Full Length Article

Reproductive Failure of *Camellia oleifera* in the Plateau Region of China Due to a Shortage of Legitimate Pollinators

Zhenghua Xie[1], Xiaoming Chen[1*] and Jianshen Qiu[1,2]
[1]*Department of Environmental Insects, Research Institute of Insect Resources, Chinese Academy of Forestry, Kunming, Yunnan 650224, China*
[2]*Guizhou Academy of Forestry, Fuyuan Road, Guiyang, Guizhou 550005, China*
*For correspondence: cafcxm@tom.com

Abstract

Decline in crop pollinators has raised concern that crop productions of agri-ecosystems could be influenced. To date, most studies were carried out at field scales or landscape scales, but little is known regarding how pollinator abundance and the corresponding pollination services vary at growing region scales. Therefore, investigations were conducted to explore the pollinator abundance and reproductive success of *Camellia oleifera* Able., between the tree's primary growing region and the plateau growing region. The abundance of solitary bee *Andrena camellia* Wu., declined from the primary region to the plateau region, whereas the abundance of social wasps increased. Honeybees and flies occasionally visited the flowers in both regions, but their visit densities were notably low. Among those species, only solitary bee was effective in transferring pollens. Pollinators deposited approximately 9 pollen grains on the stigmas in the primary region, but deposited approximately 0 pollen grains in the plateau region. *C. oleifera* trees in the plateau region underwent a higher level of pollen limitation than those in the primary region due to a shortage of the legitimate pollinator, *A. camellia*. © 2013 Friends Science Publishers

Keywords: Pollen limitation; Pollination efficiency; Pollinator decline; Growing region; Solitary bee

Introduction

Pollination services are among the most important ecological functions. Approximately 30% of global crop production is reliant on animal pollination (Free, 1993; Klein *et al.*, 2007), and the economic value of the animal pollination of global crops was estimated at €153 billion in 2005 (Gallai *et al.*, 2009). However, there has been a great decline in honeybees and wild bees during the last several decades (Watanabe, 1994; Biesmeijer *et al.*, 2006; NRC, 2007; Potts *et al.*, 2010; Levy, 2011; Winfree *et al.*, 2011). The pollinator decline raises concerns that crop production will likely be influenced due to pollination deficits (Ricketts *et al.*, 2008). But to date, the effect of pollinator decline on crop production has often been investigated at small spatial scales, such as the field scales or landscape scales (Kremen *et al.*, 2002; Ricketts, 2004; Ricketts *et al.*, 2008). Little attention has been paid to crop growing region scale.

Generally, crops are planted across a broad geographic range, and that spatial range is often divided into several growing regions based on differences in ecological factors, such as climate (Pollak and Corbett, 1993; Tonietto and Carbonneau, 2004; Hall and Jones, 2010), soil characteristics (e.g., Zhuang, 2008), crop cultivar types (Payraudeau and van der Werf, 2005) or productions (Zhuang, 2008). The abundances of pollinators are determined by the amount of suitable habitats (Carvell *et al.*, 2007; Roulston and Goodell, 2011; Winfree *et al.*, 2011). Therefore, the differences in environmental factors between growing regions could potentially drive changes in pollinator species and abundances.

Pollinator species or species groups differ greatly in terms of their effectiveness in transferring pollens. Variations in pollinator species and abundances between growing regions will thus lead to different pollination services. However, the ways in which pollinator communities affect plant pollination have been poorly understood (González-Varo *et al.*, 2009; Gómez *et al.*, 2010), even though visiting densities, the number of pollens transferred by pollinators, and fruit or seed set have often been calculated (Fenster *et al.*, 2004). The number of pollen grains deposited on the stigmas by pollinators can directly reveal the pollination effectiveness and has been used to explore the extent to which pollinator communities affected crop pollination (Kremen *et al.*, 2002; Winfree *et al.*, 2007), but how pollinator communities differ in pollination services between growing regions is still unknown.

