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The occurrence of *Xylosandrus compactus* and its associated fungi on cacao from South Sulawesi, Indonesia: A preliminary study of an emerging threat to the cacao industry

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Abstract

Xylosandrus compactus is one of the significant beetle pests on perennial crops, which has become an increasing problem on cacao productivity and sustainability in South Sulawesi, Indonesia. The beetle is also known as ambrosia beetle due to the obligate association with symbiotic fungi. The beetle and infested plant parts were collected from infested *Theobroma cacao* in the field, then the beetles identified morphologically and the associated fungi isolated. In this study, we found that the beetles attacked all stages of cocoa growing in the field such as seedling, young trees and mature trees with visible symptoms including the appearance of round bore holes with powdery frass on the tree trunk and branches. Also, we found the beetle being distributed in three main cocoa areas in South Sulawesi. According to the beetle's characteristic, the female has the black colour of the body (head and elytra), size 1–2 mm and the shining slope. Six types of fungi were isolated from infected plant parts and the beetle *X. compactus*: *Fusarium*-like colony (two isolates), *Lasiodiplodia*-like colony, *Ceratocystis*-like colony and *Diaporthe*-like colony (two isolates). Among the fungi isolated, several fungi were known as the pathogen. The beetle and its associated fungi are responsible for the typical dieback symptoms, decline and sudden death inflicted on a number of cacao trees. The occurrence of *X. compactus* on cacao tree is reported for the first time from Sulawesi, Indonesia. The beetle pest infestation will become a significant threat to the future of the cacao industry in Sulawesi, Indonesia.

Keywords Cocoa · *Xylosandrus compactus* · Ambrosia beetle · *Fusarium* · *Lasiodiplodia* · *Ceratocystis* · *Diaporthe* · Dieback · First report

Introduction

Xylosandrus compactus (Coleoptera: Curculionidae: Scolytinae), also known as black twig borer, is widely distributed around the world and one of the most significant beetles affecting trees and forests worldwide (Waller et al. 2007, Haack and Rabaglia 2013). The beetle typically inhabits

both phloem and xylem of the plant. In addition, the beetle always has an obligate association with fungi, which in some cases may be phytopathogenic (Paine et al. 1997). Severe infestation inflicted on the crop is caused by symbiotic species of the fungi that is vectored by the insect beetle. Existence of the fungi is crucial for the beetle in their life cycle due to larvae feed on fungi in the gallery (Hara and Beardley 1979).

Cocoa, which is a source of chocolate, is a very significant plant of Indonesia. Millions of people depend on their income from cocoa, especially the commodity cultivated in Sulawesi island. The island contributes more than half of the total of the national production (Directorate general of estate crops 2019). A range of pests and diseases affect the growth of cocoa, both established and emerging pests and diseases. In this work, we studied the occurrence of the *X. compactus* beetle in cacao plantations in South Sulawesi, Indonesia, and their associated fungi.

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Materials and methods

Survey, sampling and beetle identification

Surveys and observations were conducted in 2014–2019 in three main areas of cacao farm in South Sulawesi, Indonesia namely Luwu (3°01'24.0" S, 120°09'57.2" E), North Luwu (2°37'36.4" S, 120°20'53.8" E), and East Luwu (2°33'29.8" S, 120°47'53.5" E). Adult of the beetle and infested plant parts were collected from East Luwu (2°33'29.8" S, 120°47'53.5" E) and kept in plastic containers. On return to the laboratory, the beetle was taken out from the infested plant parts and preserved in vials containing 70% ethanol for morphological identification. The ambrosia beetle was characterized under a light microscope by its colour, the shape of the head, the shape of pronotum and elytra. Photographs of the infested plants and the beetles were made with a Canon PowerShot A2300 digital camera and USB digital microscope YPC-X01.

