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Investigating bark and ambrosia beetle communities in apple orchards of East Java, Indonesia

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Bark and ambrosia beetles are major pests in fruit tree orchards worldwide. In this study, we aimed to investigate bark and ambrosia beetle communities present in apple orchards in East Java, Indonesia. We sampled bark and ambrosia beetle communities using ethanol-baited traps in two different cropping systems, *i.e.*, polyculture and monoculture. We collected 353 individuals representing four tribes, 13 genera, and 16 species of both beetles. The most abundant species was *Xylosandrus morigerus* (Blanford), indicating that this species may be well established in apple orchards. Bark and ambrosia beetle abundance and species richness in apple orchards were higher in the polyculture cropping system. Nevertheless, apple orchards surrounded by a high number of forest patches (in a radius of one kilometer) were more at risk of being invaded by bark and ambrosia beetles than those surrounded by a lower number of forest patches. Continued monitoring of bark and ambrosia beetles on apple orchards could play a significant role in the early detection and the development of proactive measures for sustainable pest management.

Keywords: Scolytinae, Xyleborini, monitoring, apple orchard, ethanol-baited bottle trap

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BACKGROUND

Scolytinae (Coleoptera: Curculionidae) are generally divided into bark and ambrosia beetles. Bark beetles build breeding galleries primarily in the sapwood or phloem (Raffa et al. 2015), whereas ambrosia beetles feed on symbiotic fungi after inoculating them in galleries excavated in the wood of the tree (Rassati et al. 2016). Bark and ambrosia beetles can be important pests, especially after leaving their native range and this phenomenon is becoming more and more common due to increasing trade volumes of wood and related products (Hughes et al. 2015; Galko et al. 2019; Grousset et al. 2020; Gugliuzzo et al. 2021). Fruit trees are among the several hosts that most of these beetles can colonize and could cause damages on the infested trees, both of ambrosia beetle (*e.g.*, *Xylosandrus germanus* (Blandford) on apple trees) (Agnello et al. 2021; Agnello et al. 2015) and bark beetles (*e.g.*, *Cryphalus dilutus* Eichhoff) (Gugliuzzo et al. 2023). In Indonesia, apple is of important commodities for the local farmers (Astuti et al. 2015) and no damage caused by these bettles has been reported so far. Nonetheless, considering that climate change will likely increase susceptibility of several trees to bark and ambrosia beetle attacks, understanding which species are present in these ecosystems is of utmost important for planning management strategies (Green et al. 2020).

Differences between landscapes on diversity and abundance of species are related to the scale of the landscape mosaic that contributes to the habitat affinities or population dynamics of individual species (Debinski et al. 2001). In addition, landscapes also play a fundamental role in the abundance and diversity of insects when the size and physical arrangement of habitat patches differ (Hunter 2002). Composition and richness of ambrosia beetles in agricultural lands and forests were also influenced by landscape composition and management (Tarno et al. 2021 2022a b)

In this study we aimed to investigate bark and ambrosia beetle communities present in apple orchards in Indonesia. In particular, we implemented ethanol-baited traps method to sample apple orchards in two different cropping systems, *i.e.*, polyculture and monoculture. We compared the two cropping systems to investigate whether the abundance and species richness of bark and ambrosia beetle differ between polyculture and monoculture apple orchards. These two systems are the most two common cropping practices in East Java, Indonesia.

MATERIALS AND METHODS

Study site

The study was performed at eight apple orchards located in the central apple-producing region of East Java in Batu City, Indonesia (Fig. S1). Among them, four were managed with polyculture and the rest with monoculture. For polyculture, we referred to apple orchards in which horticultural plants were also planted (Table S1 and Fig. S1). For monoculture, we referred to orchards with grass as ground cover (Table S1 and Fig. S1). In this study, we also investigated the forest fragment, i.e. number of forest patches. The number of forest patches in each study site was characterized by a circle with a radius of one kilometer centered on the apple orchard. The forest land uses were marked using Google Earth digital maps for each circle and then manually digitized with QGIS software (version 3.26.3). The mean of forest patches in polyculture apple orchards was 19 ± 3.85, while in monoculture apple orchards it was 12 ± 0.40 (Table S1 and Fig. S2).

