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Incidence and damage of the ambrosia beetle on cocoa plants in East Luwu District, South Sulawesi, Indonesia

NURIADI[1], SYLVIA SJAM[2,♥], AHDIN GASSA[2], VIEN SARTIKA DEWI[2]
[1]Doctoral Program of Agricultural Science, Graduate School, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia
[2]Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Hasanuddin. Jl. Perintis Kemerdekaan Km.10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia. Tel./fax.: +62281242949266, ♥email: sylviasjam@yahoo.com

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Abstract. *Nuriadi, Sjam S, Gassa A, Dewi VS. 2023. Incidence and damage of the ambrosia beetle on cocoa plants in East Luwu District, South Sulawesi, Indonesia. Biodiversitas 24: 3592-3600.* The attack of ambrosia beetle on cocoa plants (*Theobroma cacao* L.) in East Luwu District, South Sulawesi Province, was first reported in 2020 at the Wotu sub-district. However, there was no information on its intensity and the number of affected sub-districts. A survey and data collection were conducted from January to July 2021 by selecting a 1 hectare area from 7 out of the 11 affected sub-districts identified in the preliminary study. The parameters observed were attack intensity, the number of galleries in stems and branches, and the number of trapped ambrosia beetle. The results showed that the attack of the ambrosia beetle on cocoa plants in the 7 sub-districts ranged from moderate to severe intensity, causing damage to mature plants and seedlings. It was observed that the number of galleries was higher on stems than on branches. The intensity of attacks on cocoa plants aged 23-27 years is higher than the cocoa aged 8-15 years. Additionally, the obtained species of ambrosia beetle are *Xylosandrus* sp.1 (Coleoptera: Curculionidae), *Xylosandrus* sp.2 (Coleoptera: Curculionidae), *Xyleborus* sp. (Coleoptera: Scolytidae) and *Hypothenemus* sp. (Coleoptera: Scolytidae). The results are expected to provide important information about the invasion of the ambrosia beetle and the prevention steps of its spread on cocoa plantations in South Sulawesi.

Keywords: Attack, galleries, invasion, *Theobroma cacao*

INTRODUCTION

Cocoa (*Theobroma cacao* L.) is one of the main commodities in Sulawesi plantations, and it plays an important role in the national economy, especially as a provider of employment, income, and foreign exchange (Cruz et al. 2018). East Luwu District, the third largest cocoa producer in South Sulawesi, covers 27,822 hectares with yields of 11,896 tons (Directorate General of Estatecrops 2021). However, pest and disease attacks on plants remain a limiting factor in the production. One such threat is the ambrosia beetle (Gregoire et al. 2014) which has been reported to attack cocoa plants in several countries such as India (Thube et al. 2022), Brazil (Macedo-reis et al. 2016), and Peru (Delgado and Couturier 2017). This beetle was reported to also attack various plant species such as *Persea americana* (Carrillo et al. 2012), *Tectona grandis* (Tarno et al. 2022), *Pinus* spp. (Ploetz et al. 2013), *Pterocarpus indicus* (Tarno et al. 2021) and *Areca catechu* L. (Thube et al. 2018), resulting in a significant decrease in population and production of the main commodity.

The population of the ambrosia beetle detected to attack teak plants in Southeast Sulawesi (Maros) included *Xyleborus affinis* (47.17%), *Xylosandrus crassiusculus* (27.64%), and *Hypothenemus* sp. (12.33%) (Tarno et al. 2022). Furthermore, the first attack of this pest on cocoa plants occurred in East Luwu District, South Sulawesi Province, in Wotu Sub-district. The preliminary study identified the presence of ambrosia beetle in 7 sub-districts. The damage caused by this pest attack is characterized by galleries in the stems and branches, with advanced symptoms of leaf wilting and yellowing (Asman et al. 2021).