Fungal isolation and identification

Associated fungi were isolated from infested bark, sapwood under gallery, and adults of the beetle. The samples of infested bark, sapwood under bark and sapwood under gallery were surface sterilized with ethanol 70% for three minutes while the samples of the insects were treated one minute, after that washed with sterile water three times and finally plated on potato dextrose agar (PDA) medium supplemented with chloramphenicol as an antibiotic. Petri dishes were incubated at 25–27 °C and observed for fungal growth every day. A small piece of fungal mycelium was taken from the growing margin with a cork borer and placed on a new petri dish containing a new PDA medium under aseptic conditions for further purification and identification of fungi. Isolated fungi were identified based on their colony characteristics and morphology under the microscope. Photograph of the fungal colony and fungal conidial were made with a Canon PowerShot A2300 digital camera.

Results

Symptoms, effects and area distribution of *X. compactus*

In recent years, we observed the beetles on cocoa trees in South Sulawesi, Indonesia, increasingly. The infestation may cause economic damages because the beetle may kill the twigs, stems, seedlings and even the whole cacao tree.

The beetle on cacao is studied to a less extent, and consequently, very little is known about the beetles distribution and its associated fungi.

Infected cacao plant parts showed the following symptoms (Fig. 1a–g) under field conditions: (i) Bore holes were small, round (1 mm in diameter) and smooth frass surrounded holes; number of holes on seedling and small branches were 1–2 holes (Fig. 1a–b); and (ii) sawdust frass was seen at the base of the tree trunk or under infected branch and powdery frass around bore holes remained attached until several days without disturbance; number of holes could reach 50 until hundreds of holes on the main trunk and at the base of the tree (Fig. 1c–d). After cutting open the infested plant parts, numerous galleries were seen through the bark in the wood (Fig. 1e). Gallery length varied dependent on plant parts attacked. On seedlings and branches galleries of the ambrosia beetle could be observed along the cambium. Also, the gallery was colonized by fungal mycelia (Fig. 1f–g).

X. compactus females had a black body (head and elytra) (Fig. 2a–c), a size of 1–2 mm and shining slope. The male had a brown body (Fig. 2c), a size smaller than the female (1 mm). The beetles were found throughout the observation location. The larvae were small, creamy white in colour and legless (Fig. 2d). Based on these characters, male and female insects were distinguished.

The pest attacked different cocoa trees of different age and obviously caused dieback and killed the tree, including seedling (Fig. 3a), mature clonal tree (Fig. 3b–c) and old hybrid tree (Fig. 3d). Also, the beetle caused disease on the branch and twig (Fig. 3e–f). The pest attacked not the only farm with lack of management (particularly pruning and sanitation) but also the well-managed farm. (The farm was applied good agricultural practices regularly on its cocoa tree, including pruning, fertilization, and sanitation.)

During the observation and sampling period, *X. compactus* was discovered in all three South Sulawesi region, namely Luwu (3°01'24.0" S, 120°09'57.2" E), North Luwu (2°37'36.4" S, 120°20'53.8" E), and East Luwu (2°33'29.8" S, 120°47'53.5" E).

Associated fungi

Six types of fungi were isolated from different source materials such as diseased bark and sapwood under bark, sapwood under gallery and insect beetle. Based on culture morphology and microscopic characteristics, the fungi were identified as *Fusarium*-like colony (two isolates), *Lasioidiplodia*-like colony, *Ceratocystis*-like colony, and *Diaphorthe*-like colony (two isolates) (Table 1).