Sampling and Identification of bark and ambrosia beetles

In this study, we baited bark and ambrosia beetles using bottle traps containing 96% ethanol (Tarno et al. 2021). The bottle trap was made from a transparent polyethylene terephthalate (PET) bottle with a volume of 1.5 L. The PET bottle was modified in the section below as a specimen container containing soap solution (water mixed with soap), and one window was cut on the side. A plastic Ziploc bag containing 96% ethanol was installed in the window part of the trap as bait. Ten traps were arranged in a transect in the middle of the apple orchard (Fig. S3). Each trap was set up at about one meter from the ground. In total, 80 traps were installed in this study. Traps were checked, samples collected, and refilled every five days from December 2022 to January 2023. The collected beetles on the field were brought to the Laboratory of Plant Pest, Universitas Brawijaya, and stored in 70% ethanol. Based on morphological characters, each specimen was identified manually using an Olympus SZ51 stereomicroscope (Olympus Optical Co., Ltd., Tokyo, Japan). Bark and ambrosia beetles were identified using the following articles Smith et al. (2020) and Johnson et al. (2022).

Data analyses

Species accumulations were generated using interpolated and extrapolated Hill number curves from individual-based abundance data. Analysis of variance (ANOVA) was used to compare the abundance and number of species of bark and ambrosia beetles between cropping systems (polyculture *vs.* monoculture), after data transformation to log(x + 1) to achieve normal distribution. Differences in bark and ambrosia beetle communities were calculated using the Bray–Curtis index (dissimilarity based on species abundance) and visualized using non-metric multidimensional scaling (NMDS). The effect of apple orchard cropping systems on bark and ambrosia beetle composition was calculated using similarity analyses (ANOSIM). The effect of the number of forest patches (explanatory variables) on the abundance and number of species of bark and ambrosia beetles was analyzed by a generalized linear model (GLM). All data were analyzed using RStudio software (R Core Team, 2018), with the ggplot2 package to provide boxplots, and the vegan package to calculate NMDS and ANOSIM (Oksanen et al. 2015).

RESULTS

Diversity of bark and ambrosia beetles in the apple orchard

In total, 353 individuals of Scolytinae beetles were collected, representing four tribes, 13 genera, and 16 species (Table 1). The Xyleborini tribe was represented by the highest number of individuals (273 individuals) and species (11 species). In this study, we collected 13 species of ambrosia beetles and three species of bark beetles. The most abundant species of ambrosia beetle and bark beetle were *Xylosandrus morigerus* and *Hypothenemus* sp.1, respectively (Table 1). The accumulation curve for species richness showed lower richness in monoculture than in polyculture apple orchards (Fig. 1).

Table 1. Diversity of bark and ambrosia beetles collected in apple orchard with two different cropping systems in Batu City, East Java, Indonesia

Tribe/Species	No. of Specimens		Total	%	Feeding guild
	Polyculture	Monoculture			
Xyleborini					
Xylosandrus discolor (Blandford)	19	12	31	8.78	AB
Xylosandrus morigerus (Blandford)	64	33	97	27.48	AB
Xylosandrus crassiusculus (Motschulsky)	5	2	7	1.98	AB
Xyleborus perforans (Wollaston)	38	17	55	15.58	AB
Xyleborinus andrewesi (Blandford)	7	2	9	2.55	AB
Cnestus aterrimus (Eggers)	1	1	2	0.57	AB
Ambrosiodmus sp.	1	-	1	0.28	AB
Euwallacea fornicatus (Eichhoff) species complex	6	1	7	1.98	AB
Diuncus haberkorni (Eggers)	28	21	49	13.88	AB
Debus adusticollis (Motschulsky)	2	2	4	1.13	AB
Eccoptopterus spinosus (Olivier)	10	1	11	3.12	AB
Ipini					
Premnobius cavipennis Eichhoff	3	2	5	1.42	AB
Scolytoplatypodini					
Scolytoplatypus sp.	1	-	1	0.28	AB

