Comparing Effectiveness of Hand Pollination, Wild Insects and Local Stingless Bees (Tetragonula laeviceps) for Pollination of Exotic Mauritius Raspberry (Rubus rosifolius)

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ABSTRACT

Mauritius raspberry (*Rubus rosifolius***) is an exotic plant cultivated in Indonesia. Studies showed that the productivity of exotic plants is reducing due to a lack of pollination agent for their origin region. Several methods could be applied to overcome this problem. The study aimed to find the most effective method for pollinating this plant among four pollination regimes: Self-pollination, wild insects, stingless bees (***Tetragonula laeviceps***), and hand-pollination. The observation was conducted on the insect pollinators' activities (visitation rate, flower constancy, and flower handling time), pollination efficiency, and quality of the fruits produced (fruit volume). The results showed a higher visitation rate (10 to 70 per hour), higher flower constancy (visited from 07.00 to 16.00), and longer flower handling time (13.6 s) of** *T. laeviceps* **on raspberry flowers than other insects. High activities related to better raspberry pollination success (96%) and bigger fruits produced. Based on this study, applying stingless bees as pollination agents for exotic plants was the best and potentially applicable to other exotic crops.**

1. Introduction

One of the emerging farming activities in many tropical countries is the cultivation of exotic fruits (Tiwari *et al.* 2021), which could alter the distribution and abundance of species (Taylor and Irwin 2004; Seebens *et al.* 2015). The most cultivated exotic fruits are berries, which are the most widely cultivated, such as strawberry, mulberry, and raspberry (*Rubus rosifolius*) (Valkenburg and Bunyapraphatsara 2001). Even though the economic value of raspberry is higher than that of other berries, this species is the least likely to be cultivated and, to some extent, has become an invasive species in highland Java (Kalkman 1993). The most common reason for the lack of intensive raspberry is low productivity

level, which is suspected to be related to genetics, soil conditions, environmental factors, and biotic interactions. In this study, the biotic interaction effect on the productivity of raspberries becomes the main subject.

Pollination is one of the important biotic interactions for fruit production (Ollerton *et al.* 2011; Burkle *et al.* 2013; Gonzalez-Varo *et al.* 2013; Reilly *et al.* 2020). Raspberry has a perfect flower with 60–120 stamens located around a central receptacle and pistils arising spirally from the receptacle and predominantly able to do self-pollination (Żurawicz 2016). This arrangement usually only allows the outer stigmas to contact the anthers (Free 1993), and insect pollination, especially that mediated by bumble bees and honey bees, could improve the yield and quality of the fruit that comply with commercial standards (Andrikopoulos and Cane 2018; Saez *et al.* 2018; Chen *et al.* 2021).

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In nature, flowering plants have developed strong relations with particular animals for pollination services. Exotic crops usually suffer from a lack of pollination due to the unavailability of their natural pollinators, and they must rely on wild native pollinators (Garibaldi *et al.* 2013, 2014; Rader *et al.* 2016; Page *et al.* 2021; Miñarro *et al.* 2023; Eeraerts *et al.* 2023; Saez *et al.* 2023). The benefits of the pollination service by wild pollinators depend on the diversity and abundance of the visiting wild pollinators and the location (Garibaldi *et al.* 2013; Kleijn *et al.* 2015; Winfree *et al.* 2015; Pérez‐Méndez *et al.* 2020; Earaerts *et al.* 2023). Diverse wild pollinators will provide better services as different insect species could complement each other and produce an additive pollination effect (Miñarro and García 2018; Winfree 2013). Even though this model is promising, accumulating evidence suggests that wild pollinator populations are declining worldwide, primarily due to farming practices (Shuler *et al.* 2005; Potts *et al.* 2010; Hallmann *et al.* 2017; Evans *et al.* 2018; Dicks *et al.* 2021). On the other hand, there needs to be more clarity about the quantity and quality of the pollen transferred by wild pollinators (Garibaldi *et al.* 2016), which makes this service relatively unstable and unreliable. Furthermore, the information on the impact of wild pollinators on the productivity of exotic crops is relatively scarce, which could lead to inappropriate conservation programs for wild pollinators as some efficient pollinators are non bees (Rader *et al.* 2016) that could be mistaken as destructive insects.

