ITS-u3 to amplify part of the second internal transcribed spacer (ITS2) of nuclear ribosomal DNA (Table C3; Cheng et al. 2016).

Table C3. Sequences of the primer pairs used in the pilot.

Name	**Region**	**Sequence 5’ – 3’**
c	*trn*L	CGAAATCGGTAGACGCTACG
h	*trn*L	CCATTGAGTCTCTGCACCTATC
ITS-p4	ITS2	CCGCTTAKTGATATGCTTAAA
ITS-u3	ITS2	CAWCGATGAAGAACGYAGC

PCR amplification and sequencing

Bioinformatics

The same bioinformatics pipeline as described in the main ‘Bioinformatics processing’ section was used.

Results

The trnL data showed poor resolution, with 98% of sequences simply classified as Magnoliopsida (dicots, the largest group of flowering plants). The remaining reads were all classified as Cactoideae, appearing in high numbers in all three samples from April and all three samples from June. In contrast, the ITS2 data did not show any sequences from the Cactoideae, but instead showed high reads from sequences from the Agavoideae (accounting for 10% of all reads) in one sample in June, and from *Ceiba sp.* (64% of all reads) in all samples from June and July. The ITS2 sequencing run produced a much lower read depth however, and the remaining 23% of reads were all from fungi, suggesting either contamination or a universality of the primers that also amplified fungal sequences.

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