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Estimating crop pollinator population using mark–recapture method

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Abstract – *Xylocopa* species are large solitary bees, which are effective pollinators of yellow passion fruit, a self-incompatible crop dependent on pollination for fruit production and economic viability. We tested the mark–recapture method to estimate these bees' population size in a passion fruit commercial orchard in Central Brazil. We collected data during 3 years and population was estimated as 118, 339, and 1,032 individuals. Population size and abundance of *Xylocopa* bees were higher in the last studied year. These bees maintained 32.5 % of natural fruit set, above economic viability levels, and higher than other commercial orchards, although population size showed no direct correlation with natural fruit set. Improvement of nesting site may explain population increase, reinforcing the importance of management. Our results showed that the mark–recapture technique can provide reliable estimates of *Xylocopa* bees population size and, thus, be useful in monitoring population trends.

Brazilian savanna / carpenter bee / Jolly–Seber method / *Passiflora edulis* f. *flavicarpa* / *Xylocopa*

1. INTRODUCTION

Mark–recapture methods are often used to estimate population size based on a single source of individual identification such as natural markings or artificial tags (Krebs 1989). There are classic mark–recapture methods for large animals as ear tags for mammals and colour bands for birds, for example (Lettink and Armstrong 2003). However, there are no standardized methods for small animals such as insects to estimate and monitor population trends, except for some groups like

butterflies (e.g. Haddad et al. 2008) and some Orthoptera species (e.g. Jamieson et al. 2000).

For wild bees, the population size has been estimated in experimental studies, as to monitor *Osmia rufa* in trap-nests (Steffan-Dewenter and Schiele 2004), or in natural populations for those species with some special features such as aggregation of nests, as in *Andrena vaga* bees, which emerge in a particular time and occupy specific places around their nests (Bischoff 2003). There are studies also involving an oligolectic bee, *A. hattorfiana*, sampled during flowering season associated to *Knautia arvensis* (Dipsacaceae) plants (Larsson and Franzén 2008). However, no mark–recapture method study has been used to estimate the population size of non-social bees that pollinate crops. Actually, information about solitary bee popula-

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tions is limited because nest sites are inconspicuous, some species have very small populations, and quantitative data on density and dispersal are difficult to obtain (Michener 2000).

Solitary bees and wild bee as a whole provide an important ecosystem service promoting pollen flow in natural areas and improving crop production. In face of the concerns about pollinators decline (e.g. National Research Council of the National Academies 2007; Potts et al. 2010), some aspects of pollinators biology need to be considered to management and conservation guidelines. These points are highlighted in the protocol to assess the pollination deficit in crop (Vaissière et al. 2011), and the lack of knowledge on these issues has been identified as an impediment on this direction. The size of pollinator population can offer some important information concerning crop pollination since population density is strictly associated to visitation and seed or fruit production (Kunin 1997).

We used the *Xylocopa* bees as a model to estimate the population size of a crop pollinator using mark–recapture method. These large solitary bees were considered the effective pollinator of yellow passion fruit, a self-incompatible crop widely cultivated in Brazil. Passion fruits are consumed fresh or used in the juice industry and have been important to the development of sustainable farming systems in the Cerrado, the savanna region in Central Brazil. The Cerrado is one of the most threatened biomes of South America because of the rapid expansion of cash crop agriculture (Klink and Moreira 2002), one of the 25 world conservation hotspots due to rapid loss of biodiversity (Myers et al. 2000). In Triângulo Mineiro region, Cerrado area is under heavy human pressure (Cavalcanti and Joly 2002). In this area, passion fruit production rely on four large species of *Xylocopa* (Yamamoto et al. 2012) but sustainable pollination and production may be hindered by those bees dwindle populations (Camillo 2003).

Previous estimates pointed out that depending on the species of bee pollinator, around 350–1,000 individuals are needed to efficiently pollinate 1.0 ha of yellow passion fruit orchard (Kavati 1998). Other estimates, considering only *Xylocopa* species

indicate that 300–350 females are required to pollinate 1.0 ha orchard with 12,000–14,000 flowers (Melo et al. 2005). Therefore, our objective was to use multiple mark–recapture technique to estimate population size of *Xylocopa* species during the yellow passion fruit flowering season, and relate this information with fruit production and economic viability.

