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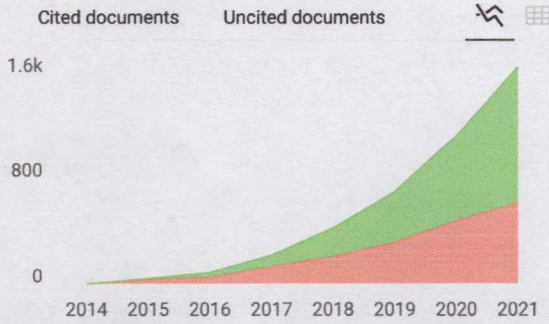
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Thesis, Dissertation:

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Information from internet: Balagadde FK, Song H, Ozaki J, Collins CH, Barnett M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. *Mol Syst Biol* 4:187. www.molecularsystembiology.com



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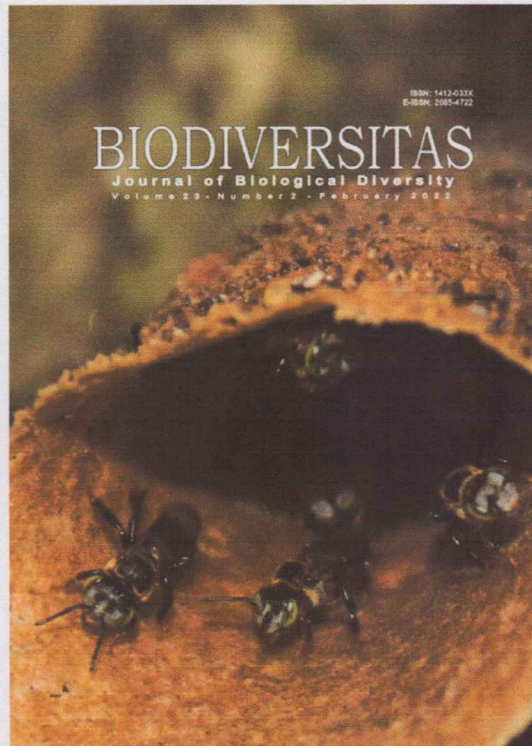


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Short Communication:

Impact of introduction of managed honey bee colony on wild bee diversity and abundance in an agroecosystem in Indonesia

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Manuscript received: 4 January 2022. Revision accepted: 28 January 2022.

Abstract. Widhiono I, Sudiana E, Suryaningsih S. 2022. Short Communication: Impact of introduction of managed honey bee colony on wild bee diversity and abundance in an agroecosystem in Indonesia. *Biodiversitas* 23: 1099-1104. Wild bees are natural pollinators of crops in agroecosystems, but managed honeybee colonies may diminish their diversity and abundance. We aimed to determine the impact of introducing managed honeybee (*Apis cerana* and *A. mellifera*) colonies on the diversity and abundance of wild bees in an agroecosystem in Indonesia. We introduced managed honeybee colonies into the plots containing four crops (tomato, green bean, chili, and green mustard). Sampling was conducted from March to July 2020 on the eastern slopes of Mount Slamet, Indonesia, at an elevation of 1241 m asl. Wild bee diversity was not affected by the introduction of managed honeybee colonies, but their abundance decreased at the end of the flowering period due to resource limitations. The most abundant wild bee species were *Amegilla cyrtandrae* and *A. burneensis*, and some wild bee species tended to be more abundant on certain crop species. We recommend that managed colonies be introduced during the early and mid-stages of crop flowering to reduce potential adverse effects on wild bee populations.

Keywords: *Amegilla burneensis*, *Amegilla cyrtandrae*, crops, Mount Slamet, phenology

INTRODUCTION

Hymenoptera is one of the four largest orders of insects (Budiaman et al. 2017). Wild bees (Hymenoptera: Apiformes). are important components of the agroecosystems because they are primary crop pollinators. The diversity and abundance of wild bee species are highly dependent on environmental conditions (Schindler et al. 2013; Cavigliasso et al. 2020; Odanaka and Rehan 2020). Wild bee species richness varies among locations and crop species. Widhiono et al. (2016) documented 9-11 species belonging to the families Apidae, Halictidae, and Megachilidae on Mount Slamet and in the surrounding area in Indonesia. Koneri et al. (2021) documented ten species belonging to Apidae, four belonging to Halictidae, and one belonging to Megachilidae in Bogani Nani Wartabone National Park, North Sulawesi, Indonesia. This suggests that wild bees remain relatively diverse in agricultural lands in Indonesia. However, given that wild bee species are highly sensitive to environmental change, their diversity and abundance should be monitored over time (Schindler et al. 2013).