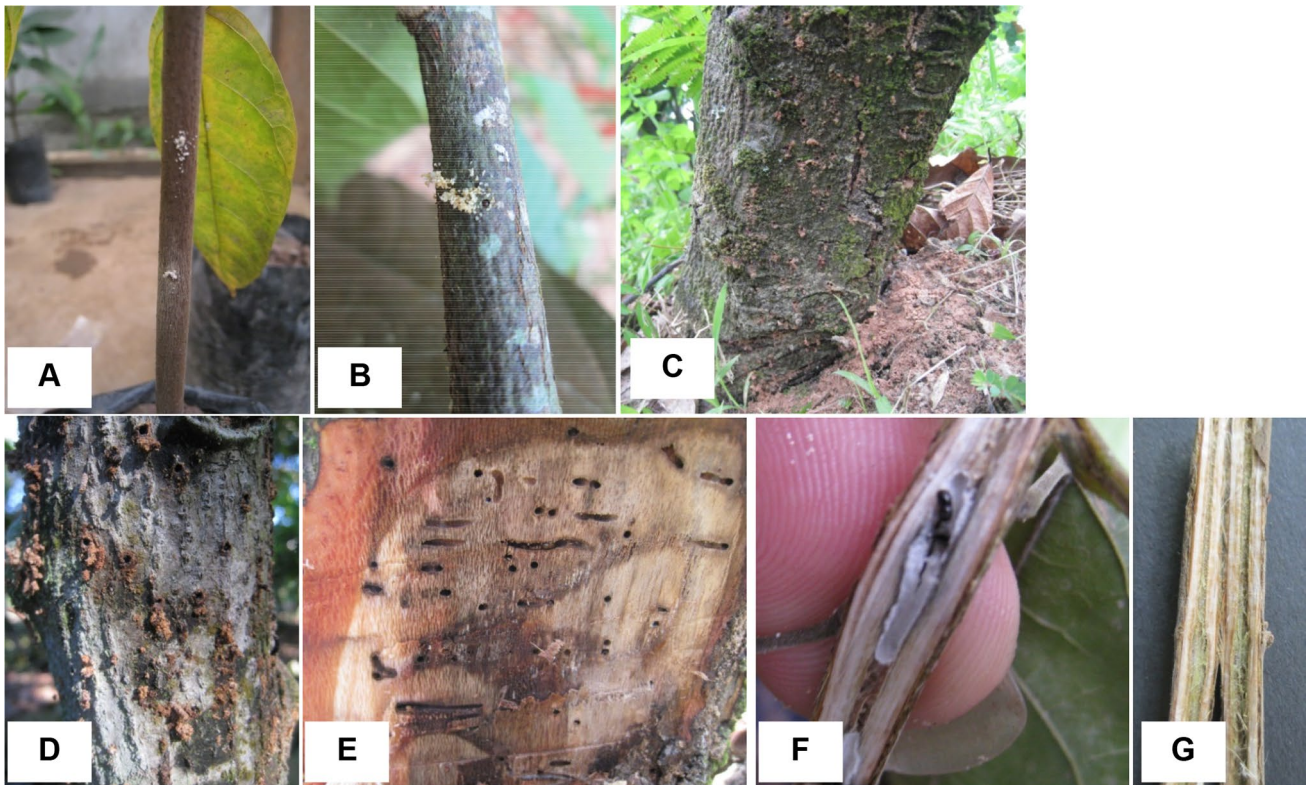


Fig. 1 *X. compactus* burrow different size of wood. **a** Bore holes with powdery frass on stem of seedling, **b** Bore holes with powdery frass on the branch of 4 years old tree, **c** Bore holes with powdery frass and

sawdust at the base of the tree of 20 years old tree, **d** Bore holes with powdery frass on stem of 15 years old tree, **e** Portion of infected galleries, **f-g** Internal gallery and fungal colonization.