The ambrosia beetle is known to cause damage to plants, resulting in wilting and death. The attack occurs by boring the stem and causing structural damage to the woody stem (Sobel et al. 2015). Specifically, it was reported to attack cocoa seedlings in Peru, causing 20.5% of deaths (Delgado and Couturier 2017). Furthermore, the *X. crassiusculus* species was reported to attack cocoa beans by 1.54% (150 out of 9750) in the plantations at Vittal, India (Thube et al. 2022). In Indonesia, the intensity of ambrosia beetle attacks on robusta coffee plants in Sukabumi ranged from 1.69 to 25.51% (Indriati et al. 2017). The impact of this pest on *Pterocarpus indicus* plants in Malang has been severe, with 3.206 trees attacked and 69.7% discovered dead (Tarno et al. 2014). Aside from direct damage to plant tissues, the ambrosia beetle can also transmit various pathogens, such as fungi, yeast, and bacteria, which can further exacerbate plant damage and mortality (Ploetz et al. 2013; Dzurenko and Hulcr 2022; Tarno et al. 2022; Thube et al. 2022).

Cocoa production in the East Luwu district of South Sulawesi, Indonesia, has been affected by the increasing rate of cocoa deaths caused by the ambrosia beetle attack. The attack of ambrosia beetle on cocoa plants in East Luwu District, South Sulawesi Province, was first reported in

2020 at the Wotu sub-district (Asman et al. 2021). There has been no report on the attack intensity of this pest on cocoa trees in Indonesia, especially in East Luwu. Therefore, this study identified the species of ambrosia beetle and analyzed the attack intensity on cocoa trees. The results are expected to provide important information about the invasion and the prevention of massive invasion in all cocoa plantations in South Sulawesi.

MATERIALS AND METHODS

Experimental Site

The study was conducted in East Luwu District, South Sulawesi Province, from January to July 2021. The location was situated at an elevation of 15-68 m.a.s.l with an average temperature, humidity, and rainfall of 26.97°C, 82.39%, and 15.57 mm, respectively. The measurement of light intensity was carried out using Lux Meter. Furthermore, the observation was conducted in cocoa plantations infested with ambrosia beetle based on a preliminary study, as shown in Figure 1. The plantations were located in the Wotu, Burau, Angkona, Mangkutana, Wasuponda, and Malili sub-districts. Table 1 summarizes the age of the plants, varieties, coordinate points and planting patterns in each District. Moreover, beetle samples were obtained from traps that were placed at the location of the affected trees, which were planted with a monoculture planting pattern.

Observation of ambrosia beetle attack intensity

To measure the attack intensity, a 1 hectare area was selected in the location affected by the ambrosia beetle in 7 sub-districts obtained from preliminary research conducted in 11 sub-districts at the East Luwu District. Within each sub-district affected by the ambrosia beetle, 250 trees were observed and divided into 5 plots of 50 trees each, selected from a population of 1035-1235 trees per hectare randomly selected. The process of observing the attacked trees and counting the number of galleries on the stem and branches took 5 months, spanning from January to June 2021. During this period, the cocoa stem was peeled off to confirm the galleries left by the beetle, and the adult beetle and its eggs were examined. The following formula was used to calculate the attack intensity:

$$I = \frac{a}{b} \text{ x } 100\% \qquad (1)$$

Where I = Attack Intensity (%), a = Number of affected plants, and b = the total number of plants. The value for each category of attack intensity ranged from 0-25 % = mild, 25-50 % = moderate, 50-75 % = severe, and > 75 % = very severe (Indriati et al. 2017).

Insect collection and identification

In July 2021, the insect was collected using 20 trap bottles installed in each plot, with a distance of 20 meters between the installations. Observations were conducted 16 times at 4 intervals in one week across all the trees simultaneously. Furthermore, the samples obtained were preserved in specimen bottles and labeled with the location and date. Ethanol 95% baited trap bottles were used as an attractant (Flechtmann et al. 2000). The material was 1.5 L transparent plastic bottles, with half of the side cut to resemble a window (Steininger et al. 2015).