Hand pollination, an assisted supplementary or exclusive pollination in which pollen is manually or mechanically applied onto flower pistils, is also applicable to ensure the pollination of exotic crops (Pinillos and Cuevas 2008; Wurz *et al.* 2021). In supplementary terms, during hand pollination, pollen is applied to augment natural pollination (Silveira *et al.* 2012), while as exclusive pollination, hand pollination is the only pollination regime since natural pollination is absent (Westerkamp and Gottsberger 2000). This method is commonly applied in plant breeding (Frankel and Galun 2012). Still, it is now seen as a solid alternative to reduce the effect of pollination limitation due to the lack of natural pollinators (Baldock 2020). The successful hand pollination technique, however, depends on the skill of the workers and the timing of pollination,

as the stigma has a short and dynamic receptive time. Further, there needs to be more information on the effectiveness of hand pollination on raspberry production.

Another possible method to ensure the pollination of exotic crops is by applying local domesticated bees. In the tropics, stingless bees play an essential role in the pollination of tropic flower-pollinator networks (Heard 1999; Santos and Absy 2010; May-Itza *et al.* 2021; Bueno *et al.* 2023; Woitowicz *et al.* 2023). This insect has been domesticated (practices called Melliponiculture) and showed high efficiency when applied as a pollination agent in the agricultural system of various local crops (Suhri *et al.* 2022; Waithaka *et al.* 2023; Wongsa *et al.* 2023). Among the available stingless bees, *T. laeviceps* is considered the best pollinator based on the results of various studies on local crops (Putra *et al.* 2014; A'yunin *et al.* 2019; Alpionita *et al.* 2021) and berries in Indonesia (Alpionita *et al.* 2021; Atmowidi *et al.* 2022).

This study tested the effectiveness of local wild pollinators, hand pollination, and domesticated stingless bees (*T. laeviceps*) in pollinating the exotic Mauritius raspberry. The information gathered could be applied to develop possible pollination strategies for other exotic crops.

2. Materials and Methods

2.1. Study Area

The study was conducted on a small-scale raspberry plantation located at Parongpong, West Bandung, from November to December 2019 (Figure 1A), dominated by vegetable farms. The study area was divided into four designated areas based on the type of pollination application consisting of (i) open pollination, (ii) Stingless bee pollination (using *T. laeviceps*), (iii) hand pollination, (iv) self-pollination (Figure 1B). Two colonies of *T. laeviceps* were installed for *T. laeviceps* pollination group.

The area is located at 1,147 meters above sea level, and precipitation is between 39 and 442 mm³. Average humidity was 55 to 100%, temperature 16 to 30°C, and maximum light intensity 120,000 lux. Raspberries were planted on the soil with an average soil temperature of 25°C, humidity between 10 to 12.5%, and pH of 6.53.

Figure 1. (A) Study area location at small-scale raspberry plantation, (B) arrangement of the study area

2.2. Pollination Study

For this purpose, 200 unbloomed flowers were randomly selected and bagged with a pollination bag $(14 \times 17$ cm) made of nylon mesh (diameter 1 mm). The flowers are divided evenly into four pollination regimes, totaling 50 flowers per pollination regime. All bags were removed when the flowers started blooming, except for the self- pollination group. The bags were opened for *T. laeviceps* and human pollination until the flower was pollinated by *T. laeviceps* and human (using a fine brush), respectively. After pollination, the flower is then bagged (until fruit is produced), and the flower stem is covered with glue to prevent further pollination by wild insects. On the other hand, the pollination bags were entirely removed for the open pollination group.

