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Pollinator and host plant interaction: association between insect pollinators on *Ficus deltoidea* Jack Plants. (Moraceae) and *Bidens pilosa* linn. (Asteraceae)

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Abstract. This study aims to explore the association between Fig wasp (Hymenoptera) and *Ficus deltoidea* (Moraceae), as well as Bidens flies (Diptera) with *Bidens pilosa* (Asteraceae). A survey method was used to observe the fruiting and flowering stages of *F. deltoidea* and *B. pilosa*, collecting samples daily to track pollinator emergence. The frequency of emerging pollinators was calculated and their sex ratio was determined. The data analysis was carried out descriptively. *Ficus* provides a place and protection for the eggs of wasps to hatch and develop into larvae to imago, which naturally within *Ficus* fruits. The female wasps role as a pollinator for the *Ficus* flower. This interaction is known as mutualistic symbiosis. Bidens flies lay eggs in the floret discs of *B. pilosa* after the pollination stage. The sex ratio of emerging bidens flies is male(♂) : female(♀) = 1:1. The interaction between Bidens flies and *B. pilosa* is an example of commensalism interaction, where *B. pilosa* benefits Bidens flies without being affected itself. The associations between *Ficus*–Fig wasps and Bidens flies–*B. pilosa* are specific to open ecosystems. The interdependence of these insects with their host plants serves as a strategic approach for ensuring successful reproduction and survival.

1 Introduction

The interactions between organisms in nature that give rise to mutually beneficial associations are recognized as symbiosis [1]. This association is established through the process of co-evolution between distinct organisms, where at least one organism becomes dependent on others within its life history [2]. Symbiotic mutualism is characterized by mutually advantageous interactions between two different species. This interaction plays a crucial role in sustaining their life cycles. Symbiotic relationships are classified into various categories depending on whether the symbiont has beneficial, harmful, or neutral effects on its host [3]. Beyond the previously discussed symbiotic mutualism, another type of symbiotic relationship involves two different species, but only one species benefits. In this case, the symbiont utilizes its host without causing any discernible benefit or detriment, constituting a commensal association.

A classic example of symbiotic mutualism is the association between flowering plants and their pollinators. In this relationship, plants act as hosts, providing resources, while symbionts consume these resources and, in turn, offer valuable services [4]. Plants and pollinators co-evolved, establishing a reciprocal association that yields mutual advantages. Amidst this interdependence, plants face challenges from their interactions with opportunistic organisms seeking sustenance and survival. Nevertheless, plants are adapted with mutual organisms for a fair give-and-take dynamic of interactions [5]. There are many studies regarding mutualistic symbiotics involving insects and specific or general host plants, regarding co-diversification, co-speciation, and co-evolution [6,7]. Among the pollinators and host plant interactions studied for a long time is the interaction between figs (*Ficus*; Moraceae) and their pollinating Fig wasps [8,9].

Comprehensive research was conducted on the pollination ecology of the mutualistic relationships between *Bidens pilosa* L. (Asteraceae) and various insect pollinators [10]. *B. pilosa*, an herbaceous plant, is abundantly found in diverse habitats including open land, roadsides, bush areas in forests, gardens, and other locations. It exhibits strong adaptability to flourish in tropical and subtropical climates [10,11]. Interestingly, this plant has a notable history of extensive interaction with several insects, believed to serve as its pollinators. However, in terms of morphology, its reproductive system generates heterogamous capitula housing sterile flower florets, alongside fertile disc florets that possess self-compatibility, enabling the process of self-pollination [10,12]. Consequently, the reliance on insects assumed to be pollinators is not the only determinant of pollination success. Notably, the Corolla tube of the *B. pilosa* flower produces nectar abundant in sucrose, amino acids, and pollen grains that indicate entomophiles [13]. Several insects, such as bees, wasps, butterflies, thrips, and flies, are known to have established associations with *B. pilosa* [10]. Notably, some of these insects, including thrips and bees, function as pollinators, particularly at the initial stages of the disc florets. The presence of fly species at specific flower stages of *B. pilosa* suggests potential mutualistic interactions. However, extensive research on the relationship between Bidens flies and *B. pilosa* has not provided clarity regarding their mutualistic interactions.