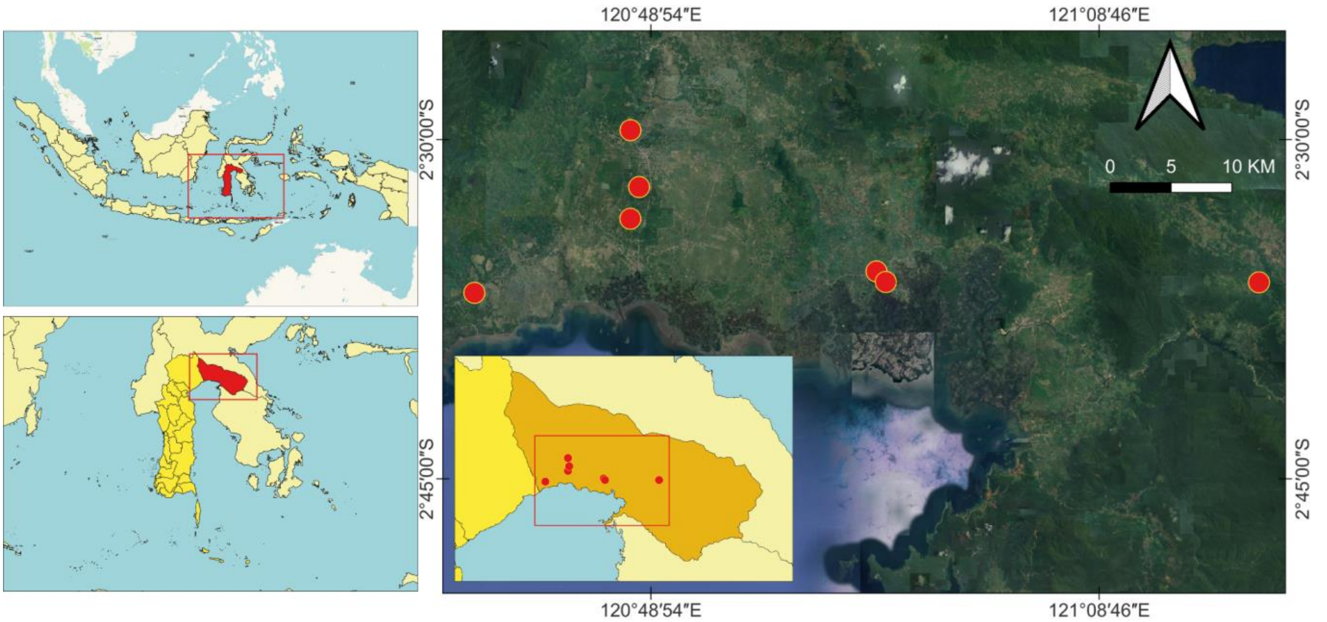

Figure 1. Observation locations of ambrosia beetle in East Luwu District, South Sulawesi Province, Indonesia

Table 1. Data of plant age, variety, planting patterns and coordinate points on cocoa plantations for each observation location in East Luwu District, South Sulawesi Province, Indonesia

Sub-district	Age plant (year)	Clone	Planting patterns	Latitude
Wotu	27	M01	Monoculture	02°33'30.24" S, 120°47'59.31" E
Burau	23	S1	Monoculture	02’36’46.54" S, 120’41’04.66" E
Tomoni	12	M01	Monoculture	02’32’06.00 " S, 120’48’22.53" E
Angkona	25	S1	Monoculture	02’35’49.91" S, 120’58’53.54" E
Mangkutana	15	BB	Monoculture	02’29’35.23" S, 120’47’59.54" E
Wasuponda	8	45	Monoculture	02’36’18.73 " S, 121’15’50.27" E
Malili	25	BB	Monoculture	02°36'17.72" S, 120°59'18.06" E

The traps were equipped with a styrofoam plate (acting as a roof to minimize rainwater entering the traps). Additionally, a plastic clip containing 5 ml of ethanol 95% and detergent water was added to precipitate the trapped insects, and the traps were tied to the tree stem using a string, as shown in Figure 2. The collected samples were identified using a Leica EZ4HD microscope, referring to the book A monograph of the Xyleborini (Coleoptera, Curculionidae, Scolytinae) of the Indochinese Peninsula (except Malaysia) and China (Smith et al. 2020). Consequently, observing the pronotum, elytra, and body length down to the genus level was necessary for identification. DNA barcoding is required to identify species.