2.3. Flower Visiting Insect Observation

Observations were conducted on the blooming flowers during the periods (1) morning (07.30-09.30), (2) noon (12.30–14.30), and afternoon (15.30-17.30). Observations were conducted by focal sampling the species and the total number of visiting insects. Insects were sampled and identified based (1) Borror *et al.* (2005) (for the general insect to family level), (2) Lien and Carpenter (2002), Lien *et al.* (2006), Michener (2007), Engel (2012), and Smith (2012) (for Hymenoptera to genus level), (3) Scudder and Cannings (2006), Carvalho and Patiu (2008), Speight (2014), Sengupta *et al.* (2016) (for Diptera to genus level), (4) Dombroskie (2011) and Baskoro *et al.* (2018) (for Lepidoptera to genus level).

Information on activity time (the time when a particular insect could be found visiting flowers), visitation rate (number of individuals visiting flowers), and handling time (time spent in the flowers) were recorded.

The level of pollination efficiency (PE) was calculated based on Keys *et al.* (1995):

 $PE = \frac{\text{Total number of flower produced fruits}}{\text{Total number of the period.}}$ Total number of observed flowers

2.4. Fruit Quality

Raspberry quality was determined by fruit volume as the fruit consisted of a collection of smaller fruits and the irregular shape of the fruit, which usually caused inconsistency in weight measurement by digital scale. Further, volume is generally applied based on the fruit's quality after post-harvest treatment (usually by drying and freezing treatment) (Stamenkovic *et al.* 2019). The volume of the raspberry was determined by the water displacement method.

2.5. Data Analysis

The data on the fruit quality showed a nonnormal distribution. Thus, the differences in the effect of pollination regimes on the fruit quality were determined by the Kruskal-Wallis test, followed by the Least Significant Difference as a post hoc test. The significance level was at P<0,05. A simple X-Y graph was produced by Microsoft Excel to show the effect of pollination efficiency and visitation rate. All statistical processes were done using the IBM Statistical Package for Social Science (IBM SPSS) ver. 22.

3. Results

3.1. Wild Flower-Visiting Insects

During this study, 16 wild insects visited raspberry flowers dominated by Hymenoptera. Interestingly, all visiting Hymenoptera belong to solitary insects. All of the wild insects did not visit the flower after 13.00,

and only 5 species had a flower handling time of more than 1 second (Table 1).

3.2. Visitation Rate and Handling Time of Stingless Bees at Raspberry Flowers

Unlike wild insects, stingless bees constantly visited, especially from 10.00 to 14.00. The peak visitation rate of stingless bees to raspberry flowers was recorded at 13.00 (Figure 2A. Stingless bees also showed significantly longer flower handling time than wild insects (See Table 1). The longest handling time was recorded at 13.00 (Figure 2B).

3.3. Pollination Efficiency

The pollination efficiency of stingless bees was the highest among other pollination regimes. This study also showed that raspberries can produce fruits by self-pollination at a relatively good level. Application of stingless bees improved the fruit production level by 12%, 20%, and 50% compared to wild insect, human-

Table 1. List of wild insects (without stingless bees) that produced the most significant fruit (ANOVA, P<0.05).
Tukey's pairwise P<0.05) (Figure 5A). Application of visited raspberry flowers during the study

Species	Ordo	Active time	Average flower handling time (second)
Vespa velutina Lasius niger Xylocopa aestuans Myzinium			$\overline{2}$ 6 5 < 1
quinquecincta Monobia quandridens Ropalidia marginata Tachypompilus	Hymenoptera	07.00-13.00	≤ 1 < 1 ≤ 1
analis Vesa affinis Lucilia sericata Sarcophaga			< 1 < 1 ≤ 1
haemorrhoidalis Simosyrphus grandicornis Eristalinus quinquelineatus	Diptera	09.00-13.00	5 < 1
Ypthima nigricans Junonia almana Hypolimnas misippus Mycalesis horsfeldii	Hymenoptera	07.00–12.00	< 1 7 < 1 < 1

assisted pollination, and self-pollination, respectively (Figure 3A). Each pollination regime had a different effect on the fruit volume produced as stingless bees pollination produced significant bigger fruits (Figure 3B). On the other hand, pollination success positively correlated to fruit volume (Figure 3C).

