BAT POLLINATION ENHANCES FRUIT CROP

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, 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. We used exclusion experiments 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 taxa 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, thus 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:* This 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 understand 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, fruit weight, seed set, *Stenocereus,* nectarivorous bats, bat pollination

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 (*Stenocereus queretaroensis*), endemic to semi-arid habitats in western central Mexico (Ibarra-Cerdeña et al. 2005). *Stenocereus 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). *Stenocereus 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.

Materials and methods

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 (Appendix S1: Table S1).

Stenocereus 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 Supplementary Information for further details). 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 (Appendix S1: Table S1). The study was carried out in six plantations, each containing all three cultivars; and six ranches with wild cacti (Fig. 1).

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. vertebrate pollinators excluded).

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 (Table S2). 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; Table S3).

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).

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, whereby models with lower AIC values were chosen. 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 this is 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 linear mixed effects model 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).

Results

Pollination dependency of *Stenocereus queretaroensis* on bats

Exclusion experiments were placed on a total of 109 *Stenocereus 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). No fruits were produced by the self-pollination treatment (closed control) or nocturnal insect pollination (Fig. 2).

Fruit set depended on pollination treatment (GLMM: $\chi^2 = 286.7$, $P < 0.0001$; Table 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). 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.

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), and significantly less sweet (13% and 14% lower sucrose concentration respectively; LMM: $\chi^2 = 9.88$, $P = 0.0196$; Fig. 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; Table S4). 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 1; Fig. 2). Fruit weight was positively correlated with seed set (LM: $F_{1,162} = 79.2$, $r^2 = 0.32$, $P < 0.0001$).

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 photos 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 visiting the flowers during filming of paired days and nights 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.

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 *Stenocereus 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.

Benefits of bat pollination

Bats are the most effective pollinators of *Stenocereus queretaroensis,* enhancing 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. Pollinator visitation rate did not affect either fruit or seed set and one visit by bats to the flower was 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).

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 meaning most individuals share self-incompatibility (SI) alleles, with a smaller number of the other cultivars present (Appendix S1, Table S1). 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).

Potential economic and socio-cultural consequences of losing bat pollination services

In Latin America, bat colonies and roosting sites are frequently destroyed to kill vampire bats (*Desmodus 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. In other areas, pitaya production is based entirely on the collection of wild fruits (Pimienta-Barrios, 1999b). 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). 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.

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).

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 *Leptonycteris 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.

Authors contributions

VZG and KSHP conceived the research, CJT and VZG 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 and declare no conflicts of interest.

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Data accessibility

Data available from the Dryad Digital Repository.

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Tables

Table 1. Outputs from Linear Mixed Models (LMMs) and Generalized Linear Mixed Model (GLMM) investigating effect of pollination treatment on fruit set, fruit weight, seed set and sucrose concentration across cultivars and wild *S. queretaroensis.* 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 Table S5 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.

	Fruit set *(GLMM:* χ^2 = 286.7, *df = 5, P* < 0.0001)			**Fruit weight** *(LMM:* χ^2 *= 51.5, df = 3, P < 0.0001)*			**Seed set** *(LMM:* χ^2 *=93.5, df =3, P < 0.0001)*			**Sucrose concentration** *(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) τ00 site residual		0.24 0.02 0.00	τ00 cactus(site) τ00 site residual		0.06 0.02 0.13	τ00 cactus(site) τ00 site residual		0.02 0.00 0.17	τ00 cactus(site) τ00 site residual		0.02 0.01 0.02