Cryphalini					
Hypothenemus sp.1	38	24	62	17.56	BB
Hypothenemus sp.2	2	1	3	0.85	BB
Cryphalus sp.	6	3	9	2.55	BB
Number of individuals	**231**	**122**	**353**	**100**	
Number of species	**16**	**14**			

Note: AB = ambrosia beetle; BB = bark beetle.

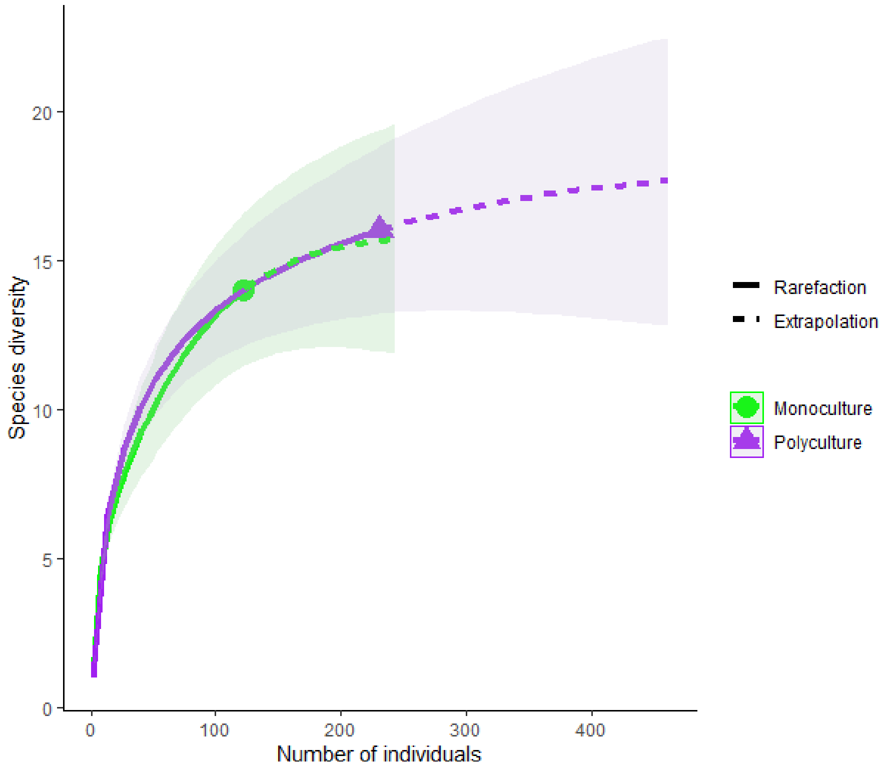

Fig. 1. Accumulation curves of Hill numbers (q = 0) represent species richness of bark and ambrosia beetles on two cropping systems of apple orchards in Batu City, East Java.

Comparison of bark and ambrosia beetle communities in apple orchards managed as polyculture *vs.* monoculture

In this study, the abundance and number of species of bark beetles showed no significant difference between polyculture and monoculture. Whereas, ambrosia beetles had the highest number of individuals (F = 11.570, p = 0.001) and number of species (F = 6.953, p = 0.01) in apple orchards managed as polyculture. The total abundance (F = 8.971, p = 0.003) and number of species (F = 5.784, p = 0.001) of scolytine beetles collected in this study were higher in polyculture than monoculture (Fig. 1).

The GLM results showed that the number of forest patches did not affect the abundance and number of species of bark beetles, ambrosia beetles, and total of scolytine beetles in this study (Table S2).

