



Hymenopteran pollinator activity and abundance on *Vicia faba* and *Passiflora miniata* in regenerating segments of the Amazonian basin in Madre de Dios, Peru

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Abstract

The huge diversity of the Amazon rainforest is currently under threat by human-induced deforestation and fragmentation. These processes are creating new portions of regenerating forest, which show potential to be converted back to apex tropical forest.

Pollinators are highly useful species in recruiting new flora to an area, so their services can offer an enhancement of natural forest succession. From this concept, the study to assess pollinator and other insect abundance in regenerating tropical forest on two floral species, *Passiflora miniata*, a native vine species and *Vicia faba*, a non-native legume species, was conducted. Study was done in Madre de Dios, Peru in the late dry season of 2022. *Passiflora miniata* was found to attract bees for significantly longer than *V. faba*. From this, it is suggested that *P. miniata* over *V. faba* should be planted to increase pollinator and insect recruitment to the area, allowing the effective catering of conservation efforts to aid successional completion.

Introduction

The Amazonian region of Madre de Dios, South-West Peru, is known for its dense cover of tropical forests, as well as its remarkable biodiversity of flora and fauna. It is believed that over 760 faunal species are endemic to the area.¹ However, the last 50 years have seen a considerable increase in deforestation, around 28.3kha in Madre de Dios alone in the last year, for economic activities such as agriculture, logging and gold mining.^{2,3,4,5} With deforestation, significant areas of the region have begun succession after abandonment, creating

regenerating forest, with slow succession back to the previous apex forest state.

With regenerating forests becoming more common habitats, and conservationists seeing these areas as viable for restoration, the need to understand what interactions are driving fast succession and ecosystem recovery is crucial to the conservation process.^{6,7} This is important economically and environmentally, as it can allow efficient restoration of the Amazon's fine balance, as well as allowing people to extract resources from the forest sustainably.⁸

The order Hymenoptera, including wasps, ants and bees, is one of the most diverse and successful insect orders and includes many necessary pollinators. With habitat fragmentation and deforestation, bees and wasps have seen reductions in their habitat range, biodiversity and abundance.⁹ However, bees, (and to some extent wasps) are vital to the seedling recruitment of new floral diversity in a plethora of habitats, with regenerating forest being no exception.⁸ With this habitat seeing high past disturbance, and therefore a disruption of plant-pollinator interactions, it is important to gain information on how these interactions function in this new ecosystem, and how this can be used to ensure the succession process is effective.

In this study, two common plant species found in the regenerating forests of Finca las Piedras, Madre de Dios, were observed to compare their pollinator abundance and visitation, as well as their foraging ecology whilst visiting. The two compared were *Passiflora miniata*, a fast-growing vine native to South America from the family *Passifloraceae* and *Vicia faba*, a non-native legume species from the family *Fabaceae*, that produces the common broad bean. (See figure 1)

The abundance of other insects present on both plant species were also recorded, as to measure the overall attraction value of each plant species to insects. With this information, it is possible to determine which plant is attracting the widest range of species, which is drawing in higher pollinator numbers for longer, and- in turn- which will

encourage recruitment of pollinators to regenerating forest areas.



Figure 1: The flowers of *Vicia faba* (left) and *Passiflora miniata* (right).

Methods

Select a plant to observe

The study was conducted in the grounds of Finca las Piedras, Peru, in regenerating forest originally disrupted by a large-scale fire in 2017. The area was surveyed for both *Vicia faba* and *Passiflora miniata* before data collection began. The area was divided into three distinct regions: the laundry area, the road leading to Monterrey, a local village, and the entrance to the Castana forest trail. Time of day was also recorded for each observation. Each region was visited once a day during data collection, and plants in each were randomly selected and observed. Observations lasted for 10 minutes, with any pollinator interaction in this time period recorded using the method described in 'Recording plant-pollinator interactions.'

Initial estimate of pollinator visitation rates

After selecting a plant, an ongoing insect count was performed in the regions of the plant observable to the onlooker. A tally of the number of insects in the direct vicinity of the plant at any time in the ten-minute observation should be done, with the recorded insect types being split into wasps, bees, and miscellaneous insects such as ants, flies or beetles. Only those having some form of interaction with the plant should be counted in these tallies, as those flying around the area cannot be considered.

Recording plant-pollinator interactions

Only pollinators in the direct vicinity of the selected plant were included in data collection. The pollinator was identified broadly by whether it was a bee or wasp, as unreliable identification data is available for this region. Any interaction with the plant, including landing on it to preen, mating rituals, resource acquisition, nesting and predation attempts in the plant's vicinity were also recorded if noticed. Observations of each insect interacting with the study plant were recorded in their entirety during the 10-minute period by a stopwatch, noting the time spent actively pollinating or non-pollinating.