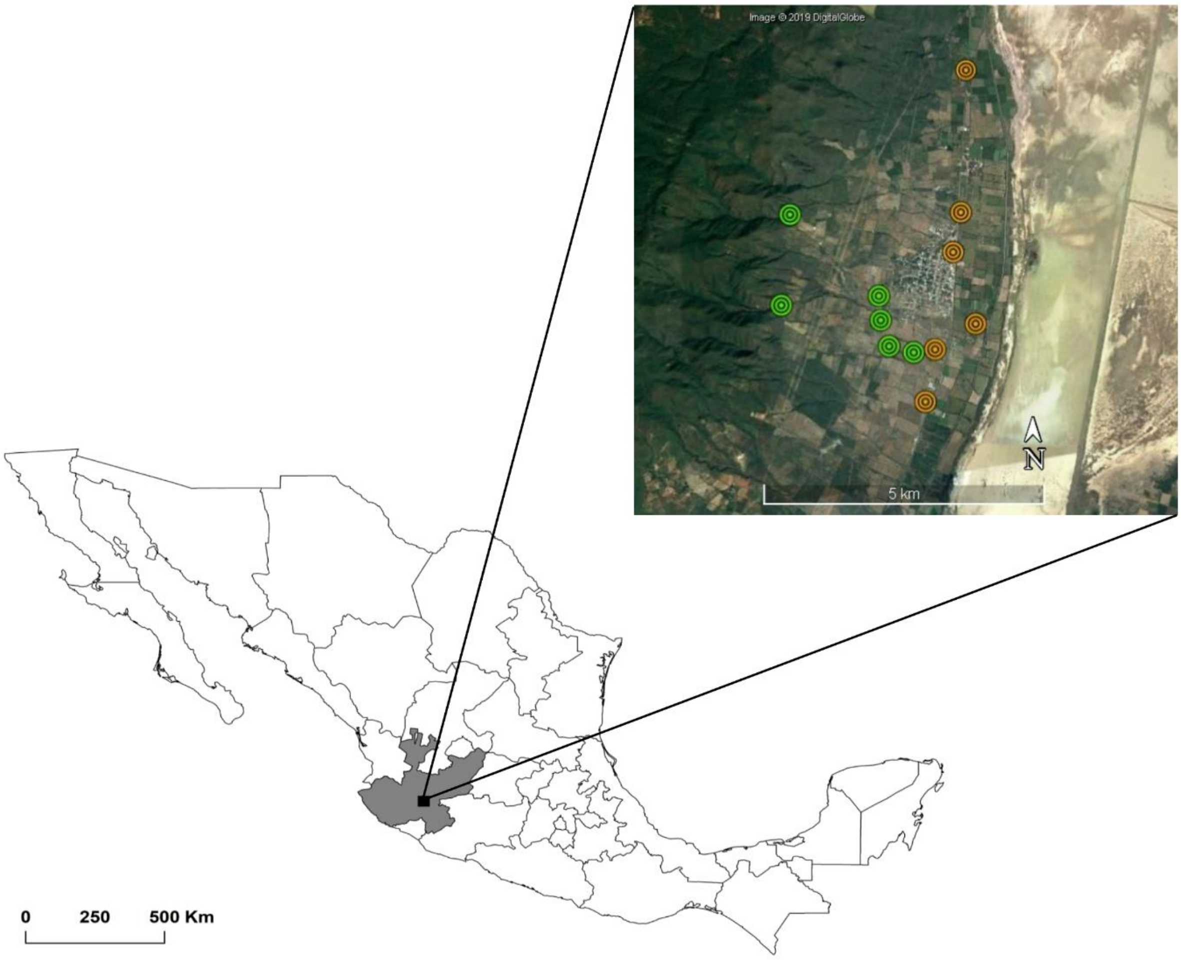

Figure 1

Figure 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 studied as cultivated sites (orange) and the six ranches studied 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. Map created using imagery from Google Earth (*Google Earth* 2019).