Camellia oleifera Able., which produces seeds that are used to produce edible tea oil, is one of the most economically important trees in China. The trees bloom from October to December (fall and winter seasons in the northern hemisphere), depending on the ambient

temperature and location. *C. oleifera* produces thousands of flowers; each flower is 3-5 cm in diameter with 40-60 stamens and approximately 220,000 pollen grains (He *et al.*, 2009). The flower visitors are solitary *Andrena camellia* Wu. (Hymenoptera: Andrena) (Wu, 1977; Ding *et al.,* 2007) and *Colletes gigas* Cockerell. (Hymenoptera: Colletidae) (Deng *et al.*, 2010). Social bees such as honeybees and wasps also occasionally visit the flowers.

The geographic range of *C. oleifera* covers a broad area and was generally divided into 9 growing regions (Fig. 1). Five years ago, several policies aiming to stimulate the development of the tea oil industry have been enacted across China. For example, a national scheme for tea oil development has been applied in 2009 (SFA, 2009). The key part of the scheme is to develop many *C. oleifera* trees across the species' geographic range, covering both the primary region and the plateau region. The differences in climate, soil characteristics and magnitude of habitat fragmentation among growing regions appear to affect the abundance of particular pollinators, but little is known to the regional variation in pollinator species, pollinator abundance, or the corresponding pollination services provided by pollinators.

The primary goal of this study is to investigate the pollinator species and abundance, their effectiveness in transferring and depositing pollens, and the pollen limitation of *C. oleifera* between the primary region and the plateau region. Three questions addressed will be: (1) do the pollinator abundance of *C. oleifera* decline from the primary region to the plateau region? (2) do the pollinators exhibit differences in effectiveness in transferring and depositing pollens?; (3) what is the strength of pollen limitation of *C. oleifera* trees between the primary region and the plateau region?

Materials and Methods

Study Regions

The primary growing region and the plateau growing region were selected (Fig. 1). The primary region was the leading region for *C. oleifera*, producing the highest amount of seeds of all regions. This region contained Hunan Province, Jiangxi Province, Zhejiang Province, most of Hujian Province and Guangxi Province. The primary region was in the subtropical monsoon climate zone with an annual rainfall of approximately 1500 mm (Zhuang, 2008). The highest temperature in July was 35°C, and the lowest temperature in January was 2°C. During flowering, the temperature was often over 18°C. Trees were planted alongside the foothills of the undulating topography, which had a mean altitude of 400 m (Zhuang, 2008). The long-term lateral and downhill transportation of clays by drainage, gravity and relief formed rich black-gray loam or clay-loam soils at the bottoms or toe slopes of hills, where solitary bees often nested (Xie *et al.*, 2013). We selected Yichun County, Jiangxi Province to be the study area in the primary region.

The plateau region in Yunnan Province had a low annual seed production and experienced the plateau monsoon climate. This region was located near the eastern Tibetan Plateau with an altitude of approximate 1200 m (Zhuang, 2008). The highest temperature was in July (mean 28°C), and the lowest was in January (mean 6°C). The annual rainfall in the region was approximately 1000 mm (Zhuang, 2008). The plateau region had a typical Karst and mixed mountainous topography. The *C. oleifera* trees in this region were planted in red or yellow sand soils. This type of soils was generally difficult for digger bees' nesting. We selected Wenshan County, Yunnan Province as the study area in the plateau region.

Pollinator Surveys

Within the primary region (Yichun County, Jiangxi Province), 18 *C. oleifera* forests were selected from an area of 19×14 km^2 (Fig. 1, bottom right). Prior investigations revealed that forest size was positively correlated with pollinator abundance; therefore the selected forests covered a broad range of areas, ranging from 0.1 to 20 ha (Fig. 1, bottom right). The 18 forests were separated by a minimum distance of 1 km, which was generally greater than the foraging distance of digger bees. Within the plateau region (Wenshan County, Yunnan Province), 18 *C. oleifera* forests were selected from an area of 80×80 km^2 (Fig. 1, bottom left), which ranged in area from 0.007 to 4 ha and represented all of the *C. oleifera* forests found in that region. The forests in the plateau region had a minimum distance of 4 km apart, which was generally greater than the foraging distance of the dominant social bees.