Data analysis

R-studio version 4.0.3 was used in data analysis. The insect count data was grouped into pollinator (wasps and bees) and non-pollinator (anything else seen) visitors for both *Vicia faba* and *Passiflora miniata*, and then analysed by a Wilcoxon test to compare visitation rates of the two categories at both

plant species. Visitor pollination proportion was calculated by adding the active pollination time with the non-pollination time and then dividing the pollination time by this sum for all observed pollinators. Pollination time was compared between the two plant species via a Wilcoxon test, as the data was not normal. An ANOVA was also conducted to determine if time of day and location were significant factors in pollination time.

Results

Although wasps were observed in this study, the sample size (N = 16) was deemed insufficient for comparison, so was not included in analysis. The overall number of bees visiting *P. miniata* and *V. faba* did not differ significantly. (Wilcoxon test; W = 1435, p-value = 0.5727, N = 164) Neither did the overall visitation by miscellaneous insects to *P. miniata* or *V. faba*. (Wilcoxon test; W = 1209, p-value = 0.3516, N = 695). Observed insects in the miscellaneous category included individuals from the orders Orthoptera, Hymenoptera, Hemiptera, Diptera and Lepidoptera. The total pollination time by bees was significantly higher at *P. miniata* than it was at *V. faba*, with a mean of 126.7 seconds (SD = 161.29) at *P. miniata* compared to 65.5 at *V. faba*- see figure 3. (SD = 101.98). (Wilcoxon Test; W = 3966.5, p < 0.05, N = 71) The time of day also significantly affected the length of time bees spent pollinating- see figure 3. (ANOVA; F = 10.03, p = 0.00184, N = 164

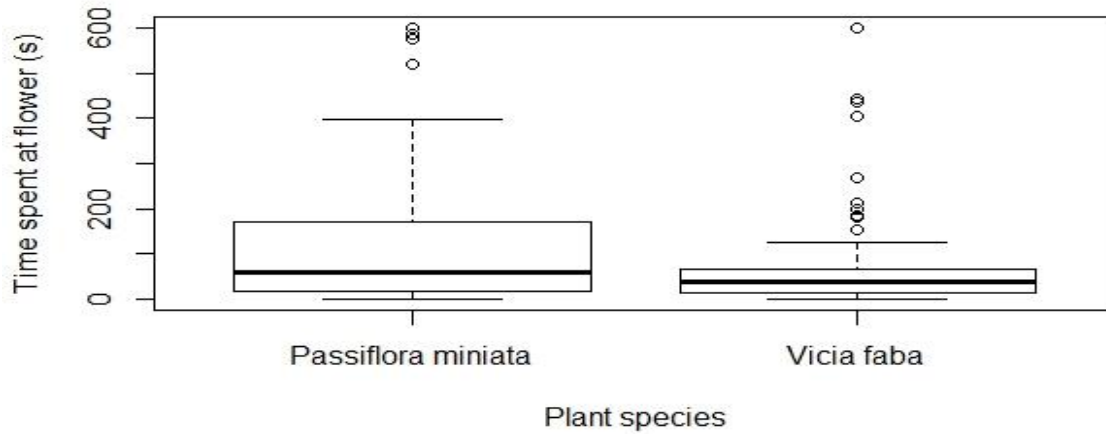


Figure 2: The distribution of times, in seconds, bees spent pollinating at the flowers of *P. miniata* and *V. faba*, respectively. This depiction excludes non-pollinating time.

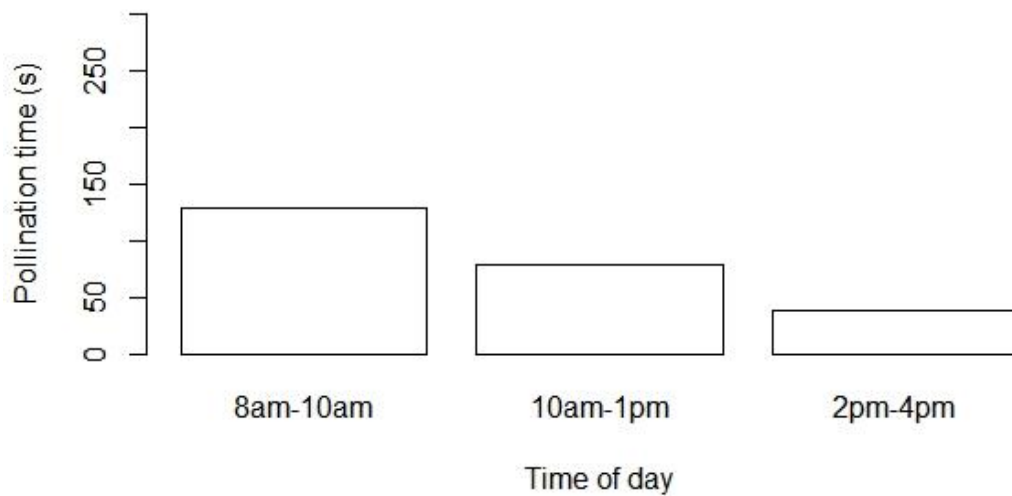


Figure 3: The mean pollination time observed for bees seen at three time periods within the day. All three periods are within peak pollination time.