2. MATERIAL AND METHODS

2.1. Study site

We conducted the study at Água Limpa Farm, Experimental Station of Universidade Federal de Uberlândia (19°05'22"S, 48°21'15"W). The farm has 104 ha of preserved area and 43 ha of orchards with a variety of fruit crops including a 0.5 ha yellow passion fruit orchard (Neto 2008). *Xylocopa* (*Neoxylocopa*) *frontalis* (Olivier, 1789), *Xylocopa* (*Neoxylocopa*) *suspecta* Moure and Camargo, 1988, *Xylocopa* (*Neoxylocopa*) *grisescens* Lepeletier, 1841, and *Xylocopa* (*Neoxylocopa*) *hirsutissima* Maidl, 1912 were the main passion fruit pollinators (Yamamoto et al. 2012). This area was used to study trap-nesting occupation by *Xylocopa* species during the years 2006 and 2007, with availability of 80 trap-nests (Mesquita and Augusto 2011), and a long-term trap-nesting experiment focused on *X. frontalis* and *X. grisescens* was carried out from 2008 to 2011 using 384 trap-nests (Junqueira et al. 2012). There is a marked difference between seasons in the region: a warmer rainy season from October to March, and a cooler and almost rainless dry season from April to September. The average monthly temperatures ranged from 18 to 24 °C and annual rainfall varied from 1,300 to 1,700 mm (Rosa et al. 1991).

2.2. Biology of *Xylocopa* species

Xylocopa bees are wood-nesting generalist pollinators of broad geographical distribution (Hurd 1978). The genus comprises about 730 species around world (Gerling et al. 1989), and 50 species are described in Brazil (Silveira et al. 2002). These bees mostly burrow nests in dead or decaying wood (Silveira et al. 2002). Females may also use pre-existing cavities such as floral stalks (Ramalho et al. 2004), and trap-nests such as wood logs or planks (Freitas and Oliveira-Filho 2001; Camillo

2003; Oliveira-Filho and Freitas 2003; Chaves-Alves et al. 2011) or bamboo canes (Chaves-Alves et al. 2011; Pereira and Garófalo 2010, Junqueira et al. 2012).

The life cycle of *Xylocopa* bees from egg to adult varies among species, and to *X. grisescens*, *X. frontalis*, and *X. suspecta* ranges from 45 to 65 days (Camillo and Garófalo 1982; Camillo et al. 1986). The longevity of females also varies widely and may be only few months to a year (O'toole and Raw 1991; Pereira and Garófalo 2010; Junqueira et al. 2012). These species present multivoltinism and produce more than one generation per year (Camillo and Garófalo 1989). Nesting occurs throughout the year with higher frequencies from December to March and from July to September (Camillo 2003). Nest foundation is solitary but nest reuse is frequent, maintaining these nests active for several months (Camillo 2003; Chaves-Alves et al. 2011). Usually there is a single mature female per nest, or at most two females (Camillo and Garófalo 1989). Foraging activities to collect larval food (pollen and nectar) occur throughout the day (Camillo and Garófalo 1982; 1989). Although bees differed a bit in size and are relatively easy to identify in the field, they use a similar guild of flowers and are important pollinators of passion fruit (Yamamoto et al. 2012).

2.3. Mark–recapture

We collected data on rainless days during the flowering period of yellow passion fruit in a 0.5-ha orchard. Sampling sessions were carried out during three different years in 2005 (29 March to 01 April, $n=4$ days), 2007 (22 to 26 January, $n=5$ days), and 2011 (01 to 08 February, $n=6$ days) with similar sample effort between years. In 2005, there was no availability of trap-nest substrate for *Xylocopa*, whilst in 2007 and 2011 there were trap-nests available for the bees around the orchard. The flowering period of yellow passion fruit occurs during the warm rainy period, showing 7–10 days peaks of high flower production scattered along the blooming period and followed by 45–50 days with a few or no flowers (Silva 2005). The weather conditions during marking sessions were similar: sunny days with temperature around $29.6^{\circ}\text{C} \pm 2.62^{\circ}\text{C}$ and $52.4\% \pm 8.43\%$ of humidity. We captured bees over 2–4 h per day (two collectors and at least an experienced one) with insect nets

walking along the orchard lines when bees were visiting flowers.

After net-collection, we put the bees in a modified plastic syringe to position and marking them (Figure 1a, b, c). We attached a numbered 2 mm $\times$ 2 mm tag on the mesothorax of bees with cyanoacrylate adhesive (Figure 1b, d). The tag had an individual alphanumeric combination printed on inkjet photo paper and was previously covered with polyvinyl chloride waterproof varnish. After the glue was dry, the marked individuals were released apparently unharmed (Figure 1e). This marking allowed identifying the bee individually in the field and thereby estimate the abundance of pollinators. Recaptured individuals had their number recorded and were released as soon as possible. We considered the abundance of pollinators as the number of marked individuals plus recaptured individuals per day. Individuals recaptured more than once a day were counted only once.