Exploring the confirmation of particular interactions between pollinator insect associations and host plants is an interesting study to investigate. Based on this, a combination of exploration and laboratory-scale research was undertaken to confirm mutualistic interactions and to find out the specific relationship between Fig wasps (Hymenoptera) and *F. deltoidea* (Moraceae), alongside the interaction between Bidens flies (Diptera) and *B. pilosa* (Asteraceae). The association between these pollinating insects and their host plants represents a crucial survival strategy for the preservation of life forms. This study holds significant importance in the realm of biodiversity, with a primary focus on fostering sustainable nature conservation.

2 Methods

This research is exploratory research with sampling techniques using survey methods and observations carried out on a laboratory scale. The objects studied were fruit *F. deltoidea* and flowers *B. pilosa* encompassed the fruiting and flowering stages, in which the appearance of pollinators was observed daily.

The object research sampling is from a natural forest located at the peak area of Mount Tangkuban Parahu (Figure 1), North Bandung City, at an altitude exceeding 700m above sea level. The area is predominantly populated by *Ficus* plants (Figure 2), which is where the *F. deltoidea* species is prominent.

(a)

Fig. 1. Sampling location [14]


(b)

Fig. 2. *Ficus deltoidea* [15]

2.1 Collection and observation of the emergence of Fig Wasp from *Ficus* fruit

The subject of observation consisted of ripe *F. deltoidea* fruit, characterized by a reddish color. Samples were systematically collected from 3 transects, with each transect yielding 100 *Ficus* fruits. Consequently, the cumulative sample size reached 300 *Ficus* fruits. Each *Ficus* fruit is enclosed in a transparent tube sealed with cotton at both ends. Subsequently, daily observations were conducted to monitor the emergence of Fig wasps including their sex type. Once no further wasps were observed, the *Ficus* fruit was dissected to examine the presence of remaining wasps and also determine their sex type.

2.2 Collection and observation of the emergence of Bidens flies from the flowering and fruiting stages of *Bidens Pilosa*

The flowering and fruiting stages of *Bidens pilosa* were obtained from the open ecosystem of the UNPAD Arboretum and *B. pilosa* in the polybags was sourced from the enclosed area of the PAU-ITB greenhouse (Figure 3). Each sample was placed in a container and covered with gauze. Daily observations were conducted to monitor the emergence and sex ratio of Bidens flies or other insects that could potentially emerge from the samples. A total of 1000 samples were collected for each stage of both flowers and fruits, namely the open ecosystem and the enclosed ecosystem.

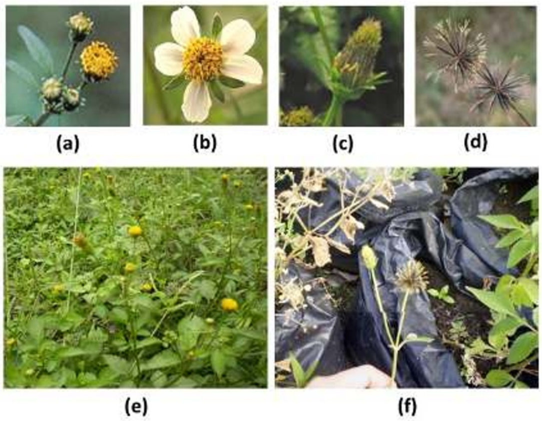

Fig. 3. Collected samples from flowering and fruiting stages of *B. pilosa*, and representatives of two distinct ecosystem types of *B. pilosa* habitats : **(a)** Flowers in the pre-pollination bud stage, **(b)** blooming flowers post-pollination, **(c)** immature fruits emerge after corolla detachment, **(d)** the blackened mature fruit that is dry-brittle, **(e)** the open ecosystem, (f) the enclosed ecosystem.

2.3 Data Analysis

The frequency of emerging pollinators was calculated and their sex ratio was determined. The data analysis was carried out descriptively.