During the peak of flowering in 2010 and 2011, pollinator species and abundances were surveyed within a 100×4 m transect placed in the 36 *C. oleifera* forests, with a distance of greater than 10 m to the forest edge. Investigators walked with similar speeds along the center line of transect from one end to the other. The duration of each transect walking was kept the same (about 20 min) to reduce the sampling effects. When pollinators were observed, they were recorded into 4 groups: digger bees, honeybees, wasps and flies. The digger bee group only included the solitary bee (*A. camellia*). The honeybee group consisted of *Apis cerena* and *A. mellifera*. The wasp groups included several species within the Vespidae family. Sticking of the pollens to the bodies of the visitors was also recorded. The pollinators were counted when they were observed or collected if identification was not possible in the field. All transects were surveyed between 10:00 am and 4:00 pm when it was sunny and the temperature exceeded 20°C.

Transfer and Deposition of Pollens

Because the principal pollinator was the solitary bee in the primary region and wasps in the plateau region (see results), more than 20 *A. camellia* and wasps visiting the flowers were caught and immediately deposited into 70% ethanol.
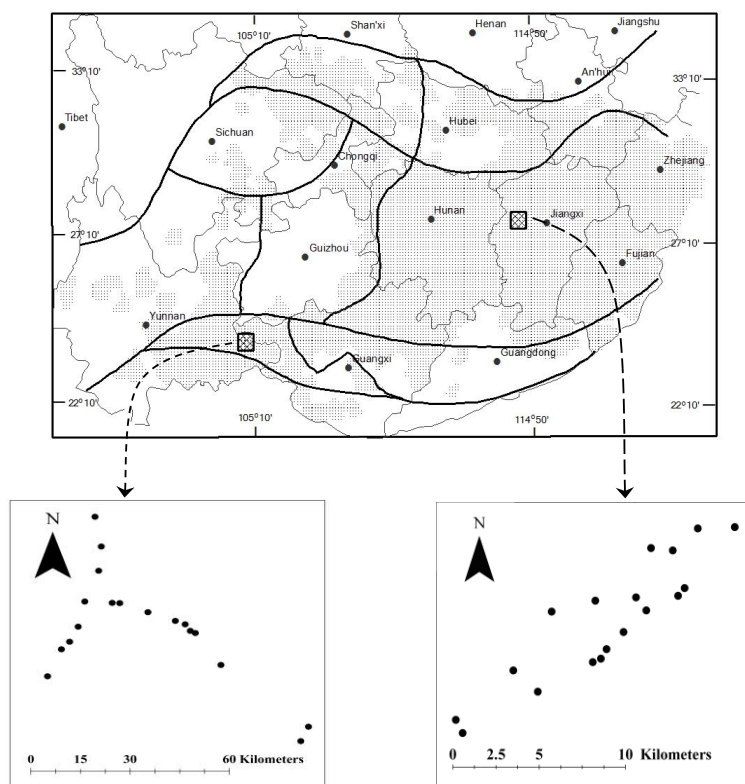
Fig. 1: The geographic distribution of *Camellia oleifera* in China. Top: The geographic distribution (stipples) was divided into 9 growing regions according to differences in climate and production (redrawn from Zhuang, 2008). The hatched squares identified the study areas at the primary region (right) and the plateau region (left) Bottom right: the sample sites (dots) within the primary region; bottom left: the sample sites (dots) within the plateau region

Pollen gains were carefully examined with a microscope under 80X magnification in the lab.

To estimate the number of pollens deposited on the stigmas by pollinators, the stamens were removed prior to anthesis from more than 50 flowers in 5 trees. Flowers were selected that were positioned at the similar heights of the trees and approximately 1.6 m above the ground. Half of the stamen-less flowers were selected randomly and were bagged with nets to prevent visitors, whereas the other half was not bagged and pollinators could freely access them. Pollinators were observed to visit the emasculated flowers (Deng *et al.*, 2010), touch the stigmas and deposit pollen gains on them. The stigmas were collected three days later and kept in 70% ethanol. The difference in the number of pollen gains deposited on the surfaces of the stigmas between the bagging treatment (CK) and natural pollination treatment was explained by the pollinators' visits, thereby providing an estimate of effectiveness in depositing pollens. This method was applied at both the primary region and the plateau region.