Based on ANOSIM, we found that the species composition of bark beetles (R = -0.072, p = 0.635) and ambrosia beetles (R = 0.093, p = 0.302) did not differ between monoculture and polyculture. We also found that the total scolytine beetles between apple orchards managed as monoculture and polyculture did not differ (R = 0.078; p = 0.327), as indicated by the overlap of plot groups within apple orchard management (Fig. 3).

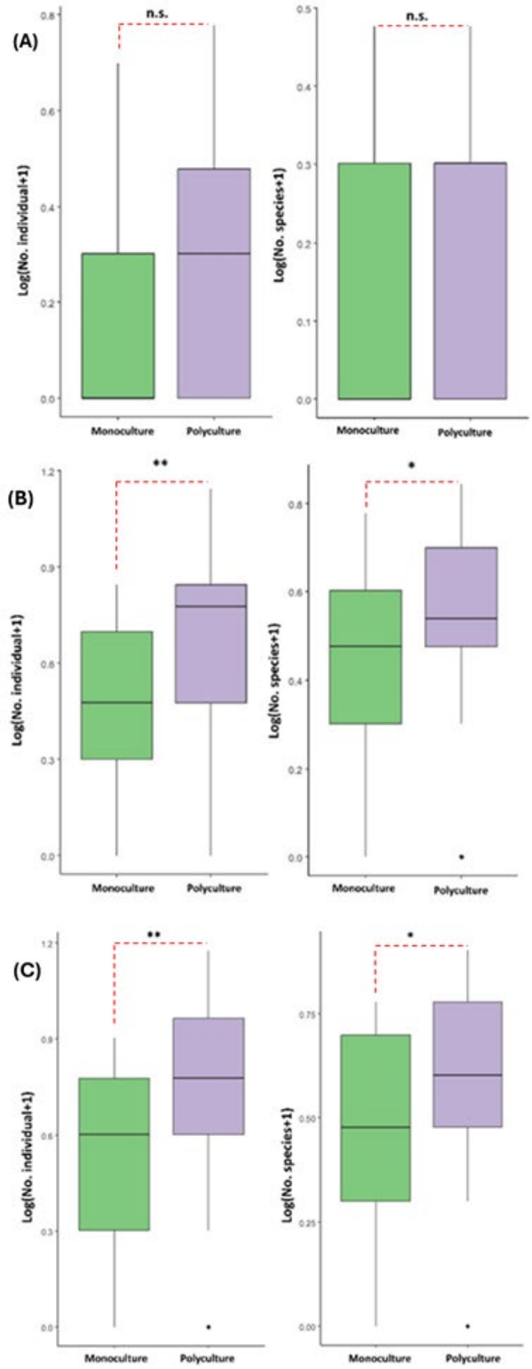

Fig. 2. The difference in the number of individuals and species of (A) bark beetles, (B) ambrosia beetles, and (C) total of scolytine beetles between monoculture and polyculture apple orchards. Number of individuals and species were transformed to log(x+1) to achieve normal distribution. According to Tukey's test, above each box plot, n.s. indicates no significant difference, [**] indicates significant differences ($p < 0.01$), and [**] indicates significant differences ($p <$

0.05). Bars represent the interquartile range with the median value. Vertical solid lines indicate the minimum and maximum values.