Data analysis

Normality data was tested using Kolmogorov-Smirnov test with the assumption that if the significance value is >0.05, then the data is normally distributed, otherwise if the significance value is <0.05, then it is not normally distributed. Based on Kolmogorov-Smirnov test, a significance value of 0.200 >0.05 was obtained, which means the data was normally distributed. The attack intensity and the number of trapped beetle were analyzed using analysis of variance (ANOVA) to determine significant differences at the 5% level. When there were significant differences (P<0.05), Duncan's test was performed at a confidence level of 95%. Student's t-test was used to compare the number of attacks on the stem and branches. Finally, data analysis was performed using IBM SPSS Statistics ver. 25 software.

RESULTS AND DISCUSSION

Results

Observations on the bottle trap installation showed that the number of ambrosia beetle trapped in each sub-district was dominated by *Xylosandrus* sp.1 with 1777 individuals, *Xylosandrus* sp.*2* with 1201 individuals, *Xyleborus* sp. with 1192 individuals, and *Hypothenemus* sp. with 184 individuals, as shown in Table 2. Beetle samples were obtained from traps placed at the location of the infected trees planted with a monoculture cropping pattern. The abundant ambrosia beetles are *Xylosandrus* sp.1 (40.81%), *Xylosandrus* sp.2 (27.58%) and *Xyleborus* sp. (27.38%) (Figure 3).

The number of ambrosia beetles between each species trapped in Wotu and Tomoni Districts was significantly different (P=0.000). Meanwhile, the number of trapped beetles in Burau and Mangkutana Districts was not significantly different (P=0.468 dan P=0.076) between *Xylosandrus* sp.1, *Xylosandrus* sp.2, and *Xyleborus* sp., but significantly different (P=0.000) with *Hypothenemus* sp. The number of trapped beetles in Angkona District was not significantly different (P=0.151) between *Xylosandrus* sp.1, *Xylosandrus* sp.2, and *Xyleborus* sp., but significantly different (P=0.000) with *Hypothenemus* sp. The number of trapped beetles in Wasuponda District was not significantly different (P=0.147) between *Xylosandrus* sp.1 and *Xylosandrus* sp.2, but significantly different (P=0.000) with *Xyleborus* sp. and *Hypothenemus* sp. While the number of trapped beetles in Malili District was not significantly different (P=0.165) between *Xylosandrus* sp.1, *Xylosandrus* sp.2., *Xyleborus* sp. (P=0.057) but significantly different (P=0.000) with *Hypothenemus* sp. (Figure 4).

Figure 2. Ambrosia beetle traps tied to cocoa tree stems

Table 2. The number of individual ambrosia beetles trapped in East Luwu District, South Sulawesi Province, Indonesia

Species	Observation location							Total (n)	%
	Wotu	Burau	Tomoni	Angkona	Mangkutana	Wasuponda	Malili		
Xylosandrus sp.1	349	237	339	245	231	191	185	1777	40.81
Xylosandrus sp.2	251	216	203	174	114	127	116	1201	27.58
Xyleborus sp.	159	228	135	195	213	106	156	1192	27.38
Hypothenemus sp.	36	34	32	28	19	14	21	184	4.23
Total	795	715	709	642	577	438	478	4354	100

Figure 3. Ambrosia beetle is abundant and attacks cocoa plants in East Luwu District, South Sulawesi Province, Indonesia. A: *Xylosandrus* sp.1 ; B : *Xylosandrus* sp.2; C: *Xyleborus* sp.)