In this study, we found some failed to produce and abort fruit from human and self-pollination groups. Both failed and aborted fruit characterized by a lack of fruit formation and irregular shape (Figure 4). Among the pollination regime, the stingless bees regime had the lowest number of failed and aborted fruit (2, 4% of total sampled flowers) followed by open pollinators (8, 16% of total sampled flowers), hand pollination (12, 24% of total sampled flowers) and self-pollination (27, 54% of total sampled flowers).

3.4. Fruit Quality

The fruit volume of the self-pollination group was significantly lower than that of other groups. Application of stingless bees as pollination agent produced the most significant fruit (ANOVA, P<0.05,

Figure 3. (A) Comparison of the pollination efficiency of four types of pollination regimes and (B) Effect of pollination efficiency on fruit volume

Figure 4. Failed and aborted raspberry fruits

Figure 5. (A) Comparison of the raspberry volume produced by four types of pollination. (*) indicated significant value with P<0.05. Box indicated the average and standard deviation of the result while the line indicated the range of the data, (B) effect of visitation rate on fruit volume

stingless bees significantly as pollinator improved the volume level of fruit by % compared to fruit produced by wild insects pollination, 134% compared to human pollination, and 285% compared to self-pollination. Further analysis of the data of the fruit volume produced by stingless bee pollination showed a strong positive correlation between visitation rate and fruit volume (Figure 5B).

4. Discussion

4.1. Development of Local Pollination Interaction by Raspberry

In their native land, raspberry flowers are pollinated mainly by bumble bees and honey bees (Willmer *et al.* 2008; Cane 2005; Lye *et al.* 2011; Sáez *et al.* 2014; Neilsen *et al.* 2017). During our study, however, only one wild bee species, *Xylocopa aestuans*, visited raspberry flowers. Further, no social bees visited the flower, which may indicate the low environmental condition of the plantation, as the social bees required a constant supply of energy from both wild and cultivated flowers.

However, this study found a high pollination efficiency provided by local insect communities and local domesticated stingless bees, albeit a lack of honeybees as significant pollinators of this fruit. The result confirmed the importance of wild insects and non-bees as pollinators of crops (Garibaldi *et al.* 2013; Rader *et al.* 2016). It seems that non-bee insects provided pollination services to raspberries from resource-gathering activities as raspberry acts as potential food resources (Drossart *et al.* 2017). Collection of food resources, especially nectar, which is abundant in the raspberry flower (Kostryco and Chwil 2022), by insects from exotic species may relate to changes in insect community and the availability of traditional food sources due to climate change and land use alteration (Villa *et al.* 2009; Potts *et al.* 2010; Williams *et al.* 2011).

Although specific studies on the importance of pollination for each wild insect species were not done, it could be assumed that insects that spent more time act as possible pollinators of raspberry. Among wild species collected in this study, it is more likely that ants and flies acted as primary pollinators for raspberry in our study area, and both of these guilds were also reported as primary nonbee pollinators (Schiestl and Glaser 2012; Cook *et al.* 2020). Further studies are required to test this hypothesis.

4.2. Stingless Bees as Pollinator of Raspberry

Our study showed constant visits of stingless bees to raspberry flowers, which are attractive to bees (Goodwin 2012; Howard *et al.* 2021). Raspberry is a generalist floral morphology (i.e., open flower, short corollas, numerous stamens) that provides easy access to stingless bees to collect nectar and pollen resources (Olesen *et al.* 2007). Raspberry provides abundant nectar sources compared to surrounding vegetation, dominated by leafy vegetables, corn, and trees that produce flowers seasonally. This condition strongly attracts wild pollinators (Krishna and Keasar 2018; Dellinger 2020; Staab *et al.* 2020; Schmack and Egerer 2023). On the other hand, nectar production of raspberry followed a clear pattern that depended on the cultivar, which may explain the longer flower handling time at 10.00 (Schmidt *et al.* 2015). Further study is needed to confirm if this pattern matches pollen quality and stigma receptivity, two major components that ensure successful pollination. This constant visit to flowers was translated into extremely high pollination efficiency at 96%, which was higher than the level of pollination efficiency by their native pollinator like bumble bees and honey bees (at 75 to 85%) (Andrikopoulos and Cane 2018).