We estimated the population size of pollinators with Jolly–Seber method recommended for open population with several occasions to mark and recapture, using the Ecological Methodology program version 7.0 (Krebs 1989). The method depends on marking a reasonable number of individuals and obtaining recaptures at all times (Krebs 1989). We used one-way ANOVA to compare the abundance of pollinators among years, additionally we used Tukey's HSD test for post-ANOVA pair wise comparisons. The abundance of pollinators had a non-normal distribution, so we log-transformed data before statistical analyses. Due to small sample sizes, we used χ^2 test to compare mean population size of pollinators along years (2005, 2007, and 2011), and to compare the proportion of recaptured bees among years.

2.4. Pollination tests

To analyze the correlation between the abundance of individuals and the population size of *Xylocopa* with natural fruit set of passion fruit we tagged some flowers daily during the same days of mark–recapture experiments in 2007 and 2011. After 15 days, we checked fruit production. To include 2005 data, we used monthly data of natural fruit set for the same area using the same methodology but not exactly in the same days of mark–recapture experiments (Yamamoto et al. 2012). We used χ^2 test to compare natural fruit

Figure 1. Marking of *Xylocopa* bees using a numbered tag **a** detail of modified syringe, **b** bee inside the syringe, **c** and **d** positioning of the bee on the syringe, and **e** a marked individual visiting yellow passion fruit flower

set among years. We analysed the correlation between the natural fruit set of passion fruit with the abundance of pollinators and pollinator population size using Pearson test with Bonferroni correction and log-transformed data. In 2011, the yellow passion fruit orchard was destroyed by *Fusarium* wilt, a disease favoured by high temperature and humidity (Liberato and Costa 2001). Many plants maintained flowers allowing bee capture and flower marking for fruit set. However, most of these plants died afterwards, possibly affecting the results of the pollination tests done during this period.

3. RESULTS

3.1. Population estimate

The abundance of *Xylocopa* bees had the same sample effort among species. Our data showed the abundance of *X. frontalis* and *X. suspecta* during sampled periods was always above 38 individuals reaching almost 160 marked bees per year. In

contrast, *X. grisescens* and *X. hirsutissima* were less abundant with no more than 10 individuals per year (Table I). Therefore, data from *X. grisescens* and *X. hirsutissima* did not allow some specific analyses.

The abundance of all marked *Xylocopa* bees collected on the passion fruit flowers was different among years ($F_{(2,12)}=9.82$, $P=0.003$) and the same was observed for *X. frontalis* and *X. suspecta* in separate ($F_{(2,12)}=8.98$, $P=0.004$ and $F_{(2,12)}=10.06$, $P=0.003$, respectively). The post-hoc test indicated significantly higher abundance in the last year (Figures 2 and 3a, b).

Collected data (detailed in Table I) allowed to estimate the population size of *Xylocopa* bees as a whole for all years and to *X. frontalis* in separate in 2007 and 2011. Data for the other species did not allow such estimate. Although *X. suspecta* was as abundant as *X. frontalis*, the irregular recapture of individuals did not allow any estimates. *X. hirsutissima* and *X. grisescens* had much lower abundance of both marked and recaptured indi-

Table I. Sums of captured individuals (Σn_i) and recaptured individuals (Σm_i) with the proportion of marked individuals recaptured by species in parenthesis, number of sampled periods (k), marked proportion ($\alpha \pm \text{SD}$), probability of survival ($\varphi \pm \text{SD}$), and estimated population size ($\Sigma B_i \pm \text{SD}$) of yellow passion fruit pollinators for each year

Years	Species	Σn_i	Σm_i	k	α	φ	ΣB_i
2005	<i>X fro</i>	59	3 (5.1 %)	4	—	—	—
	<i>X gri</i>	10	2 (20 %)	4	—	—	—
	<i>X sus</i>	38	3 (7.9 %)	4	—	—	—
	Total	107	8 (7.5 %)	4	0.13 $\pm$ 0.08	0.44 $\pm$ 0.47	118 $\pm$ 8.06
2007	<i>X fro</i>	40	11 (27.5 %)	5	0.31 $\pm$ 0.16	1.13 $\pm$ 0.62	67 $\pm$ 42.58
	<i>X gri</i>	6	2 (33.3 %)	5	—	—	—
	<i>X hir</i>	3	0	5	—	—	—
	<i>X sus</i>	56	4 (7.1 %)	5	—	—	—
	Total	105	17 (16.2 %)	5	0.20 $\pm$ 0.11	1.15 $\pm$ 0.66	339 $\pm$ 360.29
2011	<i>X fro</i>	142	55 (38.7 %)	6	0.23 $\pm$ 0.13	1.65 $\pm$ 1.87	436 $\pm$ 447.99
	<i>X gri</i>	9	3 (33.3 %)	6	—	—	—
	<i>X hir</i>	1	1 (100 %)	6	—	—	—
	<i>X sus</i>	158	18 (11.4 %)	6	—	—	—
	Total	310	77 (24.8 %)	6	0.22 $\pm$ 0.07	2.40 $\pm$ 2.84	1,032 $\pm$ 690.11