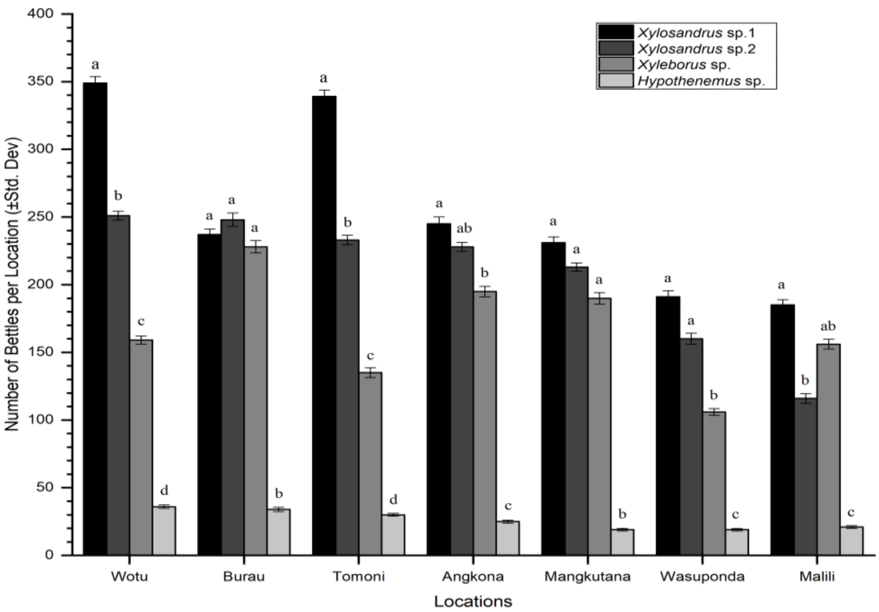

Figure 4. The number of beetle per location in East Luwu District, South Sulawesi Province, Indonesia, from each species trapped, and the values followed by the same letter are not significantly different

Characteristics of damage to cocoa plants

Based on our observations, the symptoms of ambrosia weevil-infected plants resemble those of cocoa plants in decline. Multiple cavities in the trunk and branches of the tree with an average hole diameter of 0.8-1.3 mm present symptoms. The presence of pierced cavities with frass powder (Figure 5A) was indicative of plants exhibiting decline symptoms. The predominant frass was found in the form of flour. Frass and the ambrosia beetle were discovered on all cocoa trees exhibiting decline symptoms. On the affected cocoa stem bark are black galleries and egg clusters (Figures 5B, 5C). Figure 5D demonstrated that subsequent assaults on the stems resulted in the loss of the bark's outermost layer and the withering of the cocoa stalks.

Figure 5. A. Galleries with frass on the infected 20-year-old cocoa tree stem, B. Galleries on the cocoa stem, C. Ambrosia beetle eggs on the cocoa stem, D. Symptoms of decline on the stem

Symptoms of dieback can also be observed on infected plants and seedlings, where the leaves wilt and dry out, as shown in Figures 6A, and 6B. Furthermore, the attack was observed on the seedlings, with signs such as galleries with frass on the stem (Figure 6C, 6D). On seedlings with symptoms of death (Figure 6E), splitting the stem showed traces of galleries and colonization of fungi (Figure 6F, 6G). In seedlings, the signs of death are detectable through the presence of galleries or gallery traces that are visible when the stem is split. However, fungal isolation is not carried out from dead plants.

Table 3 shows that number of galleries was higher on the stem than on the branches. The average number of galleries per tree on the stem and branches was significantly different. This suggests that insects prefer to feed on stems (lignosus) over branches.

The attack intensity in Wotu (plant age 27 years), Burau (plant age 23 years), Angkona (plant age 25 years), and Malili sub-districts (plant age 25 years) was in a severe category (53.20-61.20%), Whereas the attack intensity in Tomoni (plant age 12 years), Mangkutana (plant age 15 years), and Wasuponda (plant age 8 years), was in the moderate category (36.80-45.20%) (Figure 7). The intensity of attacks in Wotu sub-district was higher and significantly different (P=0.000) when compared to other sub-districts. However, the intensity of attacks in Wasuponda Subdistrict was lower and not significantly different (P=0.111) from the intensity of attacks in Tomoni and Mangkutana Subdistricts, but significantly different (P=0.000) compared to other subdistricts. Meanwhile, the attack intensity in Burau, Tomoni, Angkona, Mangkutana and Malili sub-districts was not significantly different (P=0.055) (Figure 7).