Honeybees and wild bees play important roles as crop pollinators (Evans et al. 2018). However, there is alarming evidence of population decline and diversity loss in wild bees. As a result, managed honeybee colonies are often introduced to ensure sufficient pollination of crops. Colonies of *Apis mellifera* or *Apis cerana* are widely used in agricultural settings. Both species can thrive in highly dense colonies (Evans et al. 2018), are generalist

pollinators (Clos et al. 2020), and are easy to manage (McCune et al. 2020). Honeybee colonies have positive impacts on crop yield but are believed to negatively affect the diversity and abundance of wild bees (Lázaro et al. 2021). Notably, even where managed colonies are introduced, wild bees continue to pollinate crops (Widhiono et al. 2016). Ultimately, wild bee abundance is driven by the availability of pollen and nectar (Roulston and Goodell 2011; McCune et al. 2020), and pollen availability is a critical factor regulating wild bee populations (Roulston and Goodell 2011). In these settings, wild bees must compete with honey bees for limited nectar and protein-rich pollen resources (Russo 2016; Alaux et al. 2019). Mallinger et al. (2017) found that competition between wild bees and honeybees increased with increasing honeybee abundance.

Although many studies have assessed the impact of managed honeybee colonies on wild bees in agricultural settings (Barcala et al. 2019; Kline and Joshi 2020; Angelella et al. 2021; Garibaldi et al. 2021) the results have been somewhat inconsistent. The majority of studies have reported negative effects of colony introduction (Mallinger et al. 2017). For example, Angelella et al. (2021) found that honeybee presence reduced the presence of wild bees on coffee flowers in coffee plantations.

Managed honeybee colonies are not widely used for pollination in Indonesia. However, given the ongoing declines in wild bee species diversity and populations, this practice may soon be necessary, and thus suitable conditions for its application should be assessed. Before

introducing managed colonies in crops, we must understand the potential negative impact on wild bees. Here, we aimed to determine these impacts in an upland agroecosystem.

MATERIALS AND METHODS

Study area

This study was conducted in Serang Village, Purbalingga Regency, Central Java, Indonesia (7°14'41" S, 109°16'49" E), at an elevation of 1240-1300 m (Figure 1). The study area was 2100 m² in size.

Sampling design and methodology

We used colonies of two honeybee species, *A. mellifera* and *A. cerana*, in our study. The colony used came from a seller in Subang, West Java, Indonesia. All colonies used are young colonies were marked with 4 combs in the nest with white combs filled with larvae and pupae. One colony was maintained in 400 m² plots that included a maximum of 360 plant stems, 90 each of *Solanum lycopersicum* (tomatoes), *Phaseolus vulgaris* (green beans), *Capsicum frutescens* (chili), and *Brassica juncea* (green mustard) and located at the center of the plots. These crop species are used in research with the consideration that they are very commonly planted by farmers in the area and produce fruit as a result of pollination. Twelve plots were placed across the study site, spaced 300-400 m apart. The determination of the distance between the plots was based on the results of previous studies conducted at this location (Widhiono et al. 2019 unpublished data). The results of our previous study showed that honeybees *A. cerana* and *A. mellifera* on land conditions rich in food sources (flowers) tended to visit flowers or take food resources closer to the nest than

those farther from the nest. Each plot included a single, centrally placed colony. Four plots contained colonies of *A. cerana*, four contained colonies of *A. mellifera*, and four had no managed bee colony (control).

Four surveyors observed 120 plant stems (n : 30 per crop species) for 30-minute periods. Observations were made between 9 AM and 12 PM. Surveyors were non-experts and were given a photo guide for wild bee identification, all wild bees found in this area were identified and confirmed to LIPI (Indonesian Science Institute) in 2017. All insects visiting the plant stems were either photographed or captured with an insect net. This sampling was repeated over three periods corresponding to phenological phases, the onset of the crop flowering period, the middle, and the end.

Statistical analyses

We assessed wild bee diversity using three indices: Shannon-Wiener (H'), Evenness (E) and Simpson's Dominance Index Indices were calculated using the software PAST 3 as:

$$H' = \sum_{i=1}^s p_i \ln p_i$$

Where: H' is the Shannon-Wiener diversity index and p_i is the number of individuals of species I , and:

$$E = \frac{H'}{\ln S}$$

Where, E is the Evenness index, H' is the Shannon-Wiener diversity index and S is the total number of wild bee species.