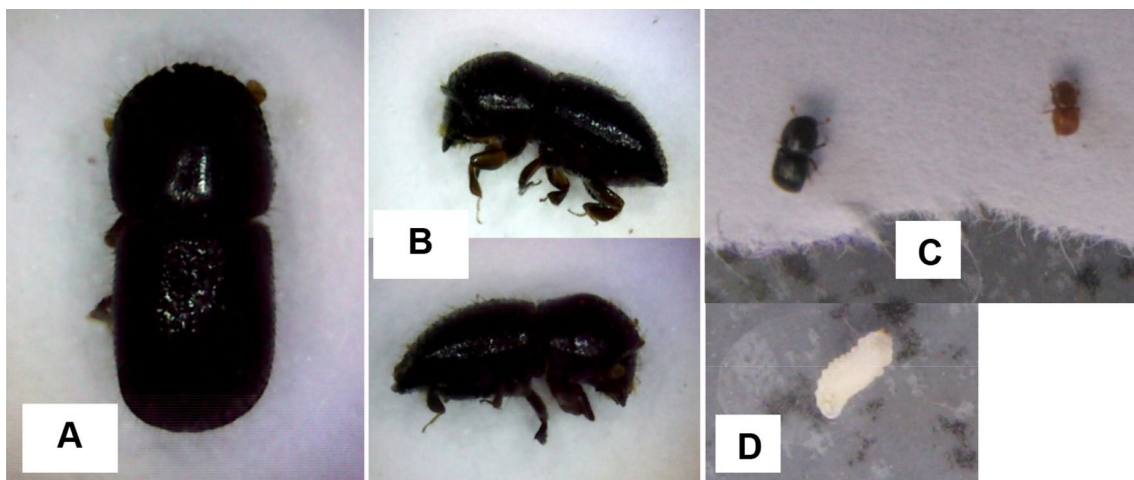


Fig. 2 *X. compactus*. **a-b** Beetle (Female), **c** Beetle, (Female-Left) and Male-Right, **d** Larvae

Discussion

In the field, *X. compactus* attacked all stages growth of cacao tree. However, the symptom appears similar to small bore holes and smooth frass surround holes. The

only different symptoms among the cacao growth phases were the number of bore holes. Bore holes on seedling and small branches were 1–2 holes while on the trunk and the base of the tree could reach 50 holes per tree. Therefore, seedling and a small branch are the most sensitive parts because the pest can kill them with only a few