Discussion

Visitation by wasps

During the duration of this study, only 16 wasps were recorded, and 6 of these were

seen to be pollinating. Because of a lack of wasp observations in the field, analysis of the data was ceased. However, the observation of any pollination carried out by wasps is interesting, as although uncommon, there is some prevalence of nectar collection by wasps. This suggests that although wasps are

not a primary pollinator of *P. miniata* or *V. faba*, they are seen to pollinate these species, albeit less commonly and at reduced efficiency to bees.

The presence of wasps was mainly on *V. faba*, with $\frac{2}{3}$ of the interactions being seen on them over *P. miniata*. Although these floral species are attracting wasps to some extent, their presence is unlikely to promote large recruitment of wasps to regenerating areas. It would be sensible to study the landscape features that do attract wasps to regenerating areas, as they could also help to promote successful seedling germination via parasitism and predation of associated herbivores.^{10,11}

Visitation by bees

The lack of difference between bee visitation rates to *V. faba* and *P. miniata* was noted within this study, with almost identical means of 1.04 and 1.15, respectively. The lack of difference suggests that a majority of species visiting both plants were generalists and will visit a wide range of species when pollinating. The equal visitation also suggests that both species have the ability to attract bees, which could promote the planting of either species within regenerating areas as to increase pollinator visitation.

Although this is true, the time individual bees pollinated *P. miniata* was significantly higher than *V. faba*, implying that although bees will visit both species readily, they have a preference for *P. miniata*. This is interesting, as *P. miniata* is a native species commonly found in the neotropics, whereas *V. faba* is non-native, likely introduced through

agricultural practices. The preference of native bees to native flora has long been described in the neotropics, so this finding would fit the rhetoric.^{12,13}

Another viable reason for bee preference to *P. miniata* could be the heightened floral rewards available at these flowers compared to *V. faba*. Since the legume is not in its native range, climate conditions and soil properties will differ to its normal range of the Mediterranean and Asia. Therefore, it may have to set aside further resources for survival than *P. miniata*, reducing its capability to invest in costly rewards such as nectar or pollen.¹⁴ This, however, would have to be tested by quantifying the floral rewards produced by each plant species.

Visitation by other insects

There was no difference seen between the number of miscellaneous insects on *P. miniata* or *V. faba*. It could be noted at both species that there was a dominance by ants, with upwards of 7 species observed on both plants over the observation period. This could be for a variety of reasons: foraging for nectar, defending territory near a nest, maintenance of a symbiosis, or just pure coincidence. The high presence of ants may not be explicable by the results of this study, but it points to the benefit of flowering plants in attracting ants. Many studies have been conducted involving the interaction of plants and ants, with many finding mutualisms between different species and their ability to influence the species pool around them.^{15,16} Therefore, the attraction of native ants could be highly important in re-establishing the lost ecosystem interactions that existed before the

disturbance. From this, it can be theorised that flowering plants in regenerating forest may generally attract more ants to an area, so to draw species like these in, specificity of plant might not matter as much as with pollinators. This theory- however- would have to be further explored to corroborate it.

Activities of coleopteran, hymenopteran, and orthopteran species on P. miniata

A wide range of insects other than bees and wasps were observed in this study, with behaviours ranging from resting to feeding. Coleopteran, (beetles) hymenopteran (ants) and orthopteran (crickets) species were seen to directly feed from the nectar of *P. miniata*. This is highly interesting, as it further highlights *P. miniata*'s attraction value to a range of species. A lack of these insect guests was seen in association with *V. faba*, which further suggests that *P. miniata* is more attractive to species that inhabit grasslands and forested areas.

The fact that *P. miniata* is a native species may also influence its attraction value to these insects, as they may show preference to inhabit native species as bees do. Although this is possible, it could be related to the comparative ease of nectar extraction at *P. miniata* than *V. faba*. *P. miniata* offers a large landing pad for nectar access, allowing larger, non-hovering species such as crickets to successfully feed on nectar. This is in opposition to *V. faba*, which offers no such landing pad, and insects wishing to extract nectar must hover. Coleopteran species, theoretically, may be able to do this, but will likely favour species with an accessible

landing area and a horizontal orientation.^{17,18,19}

Conclusion

Overall, the high presence of bees and other insects suggests that both species are relevant in drawing diversity to regenerating forest and can be useful in the context of conservation. However, from the results, it would be suggested to plant the *Passiflora miniata*, as it attracted pollinators for longer periods. Plus, there is no risk of a native species becoming invasive as there is with the non-native *Vicia faba*. Furthermore, the addition of *P. miniata* can facilitate opportunities for local communities, as their fruits can be harvested and sold. The addition of *Passifloraceae* to areas such as regenerating forest can heavily aid the attraction of pollinating species, which will, in turn, visit trees in the area and promote seedling recruitment. This can help to speed up the regenerative process, and therefore allow forest diversity to return back to an area once affected by disturbance.

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