Figure 1. Study area map showing the location of the research site in Central Java, Indonesia

In addition, Simpson's dominance index was used to assess dominance, determined by:

$$D = \sum (pi)^2 = \sum \left(\frac{ni}{N}\right)^2$$

Where, D is Simpson's dominance index, ni is the number of individuals of each species, and N is the total number of individuals. Finally, we used analysis of variance to determine differences in wild bee abundance among treatments, crop species, and flowering period. Pearson Correlation analysis was used to determine relationships between wild bee species richness and abundance with the number of flowers in each plot. These analyses were conducted using SPSS version 21 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Wild bee species richness

We recorded 1630 individuals of 11 wild bee species belonging to three families. Specifically, we photographed or captured carpenter bees (Apidae: Xylocopa), blue-banded bees, leaf-cutting bees (Megachilidae), and sweat and alkaline bees (Halictidae).

Wild bee species richness in plots with honeybee colonies did not differ significantly from control ($F 7.21 p > 0.05$). In the *A. cerana* treatment, wild bee species richness was highest on tomatoes, followed by chili, green mustard, and green beans (Table 1). In the *A. mellifera* treatment, species richness was highest on tomatoes, followed by green beans, chili, and green mustard (Table 1). Similar species diversity was recorded in the control

plots (Table 1). This lack of difference may be due to low competition for pollen and nectar between honeybees and wild bees or a shift in niche breadth between these two groups (Lázaro et al. 2021). Based on Pearson's correlation analysis showed that there is no correlation between wild bee species richness with the number of flowers ($r: 0.014 p 0.670 > 0.05$). Most wild bees are polylectic and thus can easily shift their foraging in the presence of honeybees (Valido et al. 2019).

However, competition can still occur even where there has been a shift in foraging behavior if food resources are limited, as wild bees may be driven to low-quality food sources that are insufficient for their growth (McCune et al. 2020). Renner et al. (2021) suggested that the presence of managed honeybees does not affect wild bee species diversity. However, Lindström et al. (2016) found that the introduction of *A. mellifera* colonies led to declines in wild bee populations and diversity in green mustard (*Brassica rapa*) fields. Ropars et al. (2019) found that honeybees affected wild bee populations in urban habitats. Research from the United States found an overall decrease in fruit production when *A. mellifera* colonies were introduced to agricultural fields, suggesting suppression of wild bee populations and thus their pollination services (Angelella et al. 2021). These varied results likely reflect the overall availability of floral food resources among study areas, where negative effects on wild bee populations are more likely to occur when resources are limited (Garibaldi et al. 2021). In our study, flower number and mean flower cover were positively correlated with bee species richness and abundance in all treatments and all three flowering periods. This is logical, given that sites with greater flower cover than the surrounding area typically attract greater numbers of bee species and individuals (Kratschmer et al. 2019, 2021).

Table 1. Species richness and abundance of wild bee species across three treatments and four crop types

Wild bee species	<i>Apis cerana</i>				<i>Apis mellifera</i>				Control				%	
	TM	GB	CH	GM	TM	GB	CH	GM	TM	GB	CH	GM		
<i>Xylocopa confusa</i>	4	22	0	0	5	31	0	0	6	30	2	0	100	16.3
<i>Xylocopa latipes</i>	4	22	0	0	4	20	0	0	6	25	2	0	83	5
<i>Xylocopa caerulea</i>	4	21	0	0	5	21	1	0	3	26	2	0	83	5
<i>Amegilla cyrtandrae</i>	31	22	13	14	34	26	10	14	46	31	38	41	320	19
<i>Amegilla burneensis</i>	30	23	15	17	30	21	18	17	44	24	35	34	308	18.8
<i>Megachile conjuncta</i>	4	5	14	16	4	0	13	16	15	2	7	48	144	8.8
<i>Megachile fulvifrons</i>	6	0	13	16	6	0	10	16	14	6	11	34	132	8.9
<i>Ceratina cognata</i>	8	0	11	20	8	0	13	20	8	0	15	28	131	8.03
<i>Lasioglossum malachurum</i>	7	0	14	24	7	0	12	24	4	2	9	36	139	8.5
<i>Lasioglossum leucozonium</i>	8	0	10	18	8	0	16	18	8	6	8	65	165	10.04
<i>Nomia quadridentata</i>	2	0	3	4	2	0	4	4	2	0	2	2	25	0.53
Species richness	11	6	8	8	11	5	9	8	11	9	11	8	1630	100
No. individuals	108	115	93	129	113	119	97	129	156	152	131	288		
Dominance_D	0.18	0.185	0.14	0.139	0.184	0.2061	0.1359	0.139	0.1932	0.1653	0.1879	0.152		
Simpson_1-D	0.82	0.815	0.86	0.861	0.816	0.7939	0.8641	0.861	0.8068	0.8347	0.8121	0.848		
Shannon_H	2	1.718	2.02	2.01	2	1.595	2.054	2.01	1.94	1.904	1.934	1.938		
Evenness_e^H/S	0.67	0.929	0.94	0.933	0.672	0.9854	0.8667	0.9331	0.6324	0.7459	0.6291	0.8678		