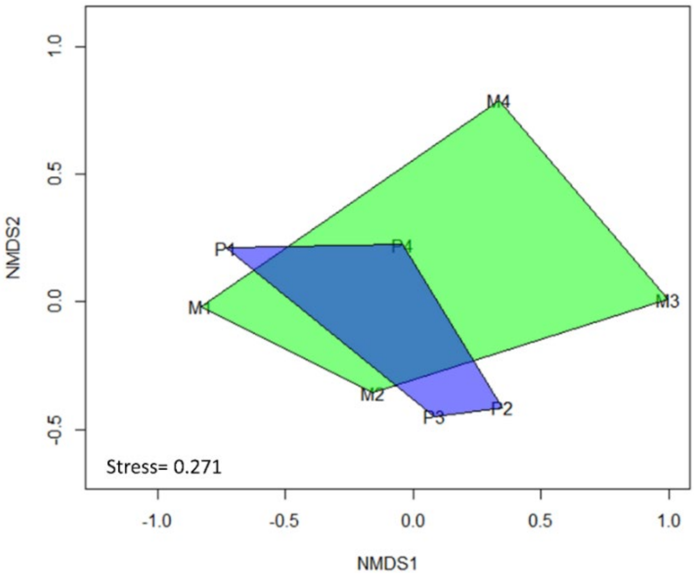

Fig. 3. Nonmetric multidimensional scaling (NMDS) of species composition of scolytine beetles between polyculture and monoculture apple orchard in Batu City, East Java based on Bray-Curtis dissimilarity index. The letters indicate cropping systems: P = polyculture and M = monoculture; and the numbers (1–4) indicate sites as replications.

DISCUSSION

We found that Xyleborini was the most abundant and species-rich tribe in the monitored apple orchards. This is not surprising as the same tribe was shown to be the most abundant in studies targeting ambrosia beetles in apple orchards in the USA (Monterrosa et al. 2022). According to Ruzzier et al. (2023), Xyleborini is widespread throughout the world's major climate regions, and many species have invaded temperate and tropical areas of the world. Several xyleborine species are reported as pests on different fruit trees, including avocado (Kendra et al. 2020), grapevines (Ruzzier et al. 2021), coffee (Greco and Wright 2015), and apple (Agnello et al. 2017). Thus, it would not be surprising to observe damage by xyleborine ambrosia beetles also on apple trees in Indonesia over time.

Among Xyleborini, *X. morigerus* was the most abundant species collected in apple orchards. This species was reported as the primary pest of coffee plants in Java, Indonesia, and occurs in a wide variety of host plants (75 species in 33 families) (Kalshoven 1961; Wood and Bright 1992). It usually breeds in twigs or small branches, but can attack larger stems up to ca. 20 cm in diameter (Ruzzier et al. 2022 2023), indicating that *X. morigerus* may be able to colonize apple trees in Indonesia. Sandoval Rodríguez et al. (2017) and Martinez et al. (2017) also collected *X. morigerus* using ethanol-baits in Brazil, and in Ecuador, respectively. In addition to *X. morigerus*, other *Xylosandrus* spp. were collected in our study, *i.e.*, *Xylosandrus crassiusculus* (Motschulsky) and *Xylosandrus discolor* (Blanford). *Xylosandrus crassiusculus* was also previously reported in fruit orchards, such as apple, peach, and avocado, in the USA (Menocal et al. 2018; Viloria et al. 2021; Monterrosa et al. 2022).

Another ambrosia beetle that was collected in abundance in this study was *Xyleborus perforans* (Wollaston). According to Smith and Hulcr (2015), the genus *Xyleborus* is broadly distributed worldwide and is a polyphyletic genus of more than 400 known species. Some species of *Xyleborus* are also reported as highly invasive and destructive to forest and orchard pests, for example, *Xyleborus glabratus* Eichhoff, is a primary vector of the pathogenic fungus that causes laurel wilt in several forest tree and avocado industries (Hughes et al. 2015). In our study, we also collected *Premnobius cavipennis* Eichhoff (Ipini), which has been reported to be abundant in plantations in Indonesia, Ecuador, and Brazil (Zanuncio et al. 2005; Martinez et al. 2017; Setiawan et al. 2018; Tarno et al. 2022b).

Hypothenemus sp.1 (bark beetle) was found to be the second most abundant species in our study. According to Vega et al. (2015), the genus *Hypothenemus* is common in tropical and subtropical areas, and most species are very small and difficult to distinguish. The majority of the *Hypothenemus* species live innocuously in twigs and only some species are reported to be important pests (Vega et al. 2015; Constantino et al. 2021).