Table 3. The average number of galleries per tree on the stem and branches in East Luwu District, South Sulawesi Province, Indonesia

Sub-district	Average number of holes	
	Stem (diameter 32.7-37.9)	**Brench (diameter 9.3-12.5)**
Wotu	42.96 ± 0.906*	10.24 ± 0.524
Burau	40.65 ± 0.928*	9.61 ± 0.405
Tomoni	38.50 ± 1.139*	11.22 ± 1.111
Angkona	36.56 ± 1.044*	9.73 ± 0.474
Mangkutana	35.93 ± 0.833*	9.26 ± 0.540
Wasuponda	33.81 ± 0.968*	8.80 ± 0.410
Malili	34.32 ± 1.032*	10.78 ± 0.457

Note: *significantly different at α 0.05

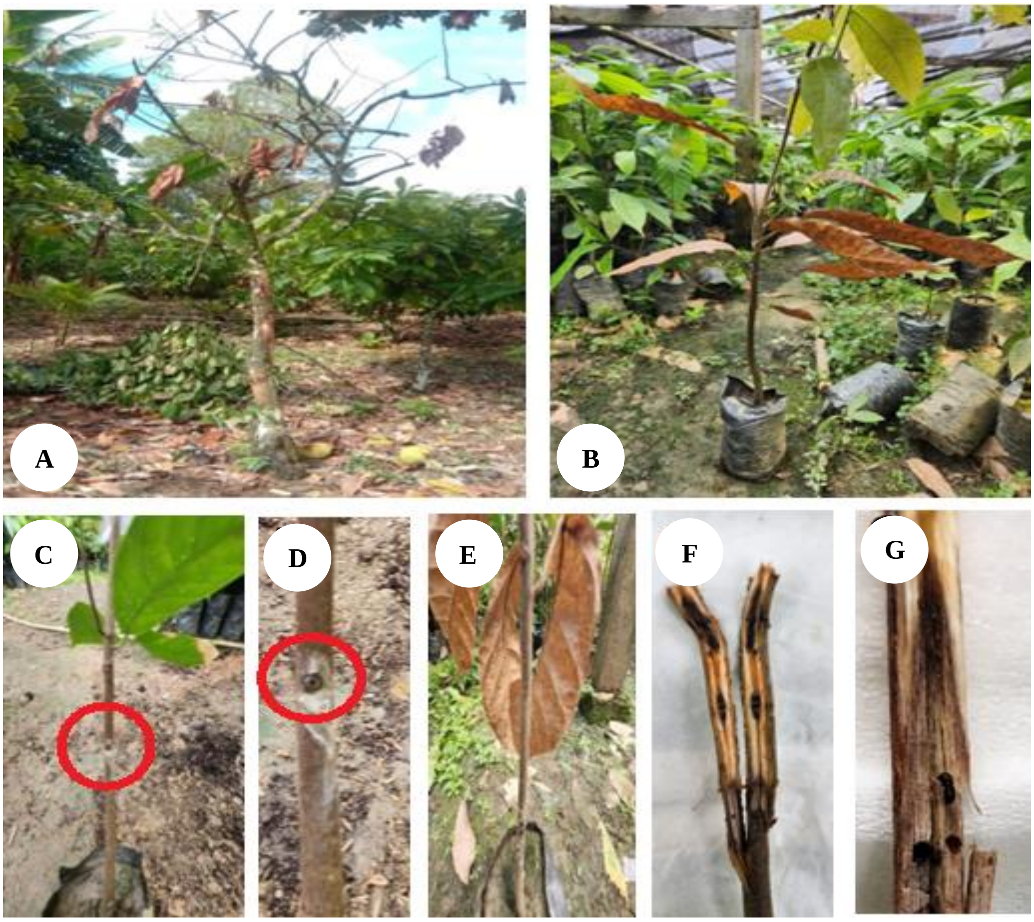

Figure 6. A. Symptoms of decline in the mature cocoa plant (15 years old); B. Dieback symptoms on cocoa seedling (4 months old); C, D. Galleries with frass on the base of seedling stem showing symptoms of death (4.5 months old); E. Seedling showing symptoms of death (4.5 months old); F, G. Internal galleries and fungal colonization