Note: TM: tomato; GB: green bean; CH: chili; and GM: green mustard. (n: 30/ plots)

Wild bee species richness among crop plants

The relative abundance of wild bees varied among the four crop species and three treatments (Table 2). *Xylocopa caerulea* and *Amegilla cyrtandrae* were dominant on tomatoes. *Xylocopa confusa*, *Xylocopa latipes*, *X. caerulea*, *Amegilla burneensis*, *Ceratina cognata*, and *Nomia quadridentata* were never observed on green bean plants. *Amegilla cyrtandrae* and *A. burneensis* were the dominant species on chili plants. Finally, the most abundant species on green mustard plants was *Lasioglossum leucozonium*, and species of *Xylocopa* were never found on this crop. *C. cognata* was also abundant on green mustard (Table 2).

There was evidence of a pattern between wild bee visitation and crop type. Specifically, species of *Xylocopa* tended to only visit tomatoes and green beans. Carpenter bees are known to visit green bean plants (Widhiono et al. 2016; Mainkete et al. 2019). It is probable that these bees extract pollen from tomato plants by buzzing (Kesar 2010). Buzzing pollination is a pollination process where the release of pollen from the anther requires vibration and sonification mechanisms. The genera of bees that are capable of buzzing include: *Halictus*, *Megachile*, *Bombus* and *Xylocopa* (Tayal and Kariyat 2021).

Wild bee abundance

The introduction of managed honeybee colonies negatively affected the total abundance of wild bees ($F_{7,21} p < 0.05$). Based on Pearson's correlation analysis showed that wild bee abundance correlate with numbers of flowers ($r: 0.57 p: 0.047 < 0.05$). In total, 445 wild bee individuals were found in the *A. cerana* plots, 458 in the *A. mellifera* plots, and 727 in the control plots. In terms of relative abundance, *A. cyrtandrae* and *A. burneensis*, two blue-banded bee species, dominated (19% and 18.8%, respectively), whereas *N. quadridentata* was the least abundant species (0.5%). The high relative abundance of the two blue-banded bee species likely reflects their abundance in the study area. Both species are broad

generalists that are widely distributed throughout the tropics (Engel 2007), and both were observed visiting all four crop species. These findings reflect those of (Kumar et al. 2017), who found that blue-banded bees visited *Solanum melongena* (eggplant), *Capsicum frutescens* (Chili), and *Solanum lycopersicum* (tomatoes). Notably, many solanaceous crops are dependent on insect pollinators for sufficient yield (Kumar et al. 2017).

The second most abundant genus in our study was *Xylocopa*, represented by three species, *X. confusa*, *X. latipes*, and *X. caerulea*. Of these, *X. confusa* was the most abundant (relative abundance of 16.7%) and the two remaining species were found at a relative abundance of approximately 5%. All three species were most abundant on green beans. Members of the genus *Xylocopa* are large carpenter bees that typically have broad geographic ranges (Sadeh et al. 2007). They forage on a wide range of food plants, have a long period of seasonal activity, tolerate high temperatures, and show activity under low light levels (Fohouo et al. 2014; Azo'o et al. 2020). These traits make them attractive candidates for agricultural pollination in hot climates, particularly in greenhouses, and of night-blooming crops (Kesar 2010). Carpenter bees are efficient pollinators of tomato (Indraswari et al. 2016), *Vigna unguiculata* (long bean) (Mainkete et al. 2019), *Cajanus cajan* (pigeon pea) (Mireille et al. 2012), and *Cucumis sativus* (cucumber) (Hashifah et al. 2020).

The third most abundant genus was *Lasioglossum*, represented by *L. leucozonium* and *L. malachurum*, with relative abundances of 10.04% and 8.5%, respectively. Both species are common in the study area, likely due to the availability of Asteraceae and Euphorbiaceae plants and the suitability of the soil for nesting (Polidori et al. 2010). Both species were found primarily on chili, green mustard, and tomato plants, similar to other research results (Rodrigo-Gómez et al. 2016). Members of the Halictidae (sweat bees), to which *Lasioglossum* belongs, are efficient pollinators of crops worldwide (Murao et al. 2017).