Our study showed constant visit of stingless bees to raspberrys, which is in accordance with Goodwin (2012) and Howard et al. (2021). Raspberry is an aggregate fruit consisting of multiple smaller fruits called drupe (Jenning 1988). The shape and cohesion of the fruit highly depend on the number of drupelets in which each of them developed from the fertilized ovary. Lack of successful pollination will significantly reduce the number of drupelets that produce smaller, lighter, and misshapen fruit (Chagnon *et al.* 1989), as shown on fruits produced by human and self-pollination groups. The structure of the reproductive part of the raspberry is not allowing wind pollination to be successful (Normasiwi *et al.* 2021), which explains the low number of drupelets for the self-pollination group. As for hand pollination, which is considered as the best pollination regime, lack of successful pollination could be caused by (1) low quality and quantity of pollen used, which could related to the pollen viability (Gonzalez *et al.* 2006), (2) source of the pollen to ensure cross-pollination between different cultivar which could be lacking at sampled raspberry plantation (Pritchard and Edwards 2006; Zurawicz 2016). The level of self-incompatibility is

varies among raspberry cultivars (Pawar *et al.* 2017; Pinczinger *et al.* 2021), (3) timing of the pollination, which related to the stigma receptivity (Pawar *et al.* 2017), and damage to the pollen and stigma during the pollination process.

Although Goldwin (2012) reported that raspberry plants produce more extensive and more fruit when visited by various insects, our study showed that single species may be able to pollinate raspberries efficiently. It seems that unconstrained visits by stingless bees are more likely to produce successful fertilization, and this study further confirms the lesser importance of the number of pollen grains deposited for raspberry pollination (Saez *et al.* 2014). The result also supports the benefit of introducing domesticated bees to the productivity of various crops, including raspberry (Chen *et al.* 2021). However, some studies showed that this activity could enhance the visitation rate of pollinators insect to flower, which, in the end, reduces the productivity of plants, especially for honey bees (Garibaldi *et al.* 2013; Saez *et al.* 2014). The decreasing productivity is most likely caused by floral damage due to insect activities on the flower. However, our study showed that heavy flower visits by stingless bees improve pollination and fruit quality without causing damage to flowers. The lack of damage to flowers could be related to the body size of stingless bees. Smaller body size prevents possible damage to flowers. It may explain better pollination efficiency by stingless bees than natural pollinators of raspberry (Saez *et al.* 2014), and smaller body size probably matches the characteristics of the raspberry flower (Naghiloo *et al.* 2021). Further studies are required to confirm this hypothesis.

4.3. Implication of Study

This study proves the importance of local ecosystem service (e.g., pollination) systems for small-scale farmers' resilience and sustainability (Stratton *et al.* 2020). Further, the result showed the possibility of the positive contribution of insect pollinators to self-fertile crops (Costa and Machado 2012; Saez 2018). In the case of pollination services, alternative or supplemental pollinators could be applied when they are proven to be a practical, economical alternative and could compensate for the level of pollination provided by wild pollinators (Hallet *et al.* 2017).

Our study showed that local domesticated stingless bees could applied as alternative pollinators for raspberry instead of importing native pollinators, which potentially significantly altered the local community and plant-insect interaction (Paini and Roberts 2005; Morales *et al.* 2013). As for small-scale farmers, the application of domesticated stingless bees could be developed into an integrative farming system in which farmers could get additional benefits from stingless bee products (e.g., honey, propolis, pollen) while practicing environmentally friendly cultivation methods due to the sensitivity of these bees to the pesticide. Further, this study could act as a base for developing a sustainable model for using pollination services by local wild and domesticated bees (Garibaldi *et al.* 2013) as a precision pollination system that ensures the crosspollination and application of suitable pollinator agents.

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