3 Result and Discussion

3.1 Association Between Fig Wasp and *Ficus deltoidea*

The observation results are shown in Table 1 and Figure 4. The total number of Fig wasps emerging from 300 fruit samples across three transects was 2433 individuals. The emergence Relative Frequency (RF) was 1.82%, peaking at the highest Importance Value Index (IVI) at 2.42% on the first day and steadily declining to 0.05% by the third day. Imago's emergence ceased entirely on the fourth day. Post the third day, larvae appeared, but their survival was limited to the pupal stage, with subsequent pupal mortality, indicating the exclusive survival and longevity of the imago stage outside the fruit. Notably, all emerged Fig wasps were female, and upon dissecting the *Ficus* fruit, a deceased male imago was identified.

Table 1. The number population and frequency rate of Fig wasp imago emerging from the collected *Ficus* fruit of third research transects

Transect	Emergence of Fig wasp (individual)*			Number Population	RF (%)	RD (%)	IVI (%)
	Day 1	Day 2	Day 3				
I	1168	252	53	1473	1.82	0.6	2.42
II	856	65	9	930	1.15	0.38	1.53
III	11	14	5	30	0.04	0.01	0.05
	2035	331	67	2433			

*Emerged from 100 samples of *F.deltoidea* fruits of each transect, with 300 total collected samples. RF=Relative Frequency; RD=Relative Density; IVI=Importance Value Index

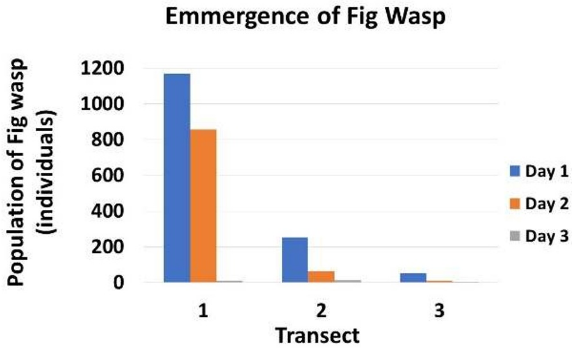

Fig. 4. Pattern of emergence of Fig wasp imago during the observation days.

Based on observations and literature, the Fig wasp and *F. deltoidea* exhibit a symbiotic mutualistic interaction [6,8,16,17]. This interaction, shaped by their co-evolution, involves specific strategies for reproductive success. *F. deltoidea* exclusively interacts with a particular wasp type crucial for pollination success, and reciprocally, the wasp only deposits eggs in *F. deltoidea*, ignoring other *Ficus* species present in the observation area where no interactions were observed. The interaction mechanism between *F. deltoidea* and Fig wasp is illustrated in Figure 5.

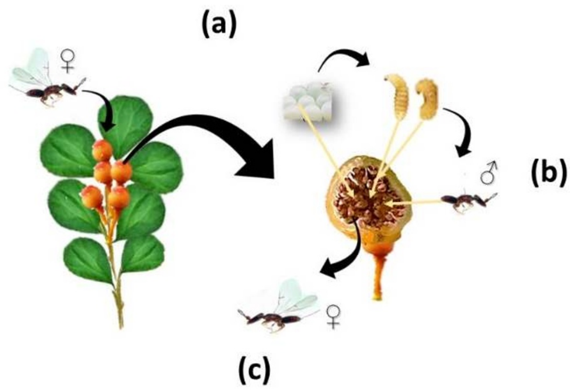

Fig. 5. Mechanism mutualistic interaction between *F. deltoidea* and Fig wasp : **(a)** The ostiole holes in mature female flowers open, then the oviposition and pollination occur by female Fig wasp when invading the female flowers, **(b)** Eggs hatch into larvae until the pupation of Fig wasp develops during the Interflora flower phase, male and female Fig wasps emerge and mate inner the Interflora, **(c)** When the male flower matures the adult female wasp is inseminated by the

male emerging out of the fruit while the male dies after mating

The presence of *Ficus* flowers and fruit significantly contributes to the survival of the Fig wasp. The developmental stages of fig larvae, progressing through pupae, and imago, occur naturally within *Ficus* flowers and fruit. The adult female wasp gathers pollen from male *Ficus* flowers and subsequently finds a shelter on new *Ficus* fruit for oviposition, aiding in the pollination of invaded female flowers. *Ficus* flowers or fruit that have ripened and undergone wasp-assisted pollination attract fruit-eating animals, which, in turn, play a pivotal role in the dispersal process of *Ficus* seeds [8,18].