X fro *Xylocopa frontalis*, *X gri* *X. grisescens*, *X sus* *X. suspecta*, *X hir* *X. hirsutissima*

viduals. The estimated population size of *Xylocopa* bees as a whole was 118, 339, and 1,032 individuals for the 0.5 ha orchard during the study period (236, 678, and 2,064 individuals/ha/year). Both *Xylocopa* bees population as a whole and *X. frontalis* in separate showed significant differences among years ($\chi^2=703.03$, $df=6$, $P<0.001$; $\chi^2=29.86$, $df=3$, $P<0.001$, respectively).

The marked proportion of bees seemed to be similar among years whereas the probability of survival increased (Table I). We observed that the proportion of recaptured individuals for all *Xylocopa* bees increased over the years (Table I) ($\chi^2=9.26$, $df=2$, $P<0.001$), as also found for *X. frontalis* ($\chi^2=24.63$, $df=2$, $P<0.001$). The recapture for *X. suspecta* and *X. grisescens* was not different among years ($\chi^2=1.19$, $df=2$, $P<0.552$; $\chi^2=4.08$, $df=2$, $P=0.129$, respectively).

Around 12.1 % of marked individuals were recaptured only once, 1.0 % twice, 1.5 % three times, and 1.5 % four times. Furthermore, on

the last day of each data collection, we still observed unmarked individuals. During all mark–recapture periods we marked only two male individuals in 2011.

3.2. Pollination tests

The natural fruit set ranged from 25.7 to 38.7 % during studied period (Table II), with no significant difference among years ($\chi^2=2.71$, $df=2$, $P=0.258$). We found no significant correlation between natural fruit set neither with the abundance of *Xylocopa* bees (Pearson coefficient $r=-0.31$, $P=0.32$ and $r=-0.07$, $P=0.88$ without 2005 fruit set data) nor with estimated population size ($r=0.28$, $P=0.55$ and $r=0.28$, $P=0.54$ without 2005 fruit set data).

4. DISCUSSION

As far as we know, this was the first study to estimate population size of solitary bees in a crop

Figure 2. Abundance of *Xylocopa* bees had significant difference ($F_{(2;12)}=9.82$, $P=0.003$) among years. Different letters indicate statistically significant differences according to one-way ANOVA followed by Tukey's test ($P<0.05$)

area, using the multiple mark–recaptures method. Our study model seemed to have succeeded in estimating the population size for the *Xylocopa* species visiting the yellow passion fruit orchard over the years, although success differed between bee species. Moreover, our findings pointed to an increment of the number of *Xylocopa* bees as a whole from a few hundred to a thousand individuals, probably driven by the availability of nesting places provided by the trap-nest experiments, which reinforces the importance of management to maintain sustainable pollination by native solitary bees.

Although the Jolly–Seber method requires a large sampling effort to obtain good results, we were able to estimate the population size of pollinators for the three study years. Some parameters contributed to apply the mark–recapture method in our model, such as the abundance of individuals in the area, the catchability of *Xylocopa* bees associated to the attractivity of passion fruit flowers. We tagged all species of *Xylocopa* recorded in region and *X. frontalis* and *X. suspecta* were the most abundant in the passion fruit orchard, which corroborates results of higher frequency of visits of these bees observed on

passion fruit flowers (Yamamoto et al. 2012). *X. frontalis* was also the most frequent in trap-nests around the area (Chaves-Alves et al. 2011; Junqueira et al. 2012).

Although it was possible to estimate population size per species only for *X. frontalis*, we may suppose that *X. suspecta* population could be equally larger, but the problem was that the individuals of this species were not recaptured enough to apply the estimation method. Our data showed that *X. suspecta* contributed with 35.5 and 53.3 % of all marked individuals during the study period, but their recapture was not superior to 11.4 % (Table 1). In contrast, we were able to estimate *X. frontalis* population size whenever we recaptured at least 27 % of marked individuals. Considering bee population, the method of mark–recapture seems to be more accurate when the number of sampling periods and the capture probability are high (Bischoff 2003). The Ecological Methodology software (Krebs 1989) we used requires recaptures at all times in order to produce population estimates.