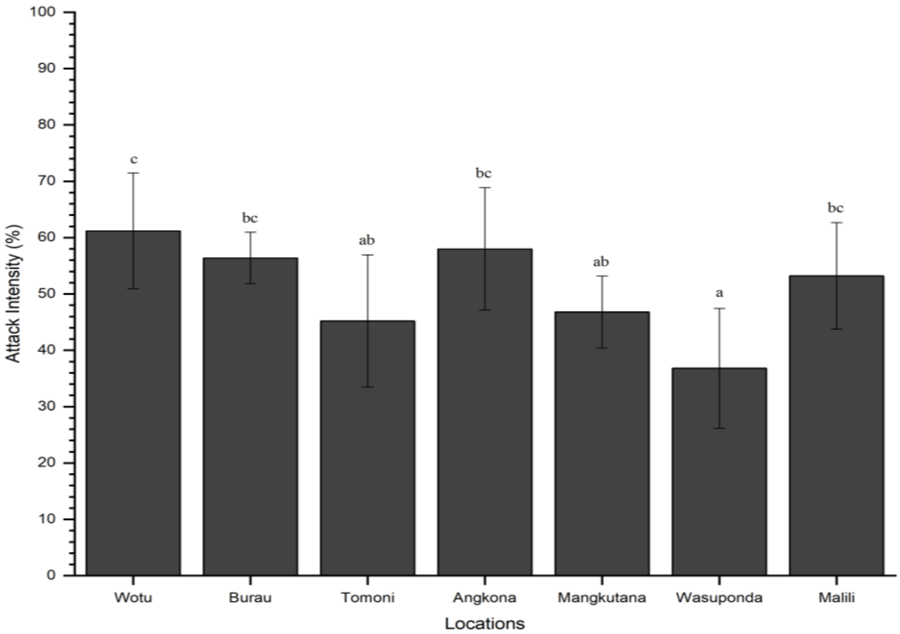

Figure 7. Attack intensity of ambrosia beetle on cocoa plants in East Luwu District, South Sulawesi Province, Indonesia, and the values followed by the same letter are not significantly different

Discussion

The age of plants affects the intensity of the attack, and the results indicated that severe attacks were discovered on cocoa trees aged 23-27 in Wotu, Burau, Angkona, and Malili sub-districts. Meanwhile, moderate attacks were observed on those aged 8-15 in Tomoni, Mangkutana, and Wasuponda sub-districts. The field observations and interviews on cocoa trees aged 20-27 showed that farmers do not perform monthly routine maintenance, such as sanitation, pruning, and shading, due to declining fruit productivity. However, it was conducted monthly on those aged 8-15 due to their high fruit productivity. This causes a difference in shading density between these categories of trees. Measurements using a lux meter at 1:00 PM local time in cocoa plantations indicated that the average sunlight intensity on trees aged 20-27 years was 568-1,829 lux, while on those aged 11-15, it was 2,574-4,235 lux. Shading density affects the low intensity of sunlight entering the plantation, as it decreases the temperature and increases the humidity, hence, impacting the attack intensity. This is related to the intensity of ambrosia beetle attacks on coffee plants. Hultman (2016) explained that the attack of *Xylosandrus compactus* on *Coffea arabica* is higher in shaded plants. Similarly, Kagezi et al. (2013) reported that *X. compactus* attack could be about 70.80% and 45.80% in fully shaded and open coffee plants, respectively.

The observations showed a significant difference in the number of galleries between the stem and branches, with a higher occurrence on the stem than branches. This means that the beetle tends to attack stems with larger diameters as they provide a greater volume of sapwood, supplying enough space for the pest (Tarno 2014). It is similar to cases of ambrosia beetle attacks on coffee plants, where a greater concentration of infestation is noted on the stem as compared to the branches. Severe damage can occur when the galleries created by the beetle harm the vascular tissue, including the phloem and xylem, and even the cambium tissue. These galleries on the branches have cut off the vascular tissue, disrupting the transportation of nutrients, leading to wilting of the branch tips, yellowing of leaves, and ultimately plant death (Indriati et al. 2017).