Pollen Limitation

To investigate the effect of pollinator abundance on pollen limitation of *C. oleifera* between the two regions, three habitat categories were further defined based on the abundance of the legitimate solitary bee *A. camellia* (see results). One habitat category was selected from the plateau region, whereas the other two were selected from the primary region. The three habitat categories were (1) the plateau region (bees rare): three *C. oleifera* forests in the plateau region where *A. camellia* was not observed (mean visit density per transect = 0); (2) the primary region (bees uncommon): two *C. oleifera* forests in the primary region where the visit densities of *A. camellia* were low (mean visit density per transect = 15); and (3) the primary region (bees abundant): three forests in the primary region where the visit densities of *A. camellia* were high (mean visit density per transect = 51).

For each of the 8 forests, three treatments were implemented on 6-10 trees that had similar dimensions in length and height and potentially belonged to the same cultivars. The three treatments were: (1) Bagging: flowers were bagged with fine mesh nets prior to anthesis to prevent insect visits; (2) Cross pollen supplementation: pollen grains from plant individuals 10 m away were manually transferred to stigmas using a fine paint brush when the stigmas were receptive; (3) Natural pollination (CK). For each treatment in each forest, at least 80 flowers (often over

100 flowers) were examined. The initial fruit set was recorded three months later, and the final fruit set was recorded in October of next year.

Statistical Analysis

The difference in pollinator abundances between the growing regions were examined using student t test after data were $\log_{10}$ (x+1) transformed to achieve potential normal distributions. The difference in the number of pollen gains attached to the bodies of the three pollinators was tested using the Kruskal-Wallis rank test, whereas the difference in the number of pollen gains deposited on the stigmas between natural pollination (CK) and bagging treatments was examined using the Mann-Whitney rank test, as those data did not meet the assumptions of normality.

Two-level Generalized Linear Mixed Models (GLMMs) were used to detect the influence of treatments and pollinator abundance on fruit set in the three habitat categories [the plateau region (bees rare), the primary region (bees uncommon), and the primary region (bees abundant)], in which the response variables, the fruit set, had a binominal distribution with a logit link function. The treatments and pollinator abundance were treated as the fixed effects and the plants, forests and regions as the random effects. The plants were nested within forests (level 1), and the forests were nested within regions (level 2). The initial fruit set and the final fruit set were analyzed, respectively. The two-level GLMMs were conducted using the lme4 package (Bates, 2010) in the R 2.11 software program (R Development Core Team, 2011).

For the eight study forests, pollen limitation was measured as the difference in initial fruit set between the natural pollination (CK) and the cross pollen supplementation treatments. A stepwise regression model was firstly applied to explore how the pollinators affected pollen limitation. The pollinator abundance observed within the 100 x 4 m transect was used to represent the visit density. In this model, pollen limitation was treated as the response variable, and the visit densities of digger bees, honeybees, wasps and flies were treated as explanatory variables after $\log_{10}$ (x+1) transformed. Because the visit density of digger bees alone explained the pollen limitation (see results), the visit density was then plotted against pollen limitation, and a simple linear regression model was fitted to the association.

Results

Pollinator Abundance

Within the primary region, a total of 426 insect visits were recorded. The most frequent pollinator was the digger bee *A. camellia*, which accounted for 93% of the visits (399 records). Honeybees, flies and wasps also visited the flowers, but they accounted for just 7% of the visits. However, in the plateau region, only 83 insect visits were

recorded. The most frequent pollinators were wasps, which accounted for 82% of visits (68 records). Honeybees, flies and digger bees accounted for the remaining 18% of visits. Only one female *A. camellia* was observed in the plateau region.

The primary region had significantly higher mean abundance of digger bees (t = 15.97, df = 19, P < 0.001) than the plateau region, but significantly lower mean abundance of wasps (t = - 8.67, df = 34, P < 0.001). No difference was examined for honeybees (t = - 1.71, df = 26, P = 0.10) or flies (t = 0.56, df = 36, P = 0.58) between the two regions. Taking all species together, the primary region also had significantly higher abundance of pollinators than the plateau region (t = 7.41, df = 29, P < 0.001) (Fig. 2).