In this study, species richness and abundance of bark and ambrosia beetles were significantly higher in polyculture than in monoculture system. This result suggests that polyculture cropping systems in apple orchards may promote the abundance and species richness of bark and ambrosia beetles. Of note, we investigated polyculture systems corresponding to apple trees with horticultural plants that are not potential hosts of the bark and ambrosia beetles. However, the higher beetle abundance and diversity may result from the higher mean number of forest patches (forest fragments) surrounding the polyculture study sites (Table S1 and Fig. S1). Macedo-Reis et al. (2019) showed that xylophagous insect abundance and richness were positively affected by forest areas in the surrounding landscape, reflecting potential dispersal distances. Sittichaya et al (2012) also reported that xyleborine ambrosia beetles are moderately strong fliers and possibly migrate from the surrounding populations.

The species composition of bark and ambrosia beetles in polyculture and monoculture apple orchards was similar, indicating that several species were found in both cropping systems. This may result from our focus on orchards in the same climatic zone and with the same host tree species. According to Sittichaya et al. (2012), ambrosia beetle composition did not differ significantly between cropping systems and study zones, possibly due to similar climatic conditions.

Additionally, a study in avocado orchards with different management systems showed that all ambrosia beetle species were collected in all orchards (Menocal et al. 2022). These studies were consistent with our result that species composition on the same tree species remained similar under different orchard management systems.

CONCLUSIONS

This study reports on the bark and ambrosia beetle communities in apple orchards of the East Java region, Indonesia. Some collected species of ambrosia beetles have been reported as important pests of several plants. It would not be surprising to observe damage by xyleborine ambrosia beetles on apple trees in Indonesia in the following years. *Xylosandrus morigerus* was a dominant species that occurred in both cropping systems, indicating that this species may be established in the apple orchards in this region. We found that the apple orchards surrounded by a high amount of forest fragments were more at risk of being invaded by bark and ambrosia beetles than those surrounded by a lesser amount of forest fragments. We also found that the species composition of bark and ambrosia beetles was similar in both cropping systems. Continued monitoring of bark and ambrosia beetles on apple orchards could play a role in the early detection and the development of proactive measures for sustainable pest management.

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Supplementary materials

Fig. S1. The location of the apple orchard for collecting bark and ambrosia beetles in Batu City, East Java, Indonesia. The letters indicated cropping systems (P and M are polycultures and monocultures, respectively), and the numbers (from 1 to 4) states apple orchards as replications in each cropping system. The two small figures on the right side are representatives of the vegetational condition of polycultures (bottom) and monocultures (above). (download)

Fig. S2. Detail of forest patches within a one-kilometer radius around the study sites. (download)

Fig. S3. Sampling design of each research site/plot. Plotting the ethanol-baited bottle traps in the research site/plot. (download)

Fig. S4. Morphological diversity of bark and ambrosia beetles collected on monocultures and polycultures of apple orchards in Batu City, East Java, Indonesia: (A) *Xylosandrus discolor* (Blandford); (B) *Xylosandrus morigerus* (Blandford); (C) *Xylosandrus crassiusculus* (Motschulsky); (D) *Xyleborus perforans* (Wollaston); (E) *Xyleborinus andrewesi* (Blandford); (F) *Cnestus aterrimus* (Eggers); (G) *Ambrosiodmus* sp.; (H) *Euwallacea fornicatus* (Eichhoff) species complex; (I) *Diuncus haberkorni* (Eggers); (J) *Debus adusticollis* (Motschulsky); (K) *Eccoptopterus spinosus* (Olivier); (L) *Premnobius cavipennis* Eichhoff; (M) *Scolytoplatypus* sp.; (N) *Hypothenemus* sp.1; (O) *Hypothenemus* sp.2; and (P) *Cryphalus* sp. (download)

Table S1. Summary of locations for collecting bark and ambrosia beetles on the apple orchard in Batu City, East Java, Indonesia. (download)

Table S2. Generalized linear models based on the abundance and number of species of bark, and ambrosia beetles to the number of forest patches. (download)