The co-evolutionary process between the Fig wasp and *Ficus* is substantiated by diverse morphological and physiological adaptations exhibited by both organisms [19], encompassing: (1) The structural design of the false fruit facilitating the wasp's ingress into the inflorescence, (2) Distinct adaptations aligned with the regeneration timeline of the fig wasp, characterized by the extreme protogyny of female flowers occurring several weeks prior to the pollen production by male flowers, (3) Inflorescence conditions conducive to Fig wasp oviposition, featuring both stalked and unstalked inflorescences with short and long styles to facilitate seed deposition, (4) Specialized morphological attributes in male and female Fig wasps, including the female's body morphology adapted for ostiole entry and possession of pollen receptacles, while the male fig wasp exhibits wingless morphology with an elongated abdomen to facilitate mating within the fruit, and (5) Specific behaviours associated with the collection and release of pollen.

This mutualistic interaction relationship exclusively manifests in natural conditions and is absent in semi-natural conditions or under human intervention. According to existing literature, ornamental *Ficus*, cultivated by humans in the form of ornamental plants, can solely propagate through cuttings, as natural reproduction is precluded [20]. This limitation arises from the absence of Fig wasps in ornamental *Ficus* plants, preventing assistance in the pollination process, and thereby hindering sexual reproduction.

3.2 Association Between Bidens Flies and *Bidens pilosa*

Based on the data analyzed recorded in Table 2 it is known that the relative frequency of appearance of Bidens flies is highest in type (b), followed by type (c). Type (d), the mature fruit of *B. pilosa*, is the lowest frequency of emergence of Bidens flies.

Table 2. The number population, frequency, and sex ratio of bidens flies imago emerging from the collected flowers and fruits *B. pilosa* from an open ecosystem

Flowering/ Fruiting Type	Emergence of Biden Flies*			RF (%)	RD (%)	IVI (%)
	Male ♂	Female ♀	Number Population			
Type (a)	0	0	0	0	0	0
Type (b)	12	12	24	2.18	0.55	2.73
Type (c)	9	10	19	1.73	0.43	2.16
Type (d)	1	0	1	0.09	0.02	0.11
Population*	22	22	44	0.01		

*Emerged from 1000 samples of flowers and fruits of *B. pilosa* for each type, with 4000 total collected samples. RF=Relative Frequency ; RD=Relative Density ; IVI=Importance Value Index

The detailed emergence of Bidens flies, derived from specimens of *B. pilosa* flower and fruit morphotypes gathered in open ecosystems, is shown in Figure 6.

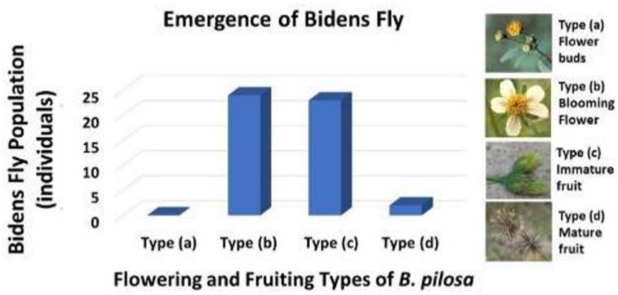

Fig. 6. The emergence of Bidens flies imago on various types of *Bidens pilosa* flowers and fruits.

In type (a) bud stage, there are no Bidens flies were emerge. It confirmed that Bidens fies has not yet invested its eggs in the stage of bud flower of *B. pilosa*. Bidens flies emerge on type (b) blooming flowers post-pollination, they emerge in type (c) immature fruits, and type (d) the mature fruit [10]. This confirms that Bidens flies are detected after the flower has been pollinated.