Transfer and Deposition of Pollens

Female *A. camellia* collected both pollen and nectar when visiting flowers and yellow pollen grains were observed on their legs and abdomens; however wasps, honeybees and flies only foraged for nectar (online resource Fig. S1). *A. camellia* carried approximately 425,000 pollen grains, wasps carried approximately 3,700 pollen grains, and honeybees carried only approximately 620 pollen grains. The numbers of pollen grains carried significantly differed among the three categories of pollinators (Kruskal-Wallis rank test: x^2 = 13.58, P < 0.001) (Table 1).

After emasculation, the stigmas of the flowers in the bagging treatment did not bear pollen in either the primary region or the plateau region (median = 0). In the natural pollination treatment, the stigmas collected 9 pollen grains (median) in the primary region but no pollen in the plateau region (median = 0). There was a significant difference in the numbers of pollen gains deposited on stigmas by pollinators between the two regions (Wilcoxon rank test: W= 891, P < 0.001) (Table 2).

Pollen Limitation

The pollen supplementation treatment had a higher initial fruit set and final fruit set than the natural pollination (CK) and bagging treatments in the three habitat categories. Although pollen limitation also occurred for natural pollination in all habitats, the degree of limitation differed. In the plateau region (bees rare), the natural pollination (CK) treatment had nearly the same initial and final fruit sets as the bagging treatment, but almost arrived at the level of the cross pollen supplementation treatment in the primary region (bees abundant) (Fig. 3 and 4). When considering the three treatments and the pollinator abundance in the two-level GLMMs, bagging negatively reduced both the initial fruit set (Model 1 in Table 3) and the final fruit set (Model 2 in Table 3), whereas pollination by insects had a positive influence on the initial fruit set (Model 1 in Table 3) and the final fruit set (Model 2 in Table 3).

Table 1: The numbers of pollen grains attached to the bodies of pollinators visiting the flowers

Pollinator species or species groups	Sample size	Pollen grains on bodies (Median and range)	Significance
Digger bees	21	425,000 (250,500 - 1,287,189)	P < 0.001
Wasps	29	3, 700 (135 - 11,000)	
Honeybees	4	620 (235 - 1,005)	

Table 2: The numbers of pollen grains deposited on the stigmas in the primary region and the plateau region

Study region	Sample size	Pollen grains on the stigma (Median and range)	Significance[§]
Primary region			P < 0.001
Bagging	28	0 (0 - 7)	
Natural pollination	30	9 (0 - 47)	
Plateau region			P = 0.45
Bagging	21	0 (0 - 3)	
Natural pollination	35	0 (0 - 7)	

[§]P values were generated by comparing the pollen numbers between bagging and natural pollination within the growing regions

Table 3: Summaries of the results for the two-level GLMMs

Models	Group comparisons[★]	Estimated Coefficient[§]	Z value	P value
Model 1: initial fruit set	natural pollination vs. bagging	-1.56	-11.82	< 0. 001
	natural pollination vs. cross-pollen supplementation	1.30	9.72	< 0. 001
	bees abundant vs. bees uncommon	-1.17	-4.15	< 0. 001
	bees abundant vs. bees rare	-2.30	-8.97	< 0. 001
Model 2: final fruit set	natural pollination vs. bagging	-1.92	-8.73	< 0. 001
	natural pollination vs. cross-pollen supplementation	0.84	6.24	< 0. 001
	bees abundant vs. bees uncommon	-0.60	-1.84	0.04
	bees abundant vs. bees rare	-1.84	-0.60	< 0. 001

[★]Group comparisons indicate how treatments or bee abundance will influence the fruit set under conditions in which the other factors were controlled
[§]+indicates relative positive influences, whereas – indicates relative negative influences

The linear regression model indicated that only the visit density of digger bees significantly affected pollen limitation. The visit density of solitary bees was negatively related to pollen limitation, with the strongest magnitude of pollen limitation in the plateau region (bees rare), where the visit density of digger bees was zero, and the lowest pollen limitation in the primary region (bees abundant), where the visit density of digger bees was highest (Fig. 5).