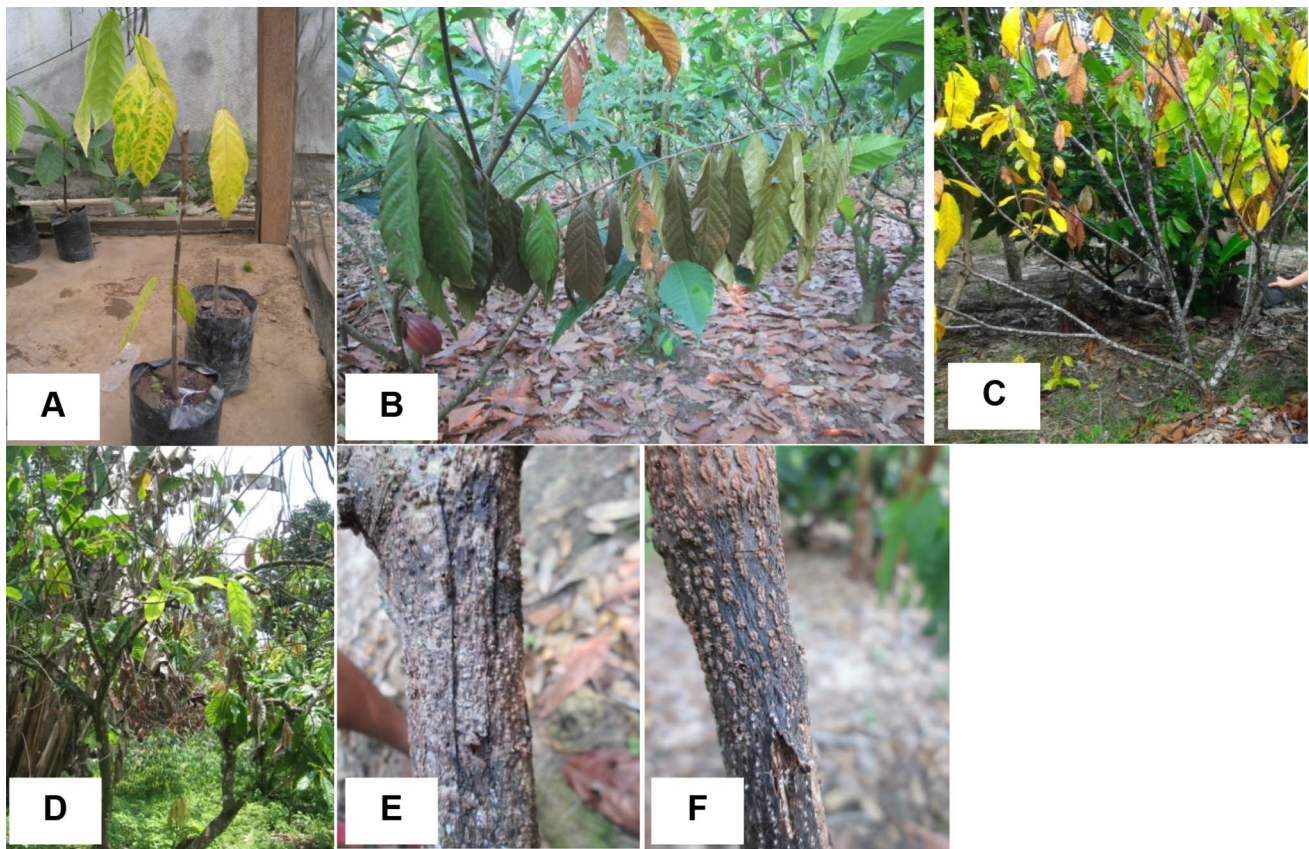


Fig. 3 Effect of *X. compactus* attack at different age of trees. **a** Death of Seedling, **b** Dieback symptoms on a branch of the clonal tree (4-year-old), **c**. Decline symptoms on a mature clonal tree (5-year-

old), **d** Decline symptoms on an old hybrid tree (20-year-old), **e–f** External symptoms of diseased branch and twig associated with *X. compactus*

bore holes in a short time. However, the beetle was able to make severe damage on older trees as well, particularly, when the beetle attacked at the base of the tree with a lot of bore holes.

Dieback and decline are the most observable symptoms of ambrosia beetle's incidence on a variety of commercial crops (Iqbal and Saeed 2012; Mendel et al. 2012; Eskalen et al. 2013). In this work, we have seen dieback and decline on the seedling, young tree and mature cacao tree infested by *X. compactus*. Cocoa tree has experienced substantial losses due to ambrosia beetles infestation in Hawaii (Hara and Beardsley, 1979). Delgado and Couturier (2017) reported incidence *X. compactus* on cacao in Peru.

We could show that *X. compactus* has established a breeding population in cocoa plantations in South Sulawesi. There were no reports of the presence of *X. compactus* on cacao in South Sulawesi previously. The beetle was found in three main cacao region separated by 60 km. In addition, we discovered that *X. compactus* attacked and killed seedlings in nurseries with UV plastic roof.

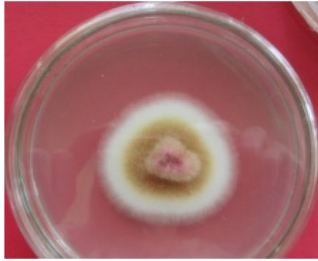
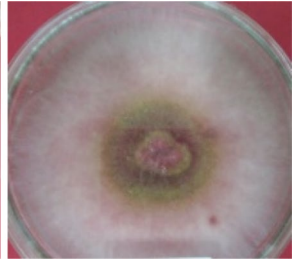


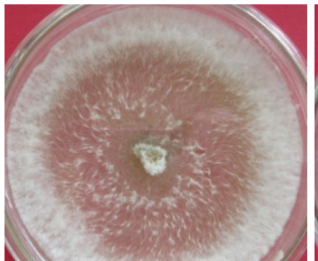

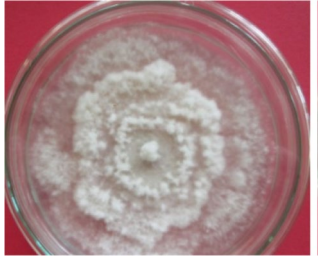
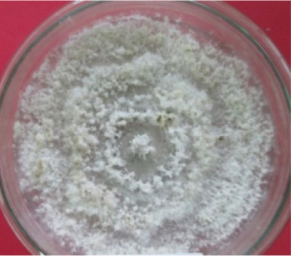


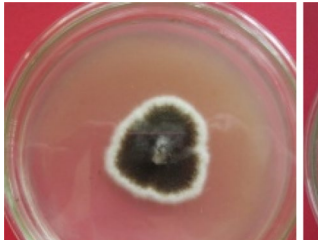

X. compactus is native in Asia. Currently, the beetle is widely distributed around the world, including Sulawesi

island, Indonesia. In South Sulawesi, *X. compactus* was first recorded that it attacked coffee trees in 1927 with a heavy infestation (Kalshoven 1981) and coffee tree remained the primary host of *X. compactus* (Waller et al. 2007, Indriati 2017).