Our data showed that *Xylocopa* species had the same proportion of recaptured individuals between

Figure 3. a Abundance of *X. frontalis* had significant difference ($F_{(2,12)}=8.98, P=0.004$) among years as observed to **b** abundance of *X. suspecta* ($F_{(2,12)}=10.06, P=0.003$). Different letters indicate statistically significant differences according to one-way ANOVA followed by Tukey's test ($P<0.05$)

years as expected by the method, except for *X. frontalis*. An explanation for the increased recapture of *X. frontalis* may be the trap-nest experiments near the orchard. The experimental trap-

nests were mostly used by *X. frontalis* (57.6 %), less by *X. grisescens* (39.0 %), and only by two individuals of *X. suspecta* (3.4 %) (Junqueira et al. 2012). Apparently, *X. suspecta* prefers other

Table II. Natural fruit set of yellow passion fruit in a Central Brazil orchard

Years	Mean of fruit set±SD (%)	Number of flowers
2005	30.7±10.75	60
2007	38.7±16.12	150
2011	25.7±12.30	130

substrates to nest instead of bamboo canes offered as trap-nests (Chaves-Alves et al. 2011). Since bees are limited to a certain foraging area within the flight range from the nest (Larsson and Franzén 2008), trap-nest using may explain the more regular and increasing visits of *X. frontalis* on passion fruit flowers. Similarly, it is conceivable that the majority of marked *X. suspecta* individuals came from the native areas surrounding the orchard, which would explain irregular visitation and recapture of individuals.

It is worth of note, however, that the increase in population size of *Xylocopa* bees also indicates reliable food resources surrounding the yellow passion fruit orchard. Since the studied site has 104 ha of preserved area (Neto 2008), available flower resources can maintain pollinator bees and nesting sites provided by trap-nests may sustain local pollination services. A recent review showed evidence that the availability of food resources directly regulates bee populations, with less clear evidence that other direct factors as nesting resources and incidental risks may be limiting (Roulston and Goodell 2011).

Our results indicated that the estimated *Xylocopa* population in the studied yellow passion fruit orchard varied from a few hundred bees to more than a thousand individuals over the three years study, probably due to nest site improvement. These pollinators helped to maintain around 32.5 % of natural fruit set at studied area, a much higher value than that considered as required for economic viability in the region (above 20 % according to Silva 2005), and higher percentage than that found in other places in Brazil, e.g. 3.27 % in São Paulo State (Camillo 2003), and 9.4 % in Bahia State (Siqueira et al. 2009). The negative effects of the *Fusarium* disease in 2011 on fruit production probably hindered any correlation between pollinator's population size and fruit set during the study. But density of *Xylocopa* bees in

the area, both estimated here and actually censused in the same orchard (Junqueira et al. 2012), indicated pollination and fruiting success without the plague would have been greater in 2011. Other results pointed that species richness of pollinators and their frequency were positively associated with reproductive efficacy of passion fruit in orchards in Central Brazil (Yamamoto et al. 2012).

Considering that our method addressed effective pollinators only, the population size estimated (236, 678, and 2,064 individuals/ha/year) was near or much higher than the expected range (300–350 individuals) to efficiently pollinate a passion fruit orchard (Kavati 1998; Melo et al. 2005). The abundance or diversity of bees in a local area may be related to the type, amount, or connectivity of surrounding natural areas (Roulston and Goodell 2011) and farmers may carry out activities of maintenance and management to ensure the presence of bees. In this sense, mark–recapture procedures can be a quick and reliable method to evaluate these actions, estimate bee density and foresee economic viability of passion fruit orchards. Despite differences in recapture rates among bee species, the low cost of marking procedures indicates this method may be used more widely with tropical solitary bees, both to estimate minimum population size for sustainable use and economic viability of bee pollination, and to detect population changes associated to management.

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Estimation de la population de pollinisateurs de fruits de la passion par l'utilisation de la méthode de capture-recapture

Savanne brésilienne / abeille charpentière / méthode de Jolly-Seber / *Passiflora edulis* f. *flavicarpa* / *Xylocopa*

Schätzung der Bestäuberpopulation für Passionsfrucht mittels einer Markierungs-Wiederfang-Methode

Brasilianische Savanne / Jolly-Seber Methode / *Passiflora edulis* f. *flavicarpa* / *Xylocopa*

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