Discussion

The decline in abundance of *A. camellia* from the primary region to the plateau region could be explained by several factors. The weather conditions are the most important factor. Yichun County, Jiangxi Province (the primary region) falls within the subtropical monsoon climate zone, whereas Wenshan, Yunnan Province (the plateau region) experiences the plateau monsoon climate. The differences in weather could influence the interactions between pollinators and flowering plants (e.g., Hegland *et al.*, 2009). For example, the flowers of *C. oleifera* generally appeared in October in the primary region, but began in the middle of September in the plateau region (Zhuang, 2008). If the pollinators did not evolve to match the early flowering of the plants in the plateau region, the phenological mismatch between pollinators and their food plants likely caused food shortages and thus drove pollinator decline.

The nesting site availability is another factor influencing the bee abundance. Digger bee *A. camellia*

preferred nesting on loose, moist and low-temperature soils (Xie *et al.*, 2013). The plateau region with Karst and mixed mountainous topography often had compacted, dry and high-temperature soil environment, in which solitary bee *A. camellia* could not nest (Xie, unpublished data). Consequently, the *C. oleifera* forests in the plateau region had smaller *A. camellia* populations. Yet another factor seems to be the degree of fragmentation of the habitat (Richetts, 2004; Winfree *et al.*, 2007; Potts *et al.*, 2010). The *C. oleifera* forests in the plateau region were cultivated in more isolated and fragmented habitats compared with the primary region. The habitat fragmentation could also perform as an important factor reducing solitary bee populations in the plateau region. In most cases, several factors often acted simultaneously (Potts *et al.*, 2010). More studies were required to explore why the legitimate solitary bees declined from the primary region to the plateau region.

A. camellia individuals carried more pollen grains than honeybees or wasps. The solitary bees were smaller in body size than wasps and honeybees, but they had dense hairs on their hind legs and abdomens. Those hairs enabled the bees to hold pollens. Yellow pollen grains were often observed on the solitary bee' bodies when they foraged for pollen (Online Resource Fig. S1). Conversely, the wasps were covered with sparse, short and stiff bristles, which carried pollen ineffectively (online resource Fig. S1). Honeybees often avoided visiting *C. oleifera* flowers because the pollens were arguably poisonous to their larvae (He *et al.*, 2009). Honeybees and wasps were observed just
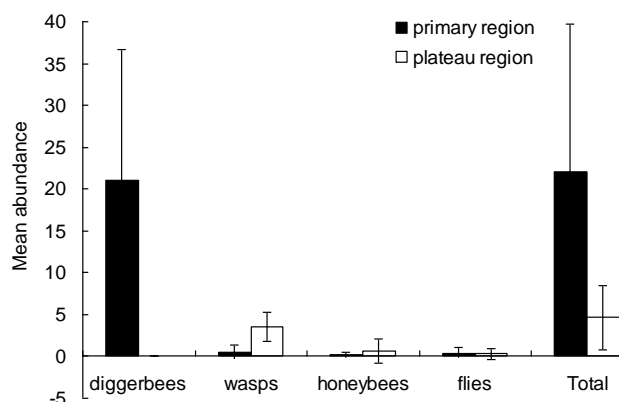
Fig. 2: The mean abundance of pollinator species groups in the 100 x 4 m² transects in the primary region and the plateau region
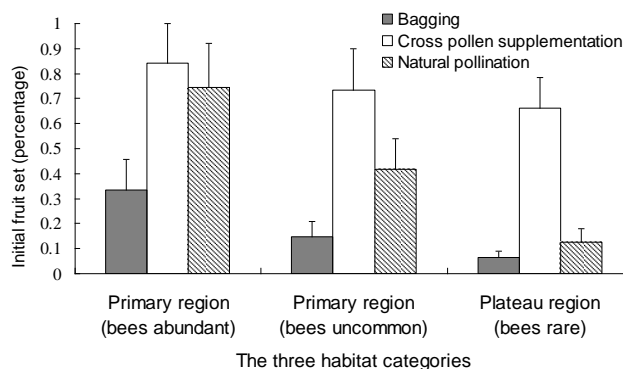

Fig. 3: The initial fruit set (mean ± SE) for the three habitat categories (bees abundant, bees uncommon and bees rare) under three treatments (bagging, cross pollen supplementation and natural pollination). Note that natural pollination had the highest initial fruit set in the primary region (bees abundant) followed by the primary region (bees uncommon) and then the plateau region (bees rare)