Previous research reported that damage caused by ambrosia beetle attacks is due to the association with fungi. Asman et al. (2021) discovered that several fungi isolated from cocoa plants in East Luwu District were attacked by ambrosia beetle, includes *Fusarium* (two isolates), *Lasiodiplodia* colony, *Ceratocystis* colony, and *Diaporthe* colony (two isolates), while the beetle species manually identified on the stem was *X. compactus*. Fungus Fusarium is a cause of dieback symptoms in cocoa plants in Ghana and Sulawesi, Indonesia (Rosmana et al. 2013). *Lasiodiplodia* spp., specifically *L. theobromae* and *L. pseudotheobromae* have been reported to cause fruit rot and stem canker disease in cocoa plants in Ghana, Philippines, and Sulawesi (Alvindia and Gallema 2017; Ali et al. 2019). *Ceratocystis cacaofunesta was* reported to cause wilting and death of cocoa plants in the Caribbean, Central and South America, as well as Colombia (Engelbrecht et al. 2007). Meanwhile, *Diaporthe* causes shoot disease and cancer symptoms in various plant species such as *Helianthus annuus* (Thompson et al. 2011), Family Rosaceae (Santos et al. 2017), *Catharanthus roseus* (Yan et al. 2018), and *Vaccinium Corymbosum* (Hilário et al. 2020). Finally, microbes that can associate with ambrosia beetle include *Aspergillus* spp., *Penicilium* spp., *Trichoderma* spp., *Fusarium* spp., *Acremonium* spp., *Gliocladium* spp. (fungi), *Streptomyces* spp. (bacteria), *Saccharomyces* spp., and *Candida* spp. (yeast) (Tarno et al. 2016).

The ambrosia beetle is considered a secondary pest because it attacks plants and creates galleries on the stem, which it uses as a site for fungal association (Hofstetter et al. 2015). After creating the galleries, it stores spores in them as a food source for larvae and adult beetles (Harrington et al. 2008). Galleries with a size of 0.8-1.3 mm are commonly discovered on the stem, but isolation is not performed on the adult beetle located on the media. Frass appears as a powder or flour-like substance.

When cocoa plants are attacked, dieback symptoms are observed, which are likely caused by galleries created and symbiotic fungi carried into the plant tissue, leading to a disruption of water transport through the xylem. These symptoms are present in trees that have been attacked by the ambrosia beetle, as well as galleries on the stem and branches. Furthermore, fungal disturbance to the xylem was indicated by a change in color in the tissue (Fraedrich et al. 2008). The ambrosia beetle trapped was dominated by *Xylosandrus* sp.1, *Xylosandrus* sp.2 and *Xyleborus* sp. Apart from these three abundant species of beetles, *Hypothenemus* sp. was also discovered. The sampling process was conducted during the rainy season for one month, and further research is needed during different seasons and for a longer period to identify additional species that may still be present on cocoa trees.

The ambrosia beetle is widely distributed in tropical areas and can cause significant damage and economic losses to plants and the timber industry (Gugliuzzo et al. 2023). *X. compactus* was reported as the primary pest of coffee plants in Indonesia, Vietnam, India, Sri Lanka, Philippines, Madagascar, West Africa, Fiji, Cuba, Brazil, Peru, Ghana, Nigeria, Cameroon, Uganda, Kenya, and Hawaii (Bukomeko et al. 2018). Meanwhile, *X. crassiusculus* is known to attack coffee, cocoa, mango, papaya, Australian pine, rubber, mahogany, tea, and teakwood in tropical Asia (Thube et al. 2022). *Xyleborus affinis* is commonly discovered in warm areas, especially in tropical and subtropical forests, where it colonizes dead or deteriorating trees in humid conditions (Cognato et al. 2011). It was first reported to attack the ornamental plant *Dracaena fragrans* in Hungary (Merkl and Tusnadi 1992), while in China, it caused damage to *Eucalyptus robusta*, *Eucalyptus* sp., *Hevea brasiliensis*, *Schefflera octophylla*, and *Sindora glabra* between 2014 and 2019 (Lin et al. 2021). *Hypothenemus* sp. is a major pest of coffee plants in Indonesia, attacking Arabica and Robusta varieties (Wiryadiputra 2014). It is also reported to attack coffee plants in Colombia, Kenya, Tanzania, and Ethiopia, making it the most destructive worldwide (Sseremba et al. 2021; Legalov and Poinar 2023).

In conclusion, ambrosia beetle attack on cocoa plants can cause moderate to severe damage, with galleries discovered on the stems and branches as well as symptoms of plant decline identified in both mature plants and seedlings. The dominant species are *Xylosandrus* sp.1, *Xylosandrus* sp.2 and *Xyleborus* sp. Therefore, the results serve as a basis for controlling the population of this pest and minimizing damage and yield losses in cocoa plants.

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