Table 2. The abundance of 11 wild bee species on four crop species

Wild bee species	TM		GB		CH		GM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<i>Xylocopa confusa</i>	4.66	1.15	27.66	4.93	0.66	1.15	0	0
<i>Xylocopa latipes</i>	4	1	22.33	2.51	0.66	1.15	0	0
<i>Xylocopa caerulea</i>	37	7.93	22.66	2.88	1	1	0	0
<i>Amegilla cyrtandrae</i>	34.66	8.08	26.33	4.50	20.33	15.37	23	15.58
<i>Amegilla burneensis</i>	7.66	6.35	22.666	1.52	22.66	10.78	22.66	9.81
<i>Megachile conjuncta</i>	8.66	4.61	2.33	2.51	11.33	3.78	26.66	18.47
<i>Megachile fulvifrons</i>	8	0	2	3.46	11.33	1.52	22	10.39
<i>Ceratina cognata</i>	6	1.73	0	0	13	2	22.66	4.61
<i>Lasioglossum malachurum</i>	8	0	0.66	1.15	11.66	2.51	28	6.92
<i>Lasioglossum leucozonium</i>	2	0	2	3.46	11.33	4.16	33.66	27.13
<i>Nomia quadridentata</i>	2	0	0	0	3	1	3.33	1.15

Note: TM: tomato; GB: green bean; CH: chili; GM: green mustard; SD: standard deviation

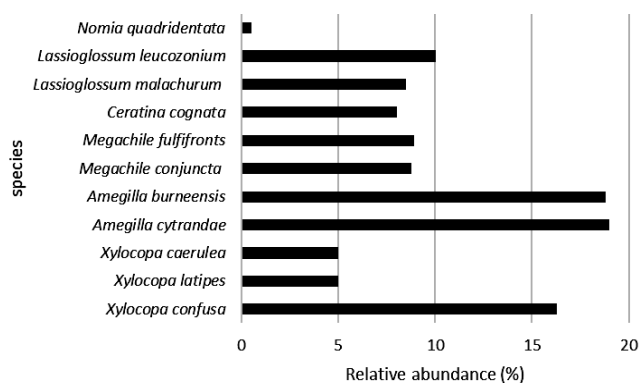


Figure 2. Relative abundance of 11 wild bee species on crop species in Mount Slamet, Central Java, Indonesia

Finally, members of the family Megachilidae (leaf-cutter bees) were abundant in our study area, represented by *Megachile relativa* (8.9%) and *M. centuncularis* (8.8%). Both species were abundant in chili and green mustard plants. This is due to the color and nectar and pollen resources of these crops (Soroka et al. 2000; Kambli et al. 2017). Leaf-cutter bees are pollinators of the Malvaceae (Ali et al. 2019). The high abundance of *Megachile* bees in our study area may also be due to the availability of suitable food resources and nesting sites (Pitts-Singer and Bosch 2010).

Flower abundance and wild bee abundance

There was variation in wild bee abundance across the three flowering periods (F 7.21 p < 0.05), with peak abundance during the middle of the flowering period and the lowest abundance during the end of the flowering period (Figure 3). Ultimately, our results indicated that wild bee abundance declined in the presence of managed honeybees and with decreased flower resources. This decline was likely due to insufficient food resources, potentially due to competition between honeybees and wild bees (Clos et al. 2020; Urbanowicz et al. 2020; Kratschmer et al. 2021).

In conclusion, the introduction of managed honeybee colonies affects the abundance, but not diversity, of wild bees in agricultural settings, especially during the end of the flowering period. Thus, we suggest that where crop pollination is augmented with managed colonies, that these colonies are introduced during the onset and middle of the flowering period to reduce pressure on wild bee populations during a period of resource limitation and the end of the flowering period.

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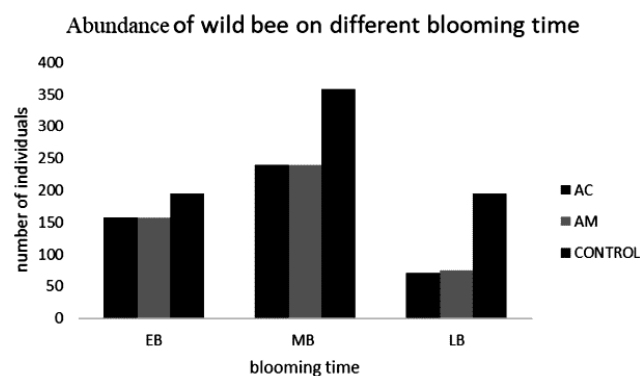


Figure 3. Wild bee abundance during three flowering periods, early blooming (EB), middle blooming (MB), and late blooming (LB), among three treatments. AC: *Apis cerana*, AM: *Apis mellifera*

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