Fig. 4: The final fruit set (mean ± SE) for the three habitat categories (bees abundant, bees uncommon and bees rare) under three treatments (bagging, cross pollen supplementation and natural pollination). Note that natural pollination had the highest final fruit set in the primary region (bees abundant), followed by the primary region (bees uncommon) and then the plateau region (bees rare)

Fig. 5: The relationship between pollen limitation (PL) and the visit densities (VD) of digger bee *A. camellia* in the plateau region (bees rare) (circles), the primary region (bees uncommon) (diamonds), and the primary region (bees abundant) (triangles). PL = 0.48 - 0.007 * VD, F=20.05, df = 6, *p* = 0.004

to imbibe nectar (Online Resource Fig. S1), but did not collect pollens. Since only the solitary bee was a legitimate pollinator (Table 2; online resource Fig. S1), a high visit density of this species reasonably resulted in the higher number of pollen grains deposited on the stigmas in the primary region. Even though the plateau region had a relative higher visit density of wasps, the plants still received low number of pollen grains because wasps were not effective in transforming pollens.

Empirical and theoretical studies showed that both insufficient pollen and resource availability both influenced the degree of pollen limitation of plants (Wesselingh, 2007; Burd, 2008). Ideally, field experiments can identify the effect of insufficient pollen on pollen limitation when the effect of resource availability is controlled. In this study, the trees with cross-fertilized flowers were also treated with bagging. The bagged flowers produced a low fruit set and did not use a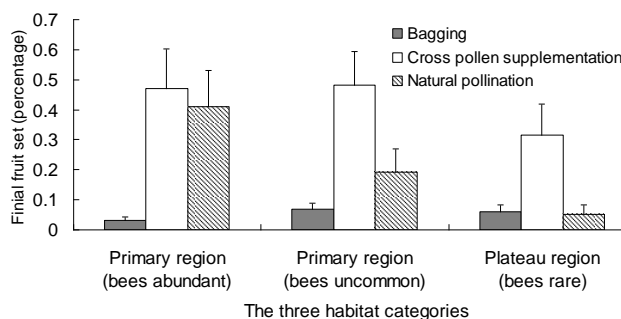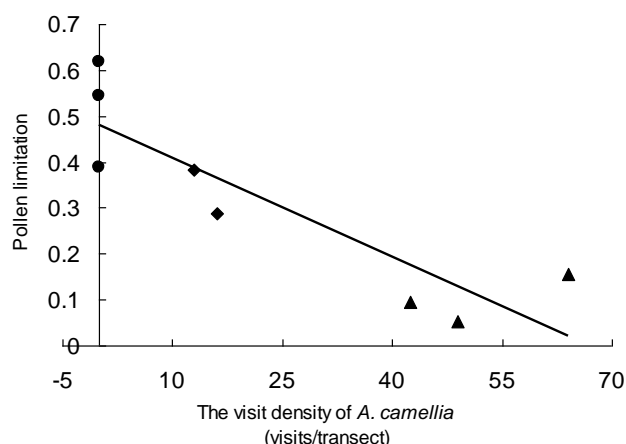s much resources as naturally pollinated flowers, which might have compensated for the resources required by the cross-fertilized flowers and thereby reduced the impact of resource limitation. Furthermore, the *C. oleifera* trees produced thousands of flowers, and approximately 70-80% of those flowers set fruits under natural pollination conditions in the primary region (Fig. 3 and 4). For each tree in the experiment, the cross-pollen fertilization treatment was applied to approximately 30 flowers. The additional flowers pollinated by the cross-pollen treatment might not be limited by resource availability.

As far as we know, this was the first study to explore the decline in pollinator abundance from the primary region to the plateau region of this crop. The role of insect pollination on fertilization success of *C. oleifera* has been

recognized for a long time (Zhuang, 2008; Deng *et al.*, 2010), but the pollination shortage of this plant in the plateau region did not receive much attention before. Results of this study indicated that the trees on the plateau region had few legitimate solitary bees, which subsequently resulted in a high level of pollen limitation. Therefore, it is necessary to take the pollination deficits of the plants into consideration when more trees are to be planted in the plateau region.

Acknowledgements

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