In the present study, *Fusarium*-like colonies, *Lasiodiplodia*-like colony, *Ceratocystis*-like colony, and *Diaporthe*-like colonies are the most prevalent fungi associated with *X. compactus* and infested plants parts. The genus *Fusarium*, *Lasiodiplodia*, and *Diaporthe* seems to be very ubiquitous and has been isolated from healthy and diseased cacao stem previously (Rubini et al. 2005; Amin et al. 2014; Asman et al. 2015; Asman et al. 2018; 2020). In this study, we were not found another common fungal associated with the ambrosia beetle, *Ambrosiella*-like colony, the fungus was frequently isolated from the mycangia of *Xylosandrus* beetle (Six et al. 2009; Bateman et al. 2015, 2016; Mayers et al. 2015). More studies should be done to clarify the identification of these fungi. Also, these isolates could be a pathogen to the tree.

The role of the isolated fungi remains difficult to describe in this study due to not testing them for

Table 1 Associated fungi with *X. compactus* and its infested plant parts

Material origin	No.	Fungi	Colony character (7-day and 40-day)	
Diseased bark and sapwood under bark	1	<i>Fusarium</i> -like colony		
	2	<i>Fusarium</i> -like colony		
	3	<i>Diaporthe</i> -like colony		
	4	<i>Diaporthe</i> -like colony		
Sapwood under gallery	1	<i>Lasiodiplodia</i> -like colony		
Insect Beetle	1	<i>Ceratocystis</i> -like colony		

pathogenicity. However, based on previous research the genera *Fusarium*, *Lasiodiplodia*, and *Ceratocystis* were reported being pathogenic on cacao and the most common fungi associated with ambrosia beetle infestation on various crops such as mango (Massod et al. 2010, Iqbal and Saeed 2012, Souza et al. 2013), Avocado (Mendel et al. 2012; Eskalen et al. 2013), Angsana trees (Bumrungsri et al. 2008). The infestation of *X. compactus* beetle may result in severe damage caused by symbiotic fungi carried by the beetle.

As shown above, the genus *Fusarium* was one of the dominant groups and a serious fungus that attacks numerous crops with a range of symptoms and across the country. *Fusarium* sp. has been reported as a causal agent of dieback symptoms on cacao in Ghana and Sulawesi, Indonesia (Adu-Acheampong et al. 2012; Rosmana et al. 2013; 2014). Meanwhile, *Lasiodiplodia* sp., a cosmopolitan fungus commonly associated with the tropical plant, is a significant fungus on cacao causing dieback, pod rot and canker symptoms around the world including South Sulawesi, Indonesia, particularly the species *Lasiodiplodia theobromae* and *Lasiodiplodia pseudotheobromae* (Adu-Acheampong et al. 2012, Mbenoun et al. 2008, Alvindia and Gallema 2017; Asman 2020, Ali et al. 2020). The genus *Ceratocystis* is known as one of the significant pathogens on cacao in the world, and the most important species is belonging to *Ceratocystis cacaofunesta* (formerly *C. fimbriata*) that causes a lethal disease of cacao in the Caribbean and Central and South America (Engelbrecht et al. 2007, Wyk Van et al. 2010). Even though the genus *Diaporthe* has no report about its pathogenicity on cacao, a number of previous research showed that the fungus causing dieback and canker symptoms diseases in other crops (Thompson et al. 2011, Dissanayake et al. 2017, Yan et al. 2018, Hilário et al. 2020).

To the best of our knowledge, the study is the first report and efforts of the beetles' incidence on cacao in Sulawesi, Indonesia. The occurrence and incidence of the beetles are the crucial concern on the economically significant cocoa crop and will threaten the cocoa industry in the future. Detailed studies on the pest's biology and management aspects are in progress.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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