Based on emerging imago, it is known that the sex ratio between male : female = 1 : 1. Morphological distinctions between male and female Bidens flies are evident in their body size and the shape of the abdominal tip. Male flies exhibit comparatively smaller and slender bodies, with a rounded tip at the end of the abdomen. Conversely, females possess a tapered abdomen with an elongated ovipositor at the abdominal tip (Figure 7)

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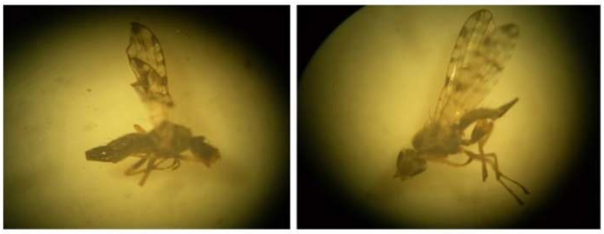

Fig. 7. The Bidens flies imago (400Ч) (a) Male (♂), (b) Female (♀)

Consequently, Bidens flies are not significantly involved in pollination and the developmental processes of the *B. pilosa* flowers stage. The transition of flowers into fruits, yielding seeds, occurs independently of Biden's flies intervention. In contrast, Bidens flies consistently appear throughout the various stages of flower and fruit development in *B. pilosa*, persisting even in the last mature stage of fruits following pollination. A comprehensive assessment of the imago population reveals a balanced distribution of male and female specimens, vital for the continuous reproduction essential to the Bidens fly generation [21]. Thus, the role of *B. pilosa* is pivotal in ensuring the successful survival and reproduction of Bidens flies.

The relationship between the Bidens flies and *B. pilosa* distinctly favors one organism, specifically the Bidens flies, which deposits its eggs in *B. pilosa* for the development of its offspring. This represents a strategic approach employed by Bidens flies to ensure the continued survival and presence of their progeny in the natural environment. For *B. pilosa* itself, the presence of Bidens flies neither cause harm nor confers benefits. These interactions are classified as commensalism interactions [22, 23].

In an enclosed ecosystem, specifically *B. pilosa* cultivated in polybags within a greenhouse, observations revealed an absence of flies emerging from all collected samples, including both fruits and flowers. This underscores that the association between Bidens flies and *Bidens* plants exclusively occurs in natural conditions. The interaction between *B. pilosa* plants and Bidens flies in plant associations is characterized as commensalism interactions. Consequently, the absence of Bidens flies in a closed ecosystem has no discernible impact on the reproductive success or seed dispersal of *B. pilosa* plants. Further investigation is warranted to ascertain the presence of natural pollinators for these plants in enclosed ecosystems. This is crucial, as the reproductive success of the *B. pilosa* plant is contingent upon the existence of its pollinators.

4 Conclusion

The association between Fig wasps and *F. deltoidea* in an open ecosystem demonstrates mutualism interaction, with Fig wasps as pollinators and *F. deltoidea* as a host plant to provide shelter and food resources for the growth and development of Fig wasps. This is known as mutualistic symbiosis. Similarly, the interaction between Bidens flies and *B. pilosa* highlights the crucial role of *B. pilosa* as an egg deposition site, supporting the growth and development of larvae into the reproductive imago phase. The presence of Bidens flies doesn't impact *B. pilosa* plants positively or negatively, making it a commensalism interaction. Combining these insects with their host plants is a strategy for ensuring survival and reproductive success.

The interaction between symbionts and hosts in mutualistic associations and commensalism holds a specific and vital role in determining the survival of individuals within natural ecosystems. Over time, this interaction has led to co-evolution and mutual adaptation, fostering ecological balance in nature. For instance, pollinator insects like the Fig wasp provide essential benefits for *Ficus* plants, contributing significantly to natural forest ecosystems and resources. Similarly, the Bidens flies rely on the *B. pilosa* plant for its existence, even though the plant doesn't receive direct benefits in return. This emphasizes the unique roles each species plays in ensuring the survival of others, maintaining the continuity of the food chain, and overall species sustainability.

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