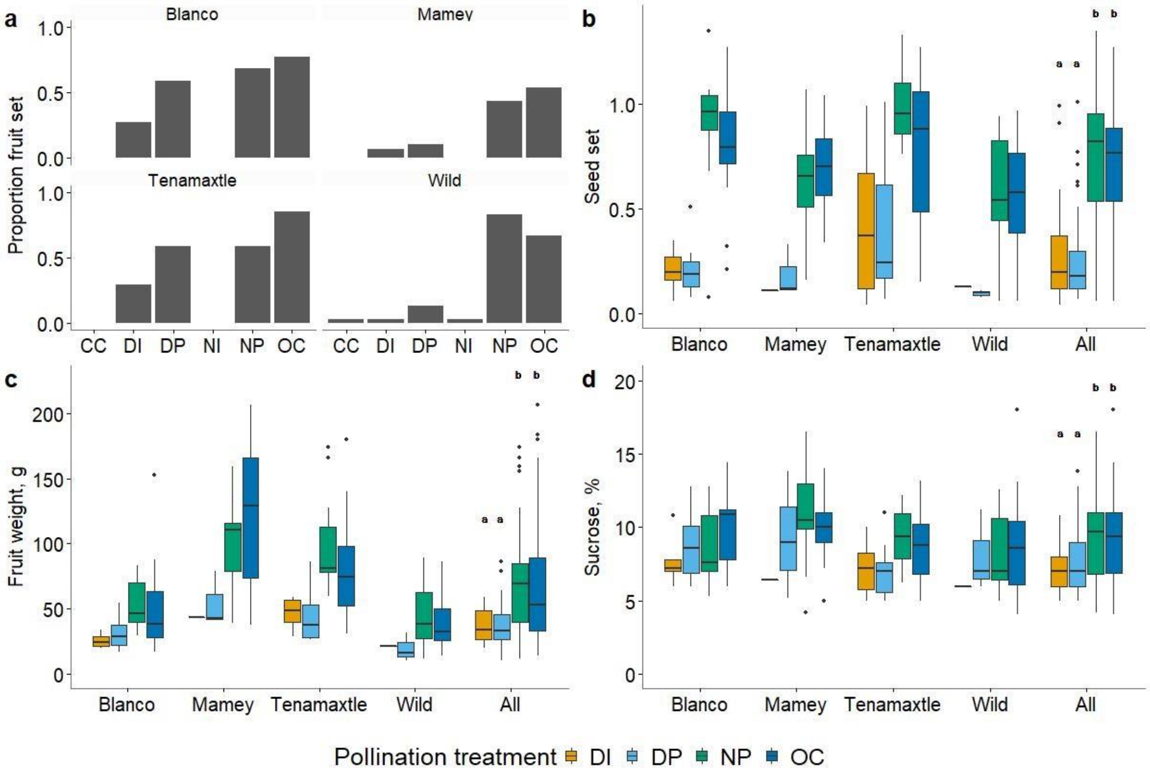

Figure 2

Figure 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).

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

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Study species

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). *Stenocereus 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). *Stenocereus 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).

Table S1. 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

Table S2. 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**
Pulp weight, g	0.64	0.82	0.85	1.00

Table S3. 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

Table S4. 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

Table S5. 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

Table S6. Feeding observations in pitaya plantations 2017. One observer stood in a central position in a pitaya plantation from 07:00 until 09:00. Interactions between animals and cactus flowers were noted in 15min segments, per species seen. Two individuals seen feeding in the same 15min segment is classed as two observations in the table. Observations shown in the table are for multiple cacti and multiple flowers simultaneously, covering the entire range of view of each observer (dozens of cacti). All bird visits to flowers seen by observers are included in the table. Each row shows the observations of one observer in one plantation.

	# 15 minute segments with individuals seen feeding at a pitaya flower			
	Amazilia violiceps (Violet-crowned hummingbird)	*Thalurania ridgwayi* (Mexican wood-nymph hummingbird)	Other hummingbird sp.	Total number of observations per hour
24 April	2	0	2	2
24 April	2	0	1	1.5
27 April	0	0	0	0
27 April	9	0	0	4.5
01 May	5	2	0	3.5